INVESTIGATING THE POTENTIAL APPLICATIONS OF LENTIL SEED COMPONENTS IN MECHANICALLY SEPARATED CHICKEN MEAT SYSTEMS

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ABSTRACT

The overall goal of this project was to explore the potential applications of lentil seed components in meat systems. Therefore, their functionality as an antioxidant and a binder were investigated in four studies. In study one, the antioxidant efficacy of water and (70% v/v) ethanol extracts of seed coat from two lentil cultivars (CDC Greenland, a large green and CDC Maxim, a small red variety) were studied in *in vitro* assays and a mechanically separated chicken (MSC) model meat system. The total phenolics (TPC) extracted in aqueous (70%) ethanol (43.96–50.46 mg gallic acid equivalents (GAE)/g) were higher ($p<0.05$) than that of water extracts (41.63 –44.30 mg GAE/g). Extracts demonstrated concentration-dependent antioxidant activity irrespective of cultivar or extraction solvent. The addition of seed coat extracts (500 ppm TPC) resulted in significant ($p<0.001$) inhibition of lipid oxidation in MSC during cooking and storage and there was an 11-fold difference in TBARS values between treated and control samples after seven days of refrigerated storage. The antioxidant capacity of seed coat was comparable to Herbalox®, sodium ascorbate, (+)-catechin, and (+/-) - α- tocopherol.

In study two, the phenolic composition and antioxidant capacity of water extracts of seven lentil cultivars (CDC Greenland, CDC Greenstar, CDC Maxim, CDC Robin, CDC SB-3, ZT-4 and 6205-ZT) were determined. TPC, flavonoids (TFC) and condensed tannins (CTC) in normal tannin seed coats ranged from 35.88 to 39.72 mg GAE/g, 3.50 to 5.14 mg catechin equivalents (CE)/g and 21.63 to 28.07 mg CE/g, respectively. Condensed tannin was absent in zero tannin cultivars and TPC was around 6-times lower than that of normal tannin cultivars. Kaempferol tetraglycoside, catechin-3-glucoside, and procyanidins were the most abundant phenolic compounds in normal tannin cultivars, whereas kaempferol tetraglycoside was dominant in zero tannin cultivars. Antioxidant activity measured by DPPH, ABTS, and ferrous ion chelation assays showed strong activity (>70%) at concentrations higher than 400 ppm of TPC. Overall, lentil cultivars with seed coat had relatively higher phenolic content and antioxidant activity compared to other cultivars. TPC, TFC and CTC concentrations were highly correlated ($r = 0.93$ to 0.98) with antioxidant activities showing their positive contribution to antioxidant capacity of seed coat. Both free radical scavenging activity and chelation mechanisms were involved in the overall antioxidant efficacy of seed components.

The objective of the third study was to evaluate the applicability of lentil seed components (flour, seed coat and seed coat water extracts) as replacements for synthetic phosphates in non-cured bologna sausage. The combination of infra-red heated (IR) lentil flour and seed coat extracts (300 ppm or 500 ppm of TPC) were able to replace the techno-functional properties of synthetic phosphates with no negative effects on water-holding, texture, and sensory properties and oxidative stability of bologna, while seed coat itself had negative impact on color, flavor and texture attributes.

The fourth study investigated the efficacy of infra-red heated lentil flour as a binder in wiener type chicken sausages developed for the Sri Lankan market. The water-holding and texture properties, and the proximate composition of the sausages containing lentil flour, were similar to those of the commercial formulation made with isolated soy protein and corn starch, and the level of lentil flour added (4% to 8%) had minimal effect. Oxidative stability of sausages formulated with lentil flour was similar to the commercial formulation, and samples with lentil flour showed no difference in oxidative stability compared to those added sodium nitrite. The cross-cultural consumer acceptability tests conducted with three consumer panels (Canadian consumers, Sri Lankan consumers living in Canada and Sri Lankan consumers living in Sri Lanka, each consisting of 60 panelists) revealed that the liking for sensory properties and overall acceptability of MSC sausages were similar between the commercial product and those with lentil flour added, demonstrating that lentil flour could be an economical and effective meat replacer product. The functionality of lentil flour as a binder were possibly attributable to its high amounts of starch and protein.

In conclusion, this study demonstrated the potential for use of lentil seed components as a potential plant antioxidant and binder in meat products without decline in sensory characteristics and nutritive value. The findings of this study will help in the development of clean label meat products, nutraceutical applications, and breeding strategies for lentil cultivars with health and functional benefits.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

6. STUDY IV: SENSORY CHARACTERISTICS, OXIDATIVE STABILITY AND CROSS-CULTURAL CONSUMER ACCEPTABILITY OF CHICKEN SAUSAGES FORMULATED WITH LENTIL FLOUR

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C	(+)-catechin
CA	Canadian consumers
CA-T	Canadian descriptive sensory panel
CE	Catechin equivalents
CFIA	Canadian Food Inspection Agency
CS	Corn starch
CTC	Condensed tannin content
DPPH	2,2-diphenyl-1-picrylhydrazyl
EG	70% aqueous ethanol extract of CDC Greenland (green) lentil seed coat
ER	70% aqueous ethanol extract of CDC Maxim (red) lentil seed coat
GAE	Gallic acid equivalents
H	Herbalox® rosemary extract
HDC	Hand deboned chicken meat
IR	Infra-red
ISP	Isolated soy protein
LC-MS	Liquid chromatography-mass spectrophotometry
LF	Infrared treated lentil flour
LP	Phospholipid peroxidation inhibition activity
MC	Fe^{2+} ion chelation activity
MDA	Malonaldehyde
MSC	Mechanically separated chicken meat
PCA	Principal component analysis

SA	Sodium ascorbate
SC	Lentil seed coat
SEM	Standard error of mean
SL	Sri Lankan consumers living in Sri Lanka
SL-CA	Sri Lankan consumers living in Canada
SL-T	Sri Lankan descriptive sensory panel
STPP	Sodium tripolyphosphate
T	$(+/-)$ α- tocopherol
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TFC	Total flavonoid content
TPA	Texture profile analysis
TPC	Total phenolic content
WBSF	Warner-Bratzler shear force
WE	Water extract of lentil seed coat
WG	Water extract of CDC Greenland (green) lentil seed coat
WR	Water extract of CDC Maxim (red) lentil seed coat

1. INTRODUCTION

1.1 Overview

Lipid oxidation is a leading cause of quality deterioration in fresh and processed meat. Quality losses in oxidized meat are generally characterized by discoloration, off-flavor, texture deterioration, loss of nutrients, and the possible formation of toxic compounds (Wang and Xiong, 1998; Püssa et al., 2009; Kong et al., 2010). Oxidative instability of meat is enhanced after cooking and during storage due to the loss of endogenous antioxidants and the release of free fatty acids from phospholipids and iron from the heme molecules (Estevez and Cava, 2004; Kristensen and Purslow, 2001; Estevez et al., 2007). During storage, unsaturated fatty acids in meat oxidize and form hydroperoxides that are subsequently decomposed to secondary products, including malonaldehydes that cause off-flavor (Frankel, 2005).

Mechanically separated chicken (MSC) is one of the cheapest sources of meat ingredients for processing. Mechanical grinding of bones results in the release of substantial amounts of fat and heme components from bone marrow, and therefore, MSC may contain more lipid than hand deboned meat (Püssa et al., 2009). A significant part of the released lipids are phospholipids rich in polyunsaturated fatty acids (Spiteller et al., 2001; Püssa et al., 2009). Hence, the fundamental problem with the utilization of MSC in meat products is their higher susceptibility to oxidation (Dawson and Gartner, 1983).

The use of antioxidant compounds is of considerable importance in preserving unsaturated lipids from oxidative deterioration (Frankel, 2005). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary-butylated hydroxyquinone (TBHQ), and propyl gallate inhibit lipid oxidation in meat products (McCarthy et al., 2001; Sebranek et al., 2005; Jayathilakan et al., 2007). In addition, nitrites and phosphates are commonly used in meat products because of their multifunctional nature, including antioxidant potency (Long et al., 2011; Xiong et al., 2012; Sebranek, 2015), whereas nitrites have beneficial effects on quality attributes such as color and flavor, as well as oxidative stability. However, the higher dietary intake

of synthetic antioxidant compounds is perceived to have deleterious consequences on health, and use of synthetic ingredients have negative implications for natural and clean label trends. The increasing consumer demand for clean label products triggers the need for naturally sourced ingredients in meat product formulations.

In this context, there is growing interest in the use of natural antioxidants, particularly those derived from plant sources (Kong et al., 2010). Plant-derived natural antioxidants exhibit various degrees of efficacy when used in different food matrices (Sebranek et al., 2005). The addition of plant extracts such as rosemary, sage, green tea, and grape skin to meat products were reported to enhance oxidative stability of lipids (Estévez and Cava, 2006; Estevez et al., 2007; Lara et al., 2011; Jia et al., 2012). Moreover, these and other related investigations revealed that the primary antioxidant compounds found in natural plant extracts are phenolic compounds.

Phenolic compounds (polyphenols) are a complex group of compounds classified as phenolic acids, stilbenes, and different types of flavonoids (Pandey and Rizvi, 2009). Phenolic compounds act as antioxidants in various ways, including binding with free radicals to break oxidation reactions and chelating transition metal ions that induce oxidation reactions (Rice-Even et al., 1996; Lopes et al., 1999). Furthermore, phenolic compounds receive considerable attention since they are recognized as reducing oxidative damage associated with many diseases including cardiovascular disease, cancer, immune deficiency-related diseases and aging (Kushi et al., 1999; Yochum et al., 1999; Kris-Atherton et al., 2002, Martin and Appel, 2010).

Lentil has gained recent attention for its health benefits in the human diet and is considered an excellent source of dietary antioxidants due in large part to its high level of phenolic compounds (Aguilera et al., 2010; Ghrachorloo et al., 2012; Zhang et al., 2015). Dueñas et al. (2002) reported that different phenolic compounds with potent antioxidant properties are concentrated in the lentil seed coat rather than in cotyledon. Seed coats of lentil have a wide range of background colors and patterns, and the variability of their phytochemical composition is likely the cause this phenotypic diversity. However, there is limited information on the water-soluble phenolic profiles of these different colored lentil seed coats and their useful antioxidant potential in food products. The lentil seed coat is a by-product of the lentil milling industry, mostly for red lentil, and the exploitation of this by-product as a source of functional compounds for their use in foods may increase the value of this by-product for the lentil industry.

Lentil is also a rich source of carbohydrates and protein, providing readily available energy (Tiwari et al., 2011). Studies of the potential for using starch and protein products from legumes in meat products found that they were effective for improving the binding capacity of water and fat, emulsifying ability and viscosity, and adhesiveness of meat batters (Prinyawiwatkul et al., 1997; Modi et al., 2004; Serdaroğlu et al., 2005; Kurt et al., 2012). The fact that lentil is a rich source of protein and starch suggests that lentil flour would be a potential binder for meat products with added useful traits, in addition to their antioxidant potential. Although a few studies have shown the benefits of adding lentil flour as a binder in beef burgers (Der, 2010; Pathiratne, 2014; Shariati-Ievari, 2016), the literature on the functionality of lentil flours and fractions in chicken meat systems, especially for emulsion type products, is not available.

Canada is the world's leading producer and exporter of lentil. The global production of lentil in 2016 was 6.3 Mt, with 51% contribution from Canada. Official forecasts indicate that Canada will continue to be the world's leading exporter of pulses, including lentil (FAOSTAT, 2017). Lentil consumption is limited in Western countries due to traditional eating customs, lack of consumer understanding of nutritional benefits and processing techniques that provide product diversity (Zhang et al., 2015). Developing diverse foods by incorporating lentil and lentil-based ingredients is an important strategy to increase the domestic consumption and export market demand. While new product development is widely recognized as the basis for most industries' profitability and success, the factor that best distinguishes the success of the new product is the consumer perception of product superiority in terms of quality or functionality relative to competitive products (Van Kleef et al., 2005). It was reported that the incorporation of pulse ingredients has different effects on the consumer acceptability of comminuted meat products (Tiwari et al., 2011). For example, chicken nuggets with cowpea flour had lower flavor scores (Prinyawiwatkul et al., 1997), while meatballs with pea flour received higher acceptability scores (Serdaroğlu et al., 2005). On the other hand, the inclusion of chickpea flour did not affect acceptability of mutton sausages (Verma et al., 1984). This emphasizes the need for an accurate assessment of consumer acceptability and preferences because it could be ingredient and product dependant. Prescott and Bell (1995) reported that sensory perception and preferences tend to differ between cultures. Prescott et al. (2002) further reported that food choice in different cultures is greatly influenced by multiple factors, including sensory appeal, convenience, health concerns, price, and ethical concerns. Therefore, it is crucial for food developers to understand how

consumers from different cultures perceive products when developing products for global markets. Understanding how consumers perceive and describe products is a starting point for identifying the products' strong and weak points to build production and marketing strategies.

The goal of this project was to investigate the feasibility and potential of utilizing lentil seed coats and flour in MSC meat systems. In the first part of this study, the antioxidant potential of seed coat was evaluated using *in vitro* assays, and raw and cooked model meat systems. The phenolic profiles of seed coats of seven lentil cultivars were also investigated. In the second part, lentil flour, or the combination of lentil flour and seed coat, was used in existing meat products (sausages and bologna) to evaluate antioxidant potential and binding properties in comparison to commercial ingredients. Finally, consumer perception of the products with lentil additives was evaluated in a cross-cultural consumer study.

1.2 Hypotheses

1. Water and aqueous ethanol (70% v/v) extracts of lentil seed coat differ in concentration of phenolic compounds and antioxidant capacity.
2. Water and aqueous ethanol (70% v/v) extracts of lentil seed coat will have the ability to delay color and lipid oxidation in raw and cooked MSC.
3. Different lentil cultivars with different seed coat colors will have different phenolic profiles, and antioxidant capacity.
4. The components of lentil seeds (flour, ground seed coat, and seed coat water extract) will deliver water-holding properties and antioxidant properties similar to those of synthetic phosphates in bologna formulated with MSC without compromising their texture and sensory properties.
5. Inclusion of infrared (IR) treated green lentil flour will improve the water-holding, textural, antioxidant, and sensory properties of wiener type MSC sausages.
6. Canadian and Sri Lankan consumers will have different perceptions toward IR treated green lentil flour incorporated wiener type MSC sausages.

4

1.3 Objectives

Based on the aforementioned hypothesis, this research project focused on the following objectives:

1. To assess the level of phenolic compounds and antioxidant potential of water and aqueous (70% v/v) ethanol extracts of lentil seed coat of two cultivars with distinct color differences compared to four other food-grade antioxidant compounds: Herbalox®, sodium ascorbate, (+)-catechin, and (+/-) - α- tocopherol.

2. To investigate the effects of lentil seed coat extracts on the oxidative stability of color and lipids in raw and cooked MSC stored under refrigerated and frozen conditions.

3. To determine the phenolic profiles and antioxidant potential of seed coat water extracts of seven lentil cultivars varying in seed coat color.

4. To explore the applicability of combinations of lentil flour, seed coat and seed coat water extracts as replacers for phosphates in MSC bologna.

5. To evaluate the feasibility and potential of utilizing lentil flour as a binder in wiener type MSC sausages.

6. To compare consumer acceptability of lentil flour containing MSC sausages between Canadian and Sri Lankan consumers.

2. LITERATURE REVIEW

2.1 Lentil

Lentil (*Lens culinaris*) is a leguminous (*Fabaceae)* plant that is classified as a pulse crop. The name lentil is derived from the lens shape of the seed. Lentil seeds are produced on bushy annual plants belonging to the family *Fabaceae* and are grown in three main ecosystems – sub-tropical savannah, Mediterranean, and northern temperate zones (Yadav et al., 2007). It is one of the first domesticated pulse crops and was first cultivated in the Mediterranean region and Asia in 8500-600 BC (Yadav et al., 2007). Global lentil production was 6.3 Mt in 2016, with about 50% contribution from Canada (FAOSTAT, 2017). Saskatchewan (95% of Canadian lentils) is the most major growing area in Canada. Canada contributes 80% of the global exports, while India, Turkey, United Arab Emirates, Bangladesh, and Sri Lanka are among the top importers of lentil (Tiwari et al., 2011).

Lentil has been incorporated into various world cuisines, especially those of Mediterranean and Indian origin (Faris et al., 2013). Lentil is categorized as a smooth seed coated pulse that requires shorter cooking time, resulting in lower nutrient losses during cooking (Satya et al., 2010). Compared to most other pulses, the cooking time of whole lentil is short (23–26 min), therefore, lentil is convenient for human consumption (Jood et al., 1998; Faris et al., 2013).

2.1.1 Market characteristics of lentil seeds

Lentil is classified into several market classes based on the size of seed, the color of cotyledons, and the colour and pattern of seed coats. In Canada, red (based on cotyledon colour) green lentil (based on seed coat colour) comprise the two major market classes of lentil. Red cotyledon lentils of different seed sizes are dehulled and marketed in unsplit or split form. Lentils with green seed coats typically have yellow cotyledon and are marketed as whole seeds of different sizes (McVicar et al., 2010). Color of seed coat is an important attribute affecting the market value of lentil. Seed coat background color has been categorized into four groups: brown, tan, gray, and

green while seed coat color patterns are categorized as absent, pointed, spotted, marbled, and complex: any combination of the previous four (Vandenberg and Slinkard, 1990). Black is considered a seed coat pattern, and zero tannin lentils have no pattern and low polyphenol content (Vaillancourt and Slinkard, 1992).

2.1.2 Nutritional value of lentil

Lentil is attracting of more interest in the field of healthy and functional food development due to its unique nutritional and functional characteristics. The nutritional composition of whole lentil is summarized in Table 2.1. Carbohydrates are the main element of lentil seeds, while the majority of the carbohydrate mass is comprised of starches (Tiwari et al., 2011). An important property of pulse starch, including that of lentil, is it low glycemic index compared to starches from cereals and tubers. The low glycemic index prevents the rapid increase in postprandial blood glucose levels and hence reduces the risk of type 2 diabetes, obesity, and cardiovascular diseases. The low glycemic index of pulse starch is strongly associated with higher amylose to amylopectin ratio, strong interaction between amylose chains, and a high amount of dietary fiber (Hoover and Zhou, 2003). According to Tosh and Yada (2010), lentil has a dietary fiber content of around 18-20%, comprising 11-17% insoluble fiber (cellulose, hemicellulose, and lignin) and 2-7% soluble fiber (oligosaccharide, pectin, and β-glucan), and suggest that this profile helps to reduce blood cholesterol and sugar levels.

Lentil is also a good source of protein, mostly located in the cotyledons in the form of storage proteins. Boye et al. (2010) reported that whole seed and cotyledon flour contained 25.9% and 29.1% of crude protein, respectively. Based on solubility properties, the lentil protein consisted of 16.8% albumins, 44.8% legumins, 4.2% vicilins, 11.2% glutelins, and 3.5% prolamins (Boye et al., 2010).

Lentil has a comparatively low amount of fat (1.1%). According to Urbano et al. (2007), 70-85% of lentil fat is unsaturated fatty acids: oleic, linoleic and linolenic and palmitic (C16:0) are the main saturated fatty acids, comprising 10-15% of total fatty acids.

Minerals play important roles in the human body's physiological functions. Lentil is rich in vital minerals, such as potassium, phosphorous, calcium, and magnesium (Urbano et al., 2007). Lentil also contains relatively high content of iron (Demirsbas, 2005), however, its bioavailability

Table 2.1 Nutrient content of whole lentil (per 100 g)

Nutrient	Unit	Whole lentil	Daily reference value
Proximate composition			
Water	g	10.4	
Energy	kJ	1477	2000
Protein	g	25.8	50
Total lipids	g	1.1	65
Ash	g	2.7	-
Carbohydrates	g	60.1	300
Starch	g	34.6	
Total dietary fiber	g	30.5	25
Minerals			
Calcium	mg	56	1000
Iron	mg	7.5	18
Magnesium	mg	122	400
Phosphorous	mg	451	1000
Potassium	mg	955	3500
Zinc	mg	4.8	15
Selenium	mg	8.3	70
Vitamins			
Vitamin C	mg	4.4	60
Thiamin	mg	0.9	1.5
Riboflavin (B2)	mg	0.2	1.7
Niacin	mg	2.6	20
Folate	µg	479	400
Vitamin A	IU	39	5000
Vitamin E (tocopherol)	mg	0.5	30IU
Tocopherol	mg	4.2	-
Vitamin K	µg	5.0	80
Choline	µg	96.4	-
Lipids			
Saturated fatty acids	g	0.2	20
Monounsaturated fatty acids	g	0.2	-
Polyunsaturated fatty acids	g	0.5	-

Source: Sotomayor et al., 1999 (starch content); Faris et al., 2013

could be decreased by the natural chelating agents such as phytic acid, tannins, and oxalate present in lentil (Faris et al., 2013).

Lentil was suggested as a target crop for the biofortification of selenium because the selenium content of lentil varies according to soil quality and agricultural practices (Thavarajah et al., 2008). Lentil cultivated in the province of Saskatchewan in Canada contain 425–673 µg Se/kg (Thavarjah et al., 2011), providing 80-120% of the recommended daily allowance in just 100 g of lentil (Faris et al., 2013). Similar to other pulses, lentil is a good source of B vitamins such as thiamin, riboflavin, niacin, and pyridoxine (Faris et al., 2013).

2.1.3 Antinutrient compounds

Molecules that interfere with the digestion process of monogastric species when seeds or flour are consumed, making them unpalatable, are described as antinutrient compounds (Domoney, 1993). Pulse crops, including lentil, contain many antinutrient compounds with a wide range of chemical characteristics and biological effects, including both protein and non-protein compounds. Protein antinutrient compounds commonly present in pulses are lectins, trypsin inhibitors, chymotrypsin inhibitors, and antifungal peptides (Ye and Ng, 2002; Roy et al., 2010). The non-protein antinutrient compounds include alkaloids, phytic acid, and phenolic compounds such as tannin and saponins. Trypsin and chymotrypsin inhibitors affect the digestion of proteins, thus reducing the availability of essential amino acids (Al-Wesali et al., 1995). Seed lectins are sugar-binding proteins as well as hemagglutinins of all human blood types (Duranti and Gius, 1997; Roy et al., 2010). Legume lectin toxicity in humans is characterized by nausea, vomiting, diarrhea, and bloating (Duranti and Gius, 1997). Roy et al. (2010) reported that heat processing of seeds and flour can reduce the toxicity of lectins to a great extent; however, insufficient cooking may not completely eliminate its toxicity. Phytic acid can form complexes with minerals and reduce the bioavailability of essential minerals, and also inhibit the activity of several enzymes (Knuckles et al., 1989). Saponins are a diverse group of compounds, and chemical structure is a steroid or triterpene group linked to sugar molecules. Depending on the pulse type, the group of saponin present varies. The toxic effect of saponin is due to its ability to lyse erythrocytes and other cells in the intestinal mucosa, thus affecting nutrient absorption (Bissinger et al., 2014).

The presence of antinutrient compounds in lentil reduces its acceptability and nutrient bioavailability. However, some of these compounds could also exhibit health promoting effects. For example, phytates, saponins, and tannins are bioactive compounds and may have ambivalent nutritional properties based on their quantity in the diet (Margier et al., 2018).

Processing of lentil eliminates or reduces the biological effect of undesirable compounds. Germination, soaking, and heat treatment are common preparation methods that improve the nutritional quality of lentil either by leaching them out or inactivating undesirable compounds (Wang et al., 2009). These treatments cause a reduction in efficacy of antinutrients and can also have a marked influence on functional or technological properties of starch and proteins of lentil seed.

2.1.4 Infrared heat treatment (Micronization)

Infrared treatment is a heating process that uses high intensity infrared (IR) radiation as the source of energy. IR radiation has been characterized based on the wavelength as near-infrared (750-3,000 nm), mid-infrared (3,000 nm - 25,000 nm), and far-infrared (Emami et al., 2010). The energy of the radiation is inversely proportional to the wavelength (Bellido et al., 2006). The range of wavelengths used in IR heating is 1,800 - 3,400 nm (Fasina et al., 2001). The material being subjected to infrared radiation should have high absorptivity to increase the efficiency of the process. The absorbed radiation causes the water molecules to vibrate, resulting in a rapid increase in temperature and vapor pressure inside the material. The IR heating process has been developed commercially for the heat treatment of bulk feeds in order to increase the digestibility and inactivate enzymes and antinutrients (Cenkowski and Sosulski, 1997).

IR treatment of food products is also used, for example, to influence the flour properties of legumes such as water absorption, foaming, gelling capacity, and pasting properties. Der (2010) demonstrated that IR heating of lentils to 135°C surface temperature results in 5.6% gelatinization of starch and 3°C decline in pasting temperature. Furthermore, Pathiratne (2014) observed higher oil absorption capacity in IR treated lentil flour compared to untreated flour, and noted 18-25% of gelatinized starch in IR treated lentil flour. Shariati-Ievari et al. (2016) investigated the physicochemical and sensory properties of IR treated lentil and chickpea flour incorporated into low-fat beef burgers, and found that the burgers with lentil flour treated at 130°C and 150°C, and chickpea flour treated at 150°C increased consumer acceptability. However, neither shear force nor

cook loss of burgers formulated with both flours was affected by the IR treatment (Shariati-Ievari et al., 2016).

In addition, IR heating has been shown to be effective for enzyme inactivation. The lipoxygenase enzyme induces the oxidation of lipids, causing off odor and flavor in food products. Der et al. (2010) observed a 100-fold reduction in lipoxygenase activity in lentil, which was micronized to a surface temperature of 135°C. Shariati-Ievari et al. (2016) investigated the effect of IR heating at 130 and 150°C on lipoxygenase activity of chickpea and green lentil flour. They reported that the IR heating at 130°C significantly decreased lipoxygenase activity in both flours. The lentil seeds heated at 150°C showed a further significant decrease in lipoxygenase activity, similar to that of chickpea flour treated at 150°C. Pathiratne (2014) reported that IR heating (at 115°C, 130°C, 150°C and 165°C) of lentil flours reduced activity of lipoxygenase (70-100%), peroxidase (32-100%), and trypsin inhibitors (up to 54%). Lentil flour heat-treated without IR was also found to protect fresh meat color and inhibit lipid oxidation in raw beef burgers compared to untreated flour, and this observation was suggested to be due to the inactivation of oxidative enzymes (Li, 2017).

2.2 Phenolic compounds

Phenolic compounds are a large and diverse group of secondary metabolites synthesized in plants. Chemically, a basic phenolic compound contains a benzene ring with one or more hydroxyl groups attached directly to the benzene ring (Shahidi and Wanasundara, 1992). Plants contain several derivatives of these basic phenolic compounds. In plants, phenolic compounds are involved in defense against stress, pathogens and predators, and also contribute color and other sensory characters in fruits and vegetables (Borochov-Neori et al., 2009; Ajila et al., 2011). Phenolic compounds are reported to have a wide range of health benefits, including anti-allergenic, anti-inflammatory, anti-microbial, antioxidant, anticarcinogenic and cardiovascular effects (Chung and Champagne, 2008; Viswanath et al., 2009) which are mostly associated with their antioxidant properties (Ajila et al., 2011).

Phenolic compounds are structurally diverse, occurring naturally as mono- and polysaccharide conjugates, and also as functional derivatives like esters and methyl esters (Ajila et al., 2011; Kebera et al, 2014). Phenolic compounds are categorized in various ways because they consist of many heterogeneous structures ranging from simple molecules to highly polymerized

compounds (Ajila et al., 2011). They are broadly divided into four classes; phenolic acids, stilbenes, flavonoids, and lignans (Pandey and Rizvi, 2009). Biogenetically, phenolic compounds originate from two metabolic pathways. Simple phenols are synthesized via the acetic acid pathway, and phenylpropanoids are the primary compounds of the shikimic acid pathway (Gaida, 2013). The two pathways are coupled to synthesize flavonoids and then nonhydrolyzable tannins are formed through condensation and polymerization phases of the flavonoid biosynthesis pathway which not well clarified (Gaida, 2013).

2.2.1 Phenolic acids

The name phenolic acid describes the phenols that have the functionality of single carboxylic acid (Ajila et al., 2011), categorized as hydroxybenzoic and hydroxycinnamic forms. Hydroxybenzoic acids have a common C6–C1 structure and include gallic, ρ-hydroxybenzoic, vanillic, syringic, and protocatechuic acids. Hydroxycinnamic acids have a three-carbon side chain (C6–C3) and the most prevalent forms are caffeic, ferulic, ρ-coumaric, and sinapic acids (Gallardo et al., 2006; Dykes and Rooney et al., 2007). The structures of the caffeic, cinnamic, ρ-coumaric, ferulic, and gallic acids are presented in Figure 2.1. Ajila et al. (2011) reported that phenolic acids were linked to various fundamental biological processes in plants, including nutrient absorption, protein synthesis, enzyme activity, and photosynthesis.

Caffeic acid   Cinnamic acid   ρ-Coumaric acid

Ferulic acid   Gallic acid
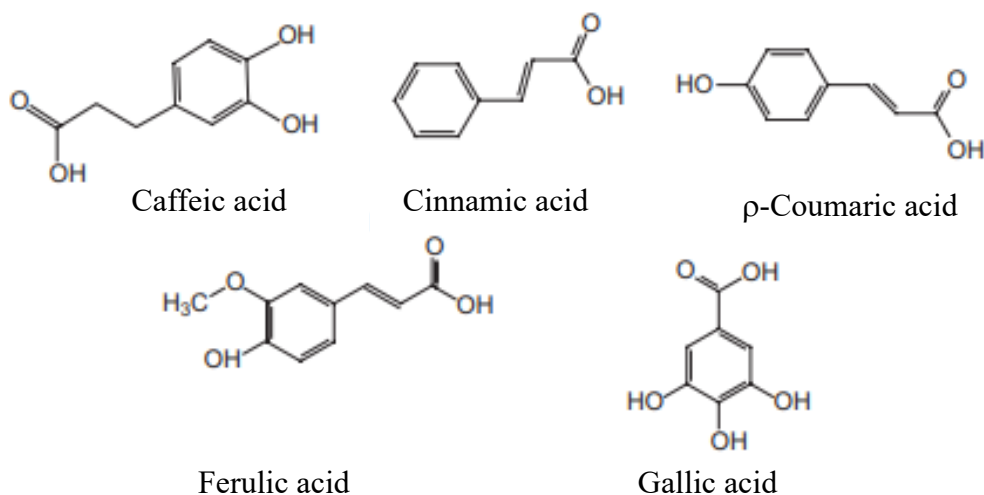
Figure 2.1 Chemical structures of phenolic acids

12

Caffeic acid is known to selectively block leukotrienes, thereby intervening in immunoregulatory diseases, asthma, and allergic responses (Yasuko et al., 1984). Furthermore, it also has demonstrated detoxification effects on carcinogenic metabolites of polycyclic aromatic hydrocarbons (Huang et al., 1996). High intake of rutin and *o*-coumaric acid was shown to suppress the high-fat dietary dyslipidemia and oxidative stress in rats (Hsu et al., 2009). However, a negative correlation was noted between the antioxidative capacity and vasodilatory effect of the phenolic acids present in wine (Mudinic et al., 2012).

2.2.2 Stilbenes

Natural stilbenes are characterized by the presence of a nucleus of 1,2-diphenylene (Shen et al., 2009; Rivière et al., 2012; Sirerol et al., 2016). Stilbenes are diverse in terms of chemical sub-units, the degree of polymerization and the pattern of oligomer construction (Shen et al., 2009). Resveratrol (Figure 2.2) is an example of a natural stilbene which is present in at least 185 plant species (Shen et al., 2009). Stilbenes are reported to have exceptional potential for cancer prevention and treatment due to their antioxidant, cell death activation, and anti-inflammatory properties associated with low *in vivo* toxicity (Sirerol et al., 2016).
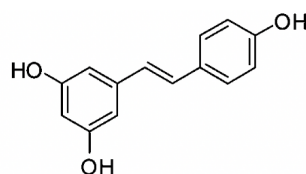
Resveratrol

Figure 2.2 Chemical structure of a common stilbene

2.2.3 Flavonoids

Flavonoids constitute the largest group of plant phenolics, with a C6-C3-C6 skeleton that consists of two aromatic rings joined by a three-carbon link. They are low molecular weight, water-soluble compounds (Kabera et al., 2014). Based on the variations in substitution patterns to ring C, they are divided into major classes (Kabera et al., 2014) i.e., flavonols, flavones, flavanones, flavanols, isoflavones, flavanonols, and anthocyanidins (Figure 2.3). Flavanols or flavan-3-ols are

Anthocyanidin   Flavones   Flavonols

Flavanones   Flavanols
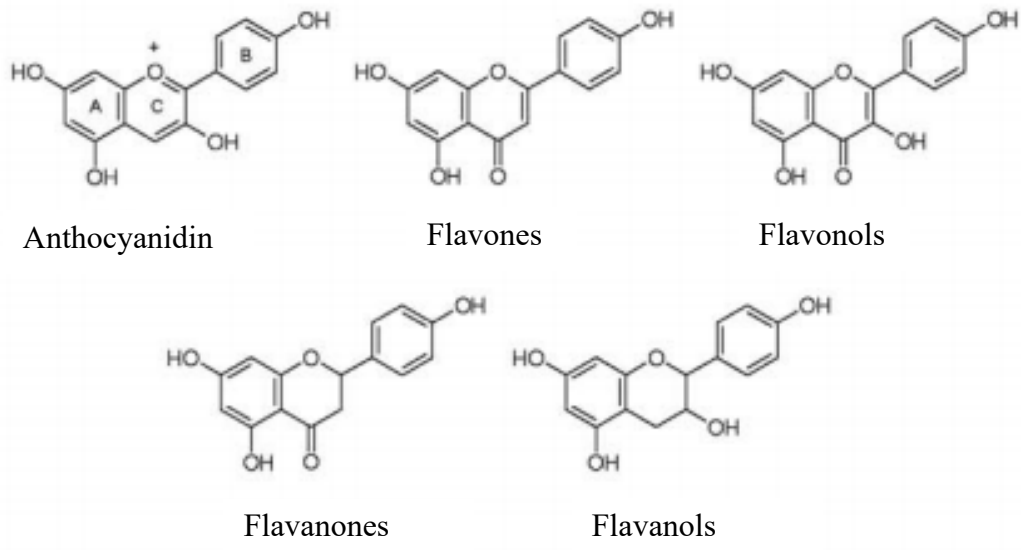
Figure 2.3 Chemical structures of flavonoids

often commonly called catechins. Unlike most flavonoids, there is no double bond between C2 and C3, and no C4 carbonyl in flavanols ring C. Hydroxylation at C3 enables flavanols to form two chiral centers on the molecule (C2 and C3); thus, four possible diastereoisomers exist (Tsao, 2010). Catechin is the *trans* configuration isomer, and the *cis* configuration is epicatechin. Catechin exists as two stereoisomers in each of these configurations, i.e. (+)-catechin, (−)-catechin, (+)-epicatechin, and (−)-epicatechin. The two isomers that are often found in food crops are (+)-catechin and (−)-epicatechin (Tsao, 2010).

Flavonoids have become popular due to the health benefits they impart. They were known to inhibit low-density lipoprotein oxidation, thus reducing the risk for the development of atherosclerosis (Lippi et al., 2010; McCullough et al., 2012). Higher intake of red wine, which contains high levels of flavonoids, mostly quercetin and rutin, may explain why the prevalence of coronary heart disease was reported to be lower in France, whose population consumes more wine compared to other Europeans, even though their cholesterol-rich food intake was greater (Wu et al., 2001).

2.2.4 Tannins

Traditionally, proanthocyanidins are referred to as tannins (Tsao, 2010), which are comprised of a wide range of oligomers and polymers. They are water-soluble except for some high molecular weight compounds (Kabera et al., 2014). Generally, they are subdivided as hydrolyzable and condensed tannins (Ajila et al., 2011), as illustrated in Figure 2.4. The hydrolyzable tannins include the esters of gallic acid (gallo- and elliagi-tannins), while the condensed tannins are the polymers of polyhydroxyflavan-3-ol monomers (Porter, 1989). Tannins may limit the bioavailability of proteins with which they form insoluble complexes (Ajila et al., 2011; Kabera et al., 2014). However, tannins are also considered the active components of plant-based medicinal products. They show therapeutic effects against diarrhea (Fujiki et al., 2012), stomach duodenal tumors (Trouillas et al., 2003; De Jesus et al., 2012), and inflammation (Park et al., 2014).

Hydrolysable tannin   Condensed tannin
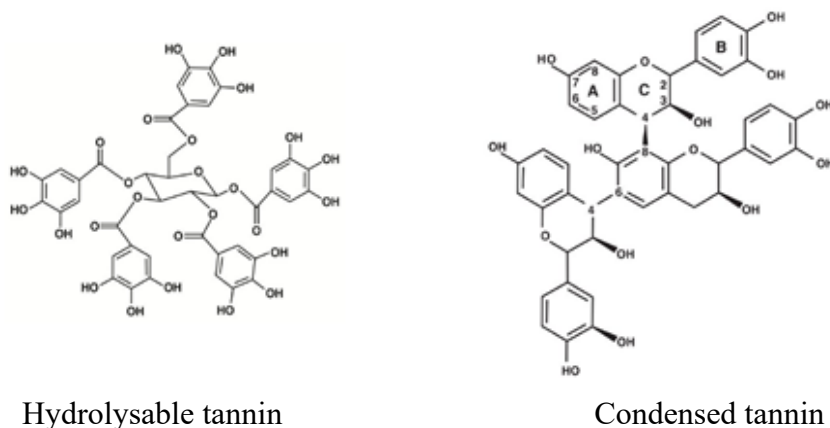
Figure 2.4 Chemical structures of hydrolysable and condensed tannins

2.2.5 Lignan

Lignans are a group of relatively simple diphenols that usually occur in the form of glycosides in plants. When ingested, plant lignans are converted by bacteria in the large intestine into two simple phenols: enterolactone and enterodiol (Figure 2.5). They are classified as mammalian lignans and constitute several important physiological properties (Adlercreutz, 2007). Experimental evidence has shown that lignans have strong anticarcinogenic effects in many types of cancer (Ezzat et al., 2018).

Enterodiol   Enterlactone
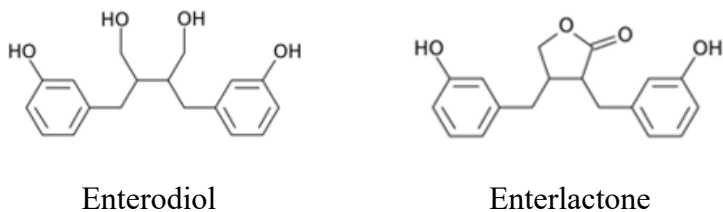
Figure 2.5 Chemical structures of lignans

2.3 Lipid oxidation

Lipid oxidation is a leading cause of quality deterioration in fresh and processed meat. Quality losses in oxidized meat are generally characterized by discoloration, off-flavor, texture deterioration and loss of nutrients. Lipid oxidation products can also damage enzymes, proteins and biological membranes and consequently pose a significant risk to human health (Ladikos and Lougovis, 1990; Candan and Bağdatli, 2017).

Lipid oxidation results in the deterioration of unsaturated fatty acids and proceeds via a free-radical chain mechanism (Ladikos and Lougovis, 1990). The free radical chain mechanism is described in terms of initiation, propagation, and termination (Figure 2.6). Initiation of lipid oxidation occurs with the abstraction of a labile hydrogen atom from a fatty acyl chain yielding a free lipid radical, which reacts rapidly with oxygen to form peroxy radicals. This peroxy radical abstracts hydrogen from another hydrocarbon chain producing a hydroperoxide and a new free radical, which can continue the chain reaction. The decomposition of lipid hydroperoxides involves further free radical mechanism and formation of non-radical products (Ladikos and Lougovis, 1990).

Radical formation

$$A\text{-}B \longrightarrow I^\bullet \qquad (2.1)$$

Initiation

$$R\text{-}H + I^\bullet \longrightarrow R^\bullet + I\text{-}H \qquad (2.2)$$

Propagation

$$R^\bullet + O_2 \longrightarrow ROO^\bullet \qquad (2.3)$$

$$ROO^\bullet + R\text{-}H \longrightarrow ROOH + R^\bullet \qquad (2.4)$$

Termination

$$ROO^\bullet + ROO^\bullet \longrightarrow ROOR + O_2 \qquad (2.5)$$

$$RO^\bullet + R^\bullet \longrightarrow R\text{-}O\text{-}R \qquad (2.6)$$

$$ROO^\bullet + R^\bullet \longrightarrow \text{Stable product} \qquad (2.7)$$

$$R^\bullet + R^\bullet \longrightarrow R\text{-}R \text{ (dimers)} \qquad (2.8)$$
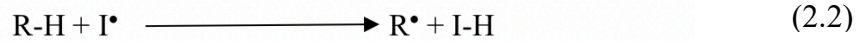
Figure 2.6 Stages of the lipid oxidation mechanism

Hydroperoxides are the primary products of lipid oxidation, and do not possess flavor (Frankel, 2005). However, their decomposition results in the formation of a wide range of organic compounds of low molecular weight, and some of these secondary oxidation products impart off-flavors to cooked and stored meat products. These breakdown products include aldehydes, ketones, hydrocarbons, esters, furans, and lactones, which impart off flavors to meat depending on their concentration and flavor threshold (Ladikos and Lougovis, 1990; Frankel, 2005).

The rate and extent of lipid oxidation in meat and meat products depend on a number of factors, one being the specific polyunsaturated fatty acids present in a particular meat. The presence of minerals such as iron, copper and cobalt are also shown to enhance oxidation of unsaturated lipids. The metal irons act as prooxidants and increase the rate of free radical formation (Ladikos and Lougovis, 1990). Both heme and non-heme proteins can function as prooxidants. Min and Ahn (2005) reported that oxidative rancidity in cooked meat can be catalyzed by both heme and non-

heme iron. Yong and Karel (1978) and Khayat and Schwall (1982) found that oxidation in fish lipid was attributable to inorganic iron and heme iron, respectively.

2.3.1 Measurement of extent of lipid oxidation

Many methods are available to measure either primary or secondary products of lipid oxidation. However, the suitability of each method depends on the type of the product and method of processing and storage (Ladikos and Lougovis, 1990). The factors that determine the effectiveness of the method include the chemical and physical properties measured, the precision and accuracy of the measurement, and how they relate to the real-life storage of food products (Fankel, 2005). Many authors have reviewed methods used to measure the extent of oxidative deterioration in muscle foods (Ladikos and Lougovis, 1990; Moore and Roberts, 1998; Fankel, 2005). The most widely used method is the thiobarbituric acid reactive substances (TBARS) test (Ladikos and Lougovis, 1990). It is simple and inexpensive and provides reliable information in well-developed simple systems (Moore and Roberts, 1998). The method is based on the spectrophotometric determination of the color complex formed between thiobarbituric acid (TBA) and secondary oxidation products of unsaturated lipids (Ladikos and Lougovis, 1990). The color complex is produced by a large number of secondary oxidation products (Frankel, 2005). This method can be performed directly on food, on an extract of lipids, or on a portion of a steam distillate of the food (Ladikos and Lougovis, 1990). Pikul et al. (1989) evaluated aqueous extraction, lipid extraction, and distillation methods for the monitoring of lipid oxidation in chicken meat. They observed that TBA values obtained by the distilled extraction method were 1.35 times higher and 1.3 times lower, respectively, compared to aqueous extraction and lipid extraction procedures. The results indicated that aqueous extraction, which involves the extraction of lipid oxidation products in perchloric acid, is an acceptable and convenient method for determining TBA values, which were also found to correlate well with sensory scores when evaluating the development of oxidative rancidity in cooked meat (Poste et al., 1986).

2.3.2 Controlling oxidative deterioration of muscle foods

The common control methods used to prevent the oxidation of meat products include the inactivation of prooxidants, packaging to minimize exposure to oxygen and application of antioxidants. Of these methods, use of low oxygen packaging was found to be less successful than treatment with antioxidants (Love and Pearson, 1971). Antioxidants control oxidation through several mechanisms, and many of the commonly used antioxidants are classified as chain-breaking antioxidants (Figure 2.7). These types of antioxidants (AH) inhibit or retard lipid oxidation by interfering with either chain propagation or initiation by readily donating hydrogen atoms to the chain carrier peroxyl radicals, ROO$^{\bullet}$, under atmospheric conditions (reaction 2.9) and to lipid radicals, R$^{\bullet}$, under conditions of limited availability of oxygen (reaction 2.10) (Frankel and Meyer, 2000). The antioxidants reacting with peroxyl radicals (reaction 2.9) were defined as chain-breaking electron donors while the antioxidants are reacting with free lipid radicals (reaction 11) as chain-breaking electron acceptors (Scott, 1997). Antioxidants can prevent the decomposition reaction by interacting with alkoxyl radicals either by donating hydrogen to form stable hydroxy compounds (reaction 2.12) or by termination reaction (reaction 2.13) with antioxidant radicals (Frankel and Meyer, 2000). Other classes of antioxidants are the initiator inhibitors or preventive antioxidants (Scott, 1997). The antioxidant mechanisms of these antioxidants include chelating metal ions, inhibiting oxidative enzymes, oxygen scavenging, and quenching singlet oxygen (Frankel and Mayer, 2000).

$$AH + ROO^{\bullet} \longrightarrow ROOH + A^{\bullet \cdot} \qquad (2.9)$$

$$AH + R^{\bullet} \longrightarrow RH + A^{\bullet} \qquad (2.10)$$

$$R^{\bullet} + A^{\bullet \cdot} \longrightarrow RA \qquad (2.11)$$

$$AH + RO^{\bullet} \longrightarrow ROH + A^{\bullet \cdot} \qquad (2.12)$$

$$A^{\bullet} + RO^{\bullet} \longrightarrow ROA \qquad (2.13)$$

Figure 2.7 Antioxidant reactions with lipid oxidation products

Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and propyl gallate are commonly used to retard or inhibit oxidation in foods. However, the worldwide trend to minimizing the use of synthetic food additives, and ,the potential benefits on anti-carcinogenicity and inhibition of biologically harmful oxidation reactions in the body, has attracted considerable interest in the use of natural antioxidants. Epidemiological studies have suggested that increased consumption of plant foods containing natural antioxidants has an inverse relationship to the incidence of several diseases, for example, cancer, aging, and cardiovascular diseases (Kushi et al., 1999; Kris-Etherton et al., 2002; Djordjevic et al., 2011). Further, the additional advantages of natural antioxidants, such as unquestioned safety by their GRAS status (Generally Recognized as Safe), large allowable concentrations, and lower volatility in heated foods, has motivated food processers to replace synthetic antioxidants with natural ones in their formulations (Frankel, 2005).

Phenolic compounds are excellent natural antioxidants due to their redox properties - they can act as reducing agents, hydrogen donors, and single-single oxygen quenchers (Rice-Even et al., 1996). The free radical scavenging ability of phenolic compounds involves the ability to donate phenolic hydrogen, as well as the stabilization of the resulting antioxidant radical by electron delocalization and/or intermolecular hydrogen bonding (Frankel, 1993). Therefore, the number and location of free-OH groups on the molecule appears to have an influence on the free radical scavenging capacity of phenolic compounds (Lupea et al., 2008). The hydroxyl and carboxyl groups in phenolic compounds are able to bind metal ions, particularly iron and copper (Michalak, 2006). Therefore, the phenolic compounds also function as metal ion chelators that can inhibit metal-catalyzed free radical formation (Salah et al., 1995; Carocho et al., 2014). The antioxidant functionality of phenolic acids is due to the ability to scavenge free radicals, while flavonoids serve as both free radical scavengers and metal chelators (Brewer, 2011). In fish and meat industries, polyphenols are used as antioxidants by dipping carcasses into polyphenolic extracts, enabling delays in oxidation (Fan et al., 2008; Kumudavally et al., 2008; Maqsood et al., 2013). Other methods were successfully tested by integrating natural extracts rich in polyphenol or pure compounds into processed products, thus avoiding the development of oxidative rancidity over a longer period of time compared to controls (Vuorela et al., 2005; Estévez et al., 2007; Haak et al., 2009; Luciano et al., 2013; Carochco et al., 2014).

2.3.2.1 Application of plant extracts as natural antioxidants in poultry products

Several studies have shown the efficacy of plant extracts as natural antioxidants in meat and poultry products. Rosemary and rosemary extracts are among the most researched natural antioxidants in meat and poultry products. Mielnik et al. (2003) investigated the use of five commercially available rosemary products as natural antioxidants in mechanically separated turkey meat (15.3% fat) which was mixed with three different levels of rosemary products and then vacuum packed in transparent polyethylene cups. Sample were stored at -25°C and TBARS were analyzed at 0, 2, 4, 5 and 7 months. They observed that type and level of rosemary product affected inhibition of lipid oxidation. The TBARS values for all rosemary treatments were lower than the control after 7 months of frozen storage.

Hassan and Fan (2005) compared the antioxidant efficacy of phenolic extracts from cocoa leaves on MSC meat against a mixture (1:1) of butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) and green tea extracts added at 200 ppm. MSC treated with various antioxidants was cooked and stored at 4°C and were analyzed for their peroxide value, TBARS and hexanal generation. Among different antioxidants tested in their study, BHA/BHT mix showed the best antioxidant effects on MSC, however, its performance was similar to the natural phenolic compounds from green tea (200 ppm) and cocoa leaf (800 ppm). At lower concentrations (200 ppm and 400 ppm), the antioxidant capacity of cocoa leaf extracts was 50-80% of the BHA/BHT mix.

Pomegranate rind is rich in tannins, anthocyanins, and flavonoids (Naveena et al., 2008a). The effect of pomegranate rind powder (10 mg tannic acid equivalent phenolics/100 g) on the oxidative stability of cooked chicken patties stored in low-density polyethylene bags for 15 days at 4°C was determined (Naveena et al., 2008b). Based on the TBARS values, samples treated with pomegranate rind powder (0.20 mg MDA/kg) had more antioxidant activity over the storage period than the control (1.27 mg MDA/kg). In the same study, they observed that the TBARS values of the cooked chicken patties treated with BHT (10 mg/ kg meat) was significantly lower (0.89 mg MDA/kg). Pomegranate rind powder had little impact on sensory quality characteristics when used at 5 to 20 mg tannic acid phenolics/100 g meat (Naveena et al., 2008a). Addition of rind powder decreased the L* values in cooked patties (56.7) compared to control (63.8). A 10-member trained sensory panel observed no differences in the off-odor, flavor and chicken flavor among the products treated with pomegranate at any of the concentrations relative to the control; however, the

flavor of chicken was slightly decreased in the products containing 20 mg tannic acid phenolic/100 g meat (Naveena et al., 2008a).

In another study, Vaithiyanandan et al. (2011) studied pomegranate fruit juice phenolic dipping solutions on the shelf life of chicken meat stored at 4°C. It was reported that TBARS values of the control samples (1.07 mg MDA/kg) were significantly higher than for the samples treated with pomegranate fruit juice phenolics (0.75 mg MDA/kg) after 28 days of storage. The sensory properties of the meat samples were evaluated by a 5-member trained panel, and the initial scores revealed that both treated and control samples had acceptable sensory quality (appearance, color, odor, off odor), however, these attribute scores of the control samples started to decline from day four while the scores of the samples treated with pomegranate fruit juice phenolics remained high.

Plum extract was evaluated for its antioxidant activity in radiation-processed (3 kGys) turkey breast rolls (Lee and Ahn, 2005). After seven days of storage at 4°C, the TBARS values of the control sample were 1.09 mg MDA/kg meat, and samples treated with 3% plum extract had reduced TBARS values of 0.84 mg MDA/kg meat. The addition of plum ingredients increased the color intensity of meat products (Lee and Ahn, 2005; Nunez de Gonzalez et al., 2008; Yildiz-Turp and Sedaroglu, 2010). The increased a* and b* values and decreased L* values were observed in samples treated with plum extract due to the original color of the extract (Lee and Ahn, 2005).

Cranberries also have high concentration of phenolic compounds (158.8 mmol of total phenolics/ g dry matter) that help to control oxidative deterioration of lipids (Vinson et al., 1998). Lee et al. (2006) investigated the effect of cranberry juice extract powder in mechanically separated turkey. Inhibition of lipid oxidation at 0.32% addition level of cranberry juice extract powder (5.1 mmol/ kg meat) was similar to that of rosemary extract (3.6 mmol/ kg meat) used at 0.04% in products stored at 2°C (14 days), and both treatments reduced TBARS values (10-fold) in comparison to control (58.8 mmol/kg meat). Further, the ability of different classes of phenolics in cranberry extracts to inhibit lipid oxidation was evaluated in mechanically separated turkey. The fraction of extract rich in flavonols showed greater inhibitory action than other fractions, which were abundant in phenolic acids, anthocyanins, and proanthocyanidins.

Several studies demonstrated the antioxidant effects of fruits and other plant materials in poultry products due to their high phenolic content. Some of these plant-based antioxidant ingredients, however, had detrimental effects on the sensory quality of products and, eventually, the product's acceptability to the consumer. Thus, it is important to take a holistic approach that

22

considers sensory changes along with antioxidant potential when selecting a plant-based antioxidant system.

2.3.3.2 Antioxidant potential of lentils

Concentration of phenolic compounds is a good indicator of potential antioxidant activity. Among the pulses, lentil contains a considerable amount of phenolic compounds in the seed (Hans and Baik, 2008). The distribution of phenolic compounds differs in the cotyledon and seed coat, and they are located mainly in the seed coat (Li, 2017). It was reported that the non-flavonoid phenolic compounds, such as free and combined hydroxybenzoic acid and hydroxycinnamic acid are mainly present in the cotyledons whereas flavonoids such as glycosides of flavonols and flavones are found in the seed coat of lentil (Amarowicz et al., 2009). Besides, Dueñas et al. (2002) and Dueñas et al. (2003) noted that trans-resveratrol-3-O-glucoside and large amounts of proanthocyanidins are present in seed coat but are absent in cotyledon. Although seed coat represents a small portion of the entire seed weight (8 to 11%), it provides a significant contribution to the overall phenolic composition of lentil (Li, 2017). Therefore, there is potential for lentil seed coat, a by-product of the lentil industry currently used as livestock feed, to be developed as a value-added product that could be used as a functional ingredient in food products as well as for the commercial extraction of phenolic compounds.

The seed coat color of pulses has been suggested to be correlated with their phenolic content (Sing and Basu, 2012). Xu et al. (2007) revealed greater phenolic content of dark colored pulses than pale colored ones. However, there are limited data on the water-soluble phenolic profiles of the seed coat from different cultivars of lentil with different colors, and there will be a high demand for this information in food applications.

2.4 Mechanically separated chicken meat (MSC)

Mechanically separated chicken meat is produced by machines which grind or crush bones and subsequently separate bone and soft tissues (muscle and fat) by forcing the tissue through a sieve to produce a meat paste (Aberle et al., 2012). Mechanical separation produces meat economically and, in a way, saves large quantities of meat that could otherwise be lost for human consumption. Yield of MSC ranges from 55 to 70% (Froning, 1981) depending on the meat-to-bone ratio of the specific parts and machine settings. Mechanical separation offers the means to

harvest functional proteins that can be used to produce a variety of processed products. Due to the fine consistency of MSC, it is commonly used for the formulation of comminuted meat products such as bologna, salami, frankfurters, and various loaf products (Froning and McKee, 2001).

2.4.1 Chemical composition of MSC

The chemical composition of MSC varies depending on the kind of raw material, bone-to-meat ratio, age of the birds, skin content, cutting method, machine type, and the operating conditions applied during the meat recovery process (Viudu-Mortos et al., 2012). Froning (1973), Grunden et al. (1973), and Essary (1979) reported that the protein, fat, and moisture contents of MSC recovered from the chicken backs and necks ranged between 9.3-14.5%, 14.4-27.2% and 63.4-66.6%, respectively.

2.4.1.1 Protein

As raw materials used in mechanical separation are rich in lipids (skin, subcutaneous fat), protein content is usually lower in MSC than in hand deboned chicken (Viuda-Martos et al., 2012; EFSA, 2013). The quality of protein of MSC has gained significant emphasis due to the potentially high amount of connective tissue proteins, primarily collagen, with poor nutritional and technological quality (Froning and McKee, 2002). However, other studies indicated that the protein quality of MSC is similar to the manually deboned meat, even if the materials used for mechanical separation were abundant in connective tissues (MacNeil et al., 1978; Babji et al., 1980). This may be because the high tensile strength of connective tissues may partially prevent its extrusion during mechanical separation (Field, 1988).

2.4.1.2 Lipids

Mechanical separation influences the lipid composition of MSC, which generally contains a higher amount of lipids compared to hand deboned meat (EFSA, 2013). These extra lipids come from subcutaneous fat, skin, or abdominal fat, but mostly from bone marrow (Trindade et al., 2004; EFSA, 2013). Due to the incorporation of phospholipids from the bone marrow and spinal tissues, lipids in MSC are rich in unsaturated fatty acids (Trinidade et al., 2004; EFSA, 2013).

The cholesterol content of MSC is also higher than that of manually deboned meat due the incorporation of bone marrow which is rich in cholesterol (Trinidade et al., 2004). According to

Ang and Hamm (1982), the cholesterol content in MSC from chicken backs contained higher cholesterol content (95 mg/100 g) than that found in manually deboned chicken (81 mg/100 g).

2.4.1.3 Calcium

MSC was closely scrutinized for the issue of possible bone content. Usually, calcium content is used as a measure of the bone content present in MSC, and bone particle size is determined by the type of deboning machine, operating pressure, and the size of filters (EFSA, 2013). During mechanical separation, bones are crushed, and therefore, a large quantity of bone particles could be found in MSC in comparison to hand-deboned meat (Trinidade et al., 2004; EFSA, 2013). The calcium content of MSC from the chicken whole back (53 mg/100 g) and neck with skin (91 mg/100 g) was higher compared to the hand deboned meat obtained from the same parts (20 mg/100 g and 17 mg/100 g, respectively). In many countries, there are regulations regarding the calcium content in mechanically separated meat. According to the Canadian Food Inspection Agency regulations, the maximum calcium level allowed in MSC is 0.027% of calcium for every 1% of protein, and no particle should be larger than 2 mm in size (CFIA, 2019).

2.4.2 Oxidative stability of MSC

The mechanical separation process causes significant cellular disruption and release of hemoglobin and lipids from bone marrow (Froning and McKee, 2001). These variables favor the auto-oxidation of polyunsaturated fatty acids in bone marrow-derived phospholipids (Moerck and Ball, 1974; Lee et al., 1975; Froning 1981; Dawson and Gartner, 1983). Polyunsaturated fatty acids in meat, such as linolenic and arachidonic acids, undergo extensive changes during storage (Igene et al., 1981). This results in the production of secondary oxidation products such as aldehydes, ketones, hydrocarbons, esters, furans, and lactones that are primarily responsible for rancid flavor and sensory deterioration in meat (Ladikos and Lougovois, 1990). Therefore, the higher susceptibility to lipid oxidation has been recognized as a significant issue for the use of MSC in processed meat products, and several studies emphasized the necessity for enhancing the storage stability of MSC. A number of researchers have studied antioxidants as a potential means of controlling lipid oxidation in MSC. Rich in antioxidant phenolic compounds, lentil products may be efficient antioxidants in controlling lipid oxidation in MSC. On the other hand, because of its

greater susceptibility to oxidation, MSC serves as an optimal medium for studying the effectiveness of lentil components to control lipid oxidation in meat systems.

2.4.3 Technological aspects

MSC is used in the formulation of comminuted meat products; therefore, functional characteristics have become an important consideration. Water holding capacity (WHC) and emulsifying capacity play a significant role in determining the technological properties of MSC (EFSA, 2013). The relatively high amount of collagen and lesser amount myofibrillar proteins present in MSC have negative effects on the functionality of meat proteins, which results in the reduction of emulsifying and water holding properties of meat during cooking and storage (Viuda-Martos et al., 2012). These characteristics, however, vary widely in terms of sources and harvesting techniques (EFSA, 2013).

Emulsifying capacity has been defined as "the amount of oil that can be emulsified by the material prior to reversion or collapse of the emulsion" (Viuda-Martos et al., 2012). This is an important attribute of MSC as it is widely used in emulsion or batter type products such as sausages, bologna and frankfurters. The emulsifying capacity of MSC is greatly affected by its composition, quality, and amount of proteins, degree of protein denaturation, freezing, and storage conditions (Trindade, 2004).

Myosin is more important for fat emulsification in processed meat products than sarcoplasmic and stromal proteins (Viuda-Martos et al., 2012). Myofibrillar proteins absorb as a thin layer at the oil-water interface lowering the interfacial tension between the two phases. Therefore, this thin layer of protein at the interface should have sufficient steric and electrostatic repulsion to obtain a stable emulsion. Abdullah and Al-Najdaw (2005) reported that the presence of skin in MSC was detrimental to its emulsifying capacity, which was primarily associated with increased fat and collagen content from the skin. Chia et al. (1999) investigated the effect of the incorporation of MSC (0-50%) on the quality of chicken sticks formulated with hand deboned chicken breast meat. The protein content and hardness of the sticks decreased with the increasing proportion of MSC, while the fat content and cooking losses increased. The products with 30 - 50% MSC was considered softer than the control. A similar effect was also reported for nuggets formulated with MSC by Chinprahast et al. (1997). The nuggets formulated with a combination of MSC and chicken breast meat were evaluated for their quality in comparison to nuggets formulated

only from chicken breast meat. The best proportion of chicken breast to MSC was determined as 60:40, respectively. Increasing amounts of MSC tended to result in inferior sensory characteristics, and a product with a very dark interior color and decreased firmness and adhesiveness resulted in the sole use of MSC.

2.5 Meat product development

The development of new products with added consumer value have a significant contribution to the growth and success of a business (Van Kleef et al., 2005). The factors that best distinguish new product success from failure is superior quality from the consumer point of view; hence, numerous studies agree that understanding consumer needs and preferences have the greatest strategic value in new product development (Van Kleef et al., 2005). Therefore, sensory testing can help the product development team to interpret consumer desires. Consumer affective tests offer the opportunity for better understanding of whether consumers like the product, prefer it over other products, or find the product acceptable based on its sensory characteristics. The important factors that influence the consumer demand for new meat and meat products are improved product quality, health benefits, differences in demographic characteristics, need for convenience, and change in relative price (Resurreccion, 2004). Therefore, sensory evaluation is a critical component in the product development process.

2.5.1 Sensory evaluation

Sensory evaluation involves a range of procedures for the accurate assessment of human responses to food while minimizing the potentially biasing effects influencing consumer perception (Lawless and Heymann, 2010). Sensory evaluation techniques include discriminative sensory analysis, descriptive sensory analysis, and consumer affective tests.

Descriptive sensory analysis involves the quantification and description of the product based on perceived intensities of the sensory attributes (Lawless and Heymann, 2010). This method is the most comprehensive and informative sensory evaluation tool, widely used for the characterization of product changes and research questions in product development. This information can also be compared with the information generated from consumer acceptance tests and instrumental analysis using statistical techniques such as multivariate regression and correlation (Lawless and Heymann, 2010).

Consumer affective tests evaluate the degree to which a consumer likes or dislikes a product and help sensory scientists to understand the behavior of different consumer groups and potential buyers of the product. Consumer sensory evaluation is usually performed toward the end of product development. The measurement of preference and the measurement of acceptance are two main approaches to consumer testing (Lawless and Heymann, 2010). These tests can be performed to obtain both qualitative and quantitative information. The quantitative tests involve focus group panels to measure subjective responses of a small group of representative consumers (Meilgard et al., 2006). The qualitative tests determine the responses of a large group of consumers regarding the preferences and liking on sensory attributes (Meilgard et al., 2006).

2.6 Processed meat products

Processed meat products are defined as "those in which properties of fresh meat have been modified using one or more procedures such as grinding or chopping, addition of seasonings, alteration of color or heat treatment" (Aberle et al., 2012). These modifications contribute to preservation, convenience, appearance, palatability, variety, and safety, giving consumers a wide choice of meat products (Aberle et al., 2012). There are several hundred different products, each with its individual product name and product characteristics, however, many of these products have great similarities based on the processing technologies used. Some processed products are prepared from large pieces of meat or whole intact cuts. These are mainly seasoned, heat processed and smoked and often they are molded, shaped or formed. Comminuted meat products are produced from small meat pieces prepared by grinding, mincing or chopping. The extent of comminution of meat varies among meat products. Products such as salami and summer sausage are prepared from coarsely comminuted meat. For other types of products, such as frankfurters and bologna, a meat mixture is prepared as a viscous mass by fine comminution with many characteristics of an emulsion, though they are not true emulsions (Aberle et al., 2012). These products usually are classified as batter type products.

2.6.1 Extenders and binders

Various extenders and binders are incorporated into comminuted meat products. Extenders commonly used in meat products are characterized by high protein content, whereas binders are high in carbohydrates (Heinz and Hautzinger, 2007; Aberle et al., 2012). A range of nonmeat proteins can be used in processed meat products as extenders, of which soy proteins and dairy are most commonly used (Xiong, 2012). In most instances, the incorporation of proteins in meat products enhances texture and sliceability of the product and reduces formulation costs (Heinz and Hautzinger, 2007; Xiong, 2012). However, the potential thermoincompatibility with muscle proteins is a technical challenge for using non-meat proteins as extenders (Xiong, 2012). For palatability and microbial safety, processed meats are usually cooked at a final temperature of >65°C. However, these temperatures are not high enough for the denaturation of most plant proteins and, therefore, limit the interactions between plant and animal proteins, which is required to produce a viscoelastic composite material framework (Xiong, 2012). However, preheat treatments may facilitate dissociation of protein subunits, as well as the partial structural unfolding (Xiong, 2012). Therefore, plant proteins subjected to preheat treatments would have improved functional properties like high emulsifying and binding properties (Feng and Xiong, 2003).

Carbohydrates are macromolecules with polyhydroxyl groups, and some may also have carboxylic and sulfate groups. Therefore, these molecules have negative charge when dissolved in water. Therefore, they have high affinity for water molecules and exhibit hydrocolloid characteristics. They absorb moisture effectively causing swelling, thickening, or gelling in the presence of water (Aberle et al., 2012; Xiong, 2012). Many carbohydrates also can emulsify fat and stabilize emulsions when they are incorporated into comminuted meat products (Xiong, 2012).

2.6.2 Application of pulses in meat products

Proteins and starches from different plant sources are widely used as extenders and binders in comminuted meat products with the intention of reducing cost of production and enhancing textural and flavor properties. Numerous studies demonstrated the successful application of pulses as an ingredient in the formulation of meat products (Prinyawiwatkul et al., 1997; Modi et al., 2004; Serdaroğlu et al., 2005; Kurt et al., 2012). Modi and group (2003) studied the effect of the addition of roasted and unroasted grain legume flours (8 g/100 g raw meat) made of soybean, black gram, chickpea, and mung bean on the quality of buffalo meat burgers. The burgers formulated with any

of these binders were organoleptically acceptable even after storage at -16°C for 4 months. However, the burger with black gram flour had better sensory quality attributes compared to the other grain legumes and also provided the highest yield, lowest percent shrinkage, and lowest fat absorption. The fat absorption, percent shrinkage, TBA values were lower for all roasted samples compared to the unroasted samples. In another study (Serdaroğlu et al., 2005), meatballs were incorporated with flours from common bean, chickpea, lentil, and wheat-based rusk at a level of 10% (w/w). Meatballs formulated with lentils showed the highest cook yield, fat, and moisture retention compared to the other products. The incorporation of pulse flour slightly increased the toughness of meatballs, and they were found to be lighter in color compared to control samples with rusk, and also had higher acceptability in terms of sensory properties. Sanjeewa et al. (2010) conducted studies on the use of desi chickpea in low-fat pork bologna formulated with 2.5 or 5% of flour. Physicochemical and cooking properties, and sensory quality were compared with the control samples prepared by adding wheat flour. Inclusion of chickpea increased the cook yield, and water holding capacity. Increasing the substitution of chickpea increased the hardness, chewiness and torsion shear stress. The flavor properties of the bologna with added chickpea, irrespective of the level of addition, were similar compared to the control samples formulated with wheat flour.

Der (2010) used flours from IR heat treated (tempered to 15% moisture and heated to a surface temperature of 135°C) and non-IR heat treated flour from whole green (yellow cotyledon) and red (red cotyledon) lentil seeds as extenders in low-fat beef burger at 6-12% (w/w) supplementation level and studied the physicochemical and sensory properties. Addition of IR heat treated lentil flour resulted in higher stability of redness in burgers between days 1 and 5 of storage at 4°C. Furthermore, increasing flour addition increased cook yield while decreasing shrinkage during cooking. The burgers incorporated with 6% (w/w) lentil flour had higher consumer acceptability than other flour treatments and were comparable to the control samples with added toasted wheat crumbs. Relatively similar results have been displayed in another study of beef burgers extended with lentil flour (6% w/w) from micronized seeds (Pathiratne, 2014). The burgers made with lentil flour from seeds tempered to 16-23% moisture level and IR heating temperature higher than 113°C displayed better effects in terms of color retention during retail storage at 4°C. Although a few studies have shown the advantages of adding lentil flour as a binder in beef burgers (Der, 2010; Pathiratne, 2014; Shariati-Ievari, 2016), there is no literature on the

functionality of lentil seed components in the chicken meat system. MSC, which has weaker water holding and emulsifying characteristics due to relatively high content of fat and collagen, would be a useful system to study the efficacy of lentil flour as a binder in emulsion type meat products.

2.6.3 Use of phosphates in meat products

Phosphates are salts of phosphoric acid available in different chemical forms (Petracci et al., 2013). Depending on the number of P atoms in the molecule, phosphates are classified as monophosphates, diphosphates, tripolyphosphates, and polyphosphates. Phosphates serve as a multifunctional ingredient in meat products due to their functional properties such as buffering effects, sequestering power, dispersing power, and capacity to improve water holding capacity (Hourant, 2004).

Addition of phosphates increases the water holding capacity of meat. The basis for the improvement of water holding capacity is related to the changes in ionic strength, pH, and cleavage of actomyosin cross-bridges. Calcium allows the formation of cross-bridges between actin and myosin to form the actomyosin complex during the development of rigor mortis, which makes the meat tougher. Phosphates have the ability to break down these bridges resulting in more tender meat (Feiner, 2006). Moreover, alkaline phosphates lead to an increase in pH within the meat product. The slight pH increase in meat products increases the difference between ultimate pH and isoelectric point. A pH movement further away from the isoelectric point causes greater electrostatic repulsive forces which expands the space between muscle fibers, allowing water to enter the muscle fiber network and then bind to charged groups on the proteins (Young et al., 2005; Feiner, 2006). Moreover, the addition of phosphates increases the ionic strength of meat, which results in severe swelling of muscle fibers and activation of protein. Increased concentrations of activated and swollen proteins promote the immobilization of water added to meat products (Long et al., 2011). Therefore, the addition of phosphates enhances the ability of meat to hold water in meat products, and consequently results in some increase in tenderness and juiciness of meat products.

Phosphates also have strong antioxidant effects against lipid oxidation in meat products. The antioxidant activity of phosphates is due to the inhibition of lipid oxidation by high pH conditions and the sequestering of metal catalysts. These metal ions do not participate in the

oxidation reaction after being sequestered by phosphates, even though they are still present in the food (Aberle et al., 2012; Cheng et al., 2007; Kılıç, 2014).

Because of their excellent water-holding and antioxidant characteristics, phosphates have become a common ingredient in meat and poultry products. Concern is increasing, however, about the use of phosphates due to indications that high dietary phosphorus intake may be a public health issue (Petracci et al., 2013).

2.6.3.1 Health aspects of phosphates

Phosphorus is essential for many biological functions. It occurs in DNA, RNA, enzymes, and co-exists, particularly with calcium and magnesium in bones. Phosphorus is necessary to develop, maintain and repair all living organism tissues and cells (Long et al., 2011).

However, high dietary phosphorus intake was found to have deleterious consequences for renal patients. Free phosphate, as found in food additives, is fully absorbed in the gastrointestinal tract and this results in elevated levels of serum phosphates in individuals with renal disease. Therefore, the high concentration of serum phosphate (hyperphosphatemia) was recognized as a good indicator of mortality caused by chronic kidney disease (Ritz et al., 2012). Moreover, recent studies have shown that phosphate appears to damage blood vessels and also induce aging processes (Shutto et al., 2011). High-normal serum phosphate concentration could be used to predict the risk of cardiovascular disease and mortality in the general population (Ritz et al., 2012). Phosphates can also reduce the bioavailability of some essential minerals, such as calcium and iron. They form insoluble salts with calcium and iron, decreasing their absorption within the intestinal tract, leading to increased risk of bone disease (Sherman and Mehta, 2009). Because of these health concerns, various food ingredients are now being tested as substitutes for phosphates to reduce or eliminate the use of synthetic phosphates in meat products.

2.6.3.2 Regulations for use of phosphates in meat products

The U.S. Food and Drug Administration has classified food phosphates as GRAS (Generally Recognized as Safe) compounds when used in compliance with good manufacturing practices (Code of Federal Regulations, 2003). According to Canadian Food Inspection Agency regulations, the maximum permitted level is 0.5% and it should be calculated as sodium phosphate dibasic added to the product (CFIA, 2019). All other permitted forms of

phosphates (sodium phosphate monobasic, dipotassium phosphate, potassium biphosphate, potassium pyrophosphate, disodium dihydrogen pyrophosphate, sodium polymetaphosphate, sodium triphosphate, sodium pyrophosphate, tetrabasic) must be converted to sodium phosphate dibasic equivalent in order to calculate their input level (CFIA, 2019).

2.6.4 Use of nitrates and nitrites in meat products

Nitrates and nitrites are traditionally used as curing agents for the production of cured meat products. The inclusion of nitrates and nitrites in cured meat has beneficial effects on quality characteristics of meat, such as color, flavor, and stability against oxidation (Aberle et al., 2012). The color of meat is an extremely important attribute for consumer acceptance. Myoglobin is the heme iron containing pigment that gives meat its color. Myoglobin has a globular protein portion (globin) and a heme portion (heme ring). Non-cured meat has three natural colors, depending on the chemical state of the iron within the heme ring. When meat is fresh and protected from contact with air (such as in vacuum packages), meat appears purple-red color, and myoglobin is in the deoxymyoglobin state. When exposed to air, meat appears bright red color and is typical for meat in retail display. Bright red color is due to the conversion of myoglobin into oxymyoglobin through the oxygenation reaction. Meat can also appear brown when the iron in the pigment becomes oxidized. Metmyoglobin is the condition in which the iron has oxidized, and this occurs when only a small amount of oxygen is present or when the meat has lost its reducing ability (Aberle et al., 2012).

The characteristic cured red color develops in meat that reacts with curing salt. The curing salts used in meat products are the potassium or sodium salts of nitrates and nitrites. Upon the addition of nitrite/nitrate to comminuted meat, myoglobin is oxidized to metmyoglobin. Metmyoglobin is then converted to nitrosylmyoglobin, an intermediate pigment, through a series of complex reactions that involve the reduction of nitrite to nitrous acid (Govari and Pexara, 2015). The globin portion of the nitrosylmyoglobin denatures and detaches from the iron atom to form a stable pigment. The nitrosylmyochromogen (nitrosylhemochrome) is the corresponding stable pigment which imparts the characteristic reddish-pink cured color. This pigment is very stable in the absence of oxygen (Aberle et al., 2012).

Curing also has a significant effect on the flavor of cured meat products. Several volatile and non-volatile compounds that contribute flavor are created as nitrites bind with proteins and lipids

(Jira, 2004; Govari and Pexara, 2015). Many different flavor compounds, such as hydrocarbons, alcohols, ketones, furans, pyrazines, and heterocyclic compounds containing sulfur and nitrogen, have been identified in cured meat products (Govari and Pexara, 2015).

Nitrites and nitrates further affect the flavor of meat products by acting as powerful antioxidants. A stable complex is formed between heme-bound iron and nitrite, inhibiting the release of ferrous ions. This makes free ferrous ions unavailable to initiate lipid peroxidation (Aberle et al., 2012; Govari and Pexara, 2015). It is also reported that nitric oxide can act as a free radical acceptor and is therefore involved in the termination of free radical chain reactions inhibiting the progression of lipid oxidation (Aberle et al., 2012).

Nitrites have bacteriostatic effects on microorganisms, inhibiting the growth of spoilage bacteria and pathogens in cured meat products (Govari and Pexara, 2015). The antimicrobial effects of control of *Clostridium botulinum* were found to be the most important - botulism is the most lethal foodborne disease. C. *botulinum* is a gram-positive, anaerobic spore-forming bacteria (Hezam et al., 2019), which can grow in foods with low acid such as meat. This organism produces toxins that can cause the disease known as botulism. The antimicrobial effect of nitrites is related to the formation of NO or nitrous acid, which damages the bacterial cells (Møller and Skibsted, 2002). Reddy et al. (1983) reported that the inhibition of *C. botulinum* is probably due to the inactivation of iron-sulfur enzymes (especially ferredoxin) present in vegetative cells.

2.6.4.1 Health aspects of nitrites and nitrates

Nitrates and nitrites could be a source of nitrosating compounds which contribute to the subsequent development of carcinogenic N-nitroso compounds such as N-nitrosamines (Aberle et al., 2012). Nitrosamines are formed as the products of the reactions between nitric oxide and secondary or tertiary amines under certain conditions, such as high temperature or low pH (Scanlan, 1983; Aberle et al., 2012). The manufacturing method, preparation by the consumer, or digestion step in the stomach are critical points for the formation of nitrosamines (Bauer, 2014). Nitrosamines are suspected to be potent carcinogens that can induce tumour growth in humans (Cassens, 1979). The International Agency for Research on Cancer recently classified processed meat as carcinogenic to the human (Riel et al., 2017). Other than potential carcinogenic effects, nitrite is toxic if consumed in excessive amounts. A single dose of nitrite in excess of 15-20 mg/kg body

weight can be lethal (Aberle et al., 2012). These potential health concerns associated with nitrites in meat products require a significant reduction in the use of nitrites in meat products.

2.6.4.2 Regulations for use of nitrates and nitrites in meat products

Slow or rapid curing methods can be used for the curing of meat products. Generally, nitrate and/or nitrite salts are used in the slow curing processes, whereas nitrites are used in the rapid curing of meat products (CFIA, 2019). The calculations for nitrate and/or nitrite in products are made at the input level. As per Canadian Food and Drug Regulations, in products other than side bacon, the maximum input level of sodium nitrite salts is 200 mg/kg (200 ppm) of meat product. In the curing of side bacon, the maximum input level of sodium nitrite salts permitted is 120 mg/kg (120 ppm) of pork bellies (CFIA, 2019).

3. STUDY I: PHENOLIC COMPOSITION AND ANTIOXIDANT POTENTIAL OF LENTIL SEED COAT EXTRACTS ON OXIDATIVE STABILITY OF RAW AND COOKED MECHANICALLY SEPARATED CHICKEN DURING REFRIGERATED AND FROZEN STORAGE

3.1 Abstract

Lentil seed contains phenolic compounds with potent antioxidant activity, which are concentrated in the seed coat. Water and 70% (v/v) aqueous ethanol extracts of the seed coats of two lentil cultivars (CDC Greenland and CDC Maxim) were analyzed for their phenolic composition and antioxidant potential. The total phenolic content (TPC) extracted in aqueous ethanol (43.96 – 50.46 mg GAE/g) were higher ($p<0.05$) than that of water extracts (41.63 – 44.30 mg GAE/g). The total flavonoids, tartaric esters, and flavonols in lentil seed coat ranged from 4.83 to 6.27 mg CE/g, 0.73 to 0.31 mg caffeic acid equivalents/g and 1.17 to 3.15 mg quercetin equivalents/g, respectively. The antioxidant activity determined using DPPH, ABTS, Fe^{2+} chelation, and phospholipid peroxidation inhibition assays showed significant ($p<0.001$) positive correlation ($r > 0.79$) with total phenolic concentration and the overall activity was 72%, 87%, 78%, and 57%, respectively at 500 ppm of TPC concentration. The seed coat extracts exhibited significant ($p<0.001$) antioxidant activity against lipid oxidation in cooked mechanically separated chicken that was higher than that for the control meat sample without added antioxidants and equivalent to other food-grade antioxidant compounds: Herbalox®, sodium ascorbate, (+)-catechin and (+/-)-α-tocopherol. The addition of seed coat extracts (500 ppm of TPC) resulted in 90% decrease in TBARS values in cooked chicken meat stored at 4ºC for seven days compared to the control, and no significant differences were reported between cultivars and type of extraction solvent. During storage at -18ºC, TBARS values of cooked meat treated with seed coat extracts remained stable and below 0.66 mg MDA/kg for three months, whereas the corresponding values for control were around 1.65 mg MDA/kg meat. In contrast, the seed coat extracts had no significant effect on lipid oxidation or the color of raw meat. These results demonstrated that lentil is a potential source of natural antioxidants that could enhance the quality of cooked meat products.

3.2 Introduction

Lipid oxidation is a major quality deteriorative reaction in fresh meat and processed meat products. Oxidative deterioration in meat causes discolouration, off-flavor development, toxic compound formation, and loss of nutrients (Dawson and Gartner, 1983; Palmieri and Sblendorio, 2007; Contini et al., 2014). The susceptibility of meat to oxidative deterioration depends on several factors including the concentration of unsaturated lipids, heme pigments, metal catalysts and other oxidizing agents in the muscle tissue (Falowo et al., 2014). Mechanically separated chicken (MSC) is a co-product of chicken processing obtained by grinding or crushing chicken frames left after the bulk meat has been removed manually. MSC is highly susceptible to lipid oxidation due in part to the disruption of tissue structure by grinding and subsequently separating soft tissues by forcing through a sieve, which makes membrane lipids more accessible to oxidation catalysts (Spiteller et al., 2001; Püssa et al., 2009). Moreover, the mechanical deboning process results in excessive stress and aeration and the release of a considerable amount of fat and heme components from the bone marrow (Trinidade et al., 2004). MSC would, therefore, be a useful model for the measurement of the efficacy of antioxidants.

Antioxidants can be of synthetic or natural origin. Several synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ) and propyl gallate (PG), have been reported to be effective in controlling lipid oxidation in meat products (Chastain et al., 1982; Crackel et al., 1988; Lai et al., 1991; Movileanu et al., 2013). However, in response to the growing customer demand for clean label ingredients in foods, natural antioxidants are of great interest. In addition, the use of natural antioxidants has several benefits over the use of synthetic antioxidants, including unquestioned safety due to their GRAS status (generally recognized as safe), higher concentrations permitted, worldwide acceptance, and lower volatility in cooked foods (Frankel, 2005).

Extracts derived from plant materials such as rosemary (Lai et al., 1991; Nissen et al., 2004; Sebranek et al., 2005; Akarpat et al., 2008), oregano (Colindres and Brewer, 2011) green tea (Wójciak et al., 2011), grape seed (Sasse et al., 2009; Colindres and Brewer, 2011; Kulkarni et al., 2011), pomegranate peel (Devatkal et al., 2014), curry leaf and mint leaf (Biswas et al., 2012), ginger, onion and garlic (Cao et al., 2013) have shown positive effects on the inhibition of lipid oxidation in meat and poultry products. The antioxidant properties of these plant extracts were apparently related to their phenolic compounds. Phenolic compounds are a complex group

classified as phenolic acids, flavonols, flavones, isoflavones, lignans, procyanidins and anthocyanins (Xu and Chang, 2007). In particular, the ability of plant phenolics to inhibit lipid oxidation can be ascribed to their free radical scavenging activities (Robak and Gryglewski, 1988; Jovanovic et al., 1996), ability to chelate metals (Morel et al., 1993; Hider et al., 2001), and ability to deactivate ferrylmyoglobin (Hu and Skibsted, 2002) and transferrin (Brunet et al., 2002). These antioxidant properties appear to depend on the type and concentration of phenolic compounds (Rice-Evans et al., 1996; Wojdyło et al., 2007), thus, the plant source is an important variable to consider for the extraction of natural phenolic antioxidants. Plant extracts are prepared by using different solvents and extraction methods and it was found that the activity of a plant extract is affected by the extraction method and the solvent used. Differences in compound polarity are known to affect their solubility and antioxidant activity in aqueous, lipid or aqueous-lipid systems (Hu et al., 2000; Wangensteen et al., 2004; Arbashahi-Delouee and Urooj, 2007).

Lentil (*Lens culinaris*) is a pulse crop with seed coats that have a high level of phenolic compounds. A study comparing the phenolic content and antioxidant activity of seeds of grain legumes including pea, lentil, chickpea, common bean and soybean showed that lentil has the highest concentrations of phenolic content and highest antioxidant activity (Xu and Chang, 2007). The distribution of phenolic compounds differs in the cotyledon and seed coat (Li, 2017). According to Li (2017), around 90% of the total seed phenolics were concentrated in seed coats. The seed coat is often removed during seed processing, especially for red lentil, but is currently in limited use as a food ingredient. Therefore, lentil seed coats represent a potential source for value-added healthy products by incorporating them into food products, or in sustainable extraction of phytochemicals. Aqueous extracts of seed coat contain soluble compounds that eliminate fiber components of seed coat while providing chemical components that may be more active as antioxidants and readily available for chain reaction breaking in lipid oxidation.

The objectives of this study were to (i) assess the phenolic contents and antioxidant potential of water and aqueous (70% v/v) ethanol extracts of seed coat of two contrasting cultivars compared with that of four food grade antioxidant compounds: Herbalox®, sodium ascorbate, (+)-catechin, (+/-) - α- tocopherol (ii) investigate the effects of lentil seed coat on the oxidative stability of color and lipids in raw and cooked mechanically separated chicken stored under refrigerated and frozen conditions.

Hypothesis:

I.   Water and aqueous ethanol (70%) extracts of lentil seed coat of contrasting types have different phenolic concentrations, and antioxidant capacity as the solubility of phenolic compounds could be influenced by their polarity.

II. Water and aqueous ethanol (70%) extracts of lentil seed coat will have the ability to delay oxidative changes in raw and cooked MSC because of the potent antioxidant capacity of their phenolic compounds

3.3 Materials

Lentil: Whole seed samples of two cultivars/ genotypes (CDC Greenland and CDC Maxim) from the 2014 harvest were obtained from a certified seed grower in Saskatchewan, Canada. CDC Greenland and CDC Maxim belong to the categories large green (yellow cotyledon, green seed coat) and small red (red cotyledon, gray seed coat), respectively, and are the two most commonly grown lentil cultivars/genotypes in Saskatchewan. Three batches of seeds were obtained from different fields in the Moose Jaw area in Saskatchewan (Canada). Incoming seeds were stored in plastic bags and stored at room temperature prior to use.

Meat: Mechanically separated chicken meat (MSC) was obtained from Prairie Pride Natural Foods Ltd. (Saskatoon, Canada) on the same day of deboning. The receiving temperature of MSC was between 8-11°C. Upon receiving, meat was stored at 1°C (within 30 min after receiving) and sample processing was done on the following day.

Chemicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid monosodium salt hydrate (FerroZineTM), ferrous chloride, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridy-*s*-triazine (TPTZ), gallic acid, Folin-Ciocalteu reagent, potassium persulfate, butanol, ferric ammonium sulfate, aluminum chloride, sodium hydroxide, boric acid, sulfuric acid, ethanol, trichloroacetic acid, thiobarbituric acid, caffeic acid and quercetin used were all chemical analysis grade. Phosphatidylcholine (from fresh egg yolk), sodium ascorbate, (+/-)-α-tocopherol and (+)-catechin were purchased from Sigma Aldrich. Herbalox® rosemary extract was from Kalsec Inc. MI, United States.

3.4 Methods

3.4.1 Determination of proximate composition

Moisture, protein, fat and ash content of lentil samples were measured according to the methods of AOAC 950.46, 981.10, 960.39a and 920.153, respectively (AOAC, 1990). To calculate the protein content, a nitrogen conversion factor of 6.25 was used.

3.4.2 Preparation of lentil seed coat

Seeds were decorticated using an abrasive mill (Satake, Japan) and seed coats were separated using sieves and a column blower (Crop Development Centre, University of Saskatchewan). The seed coat fraction was then milled to pass through 250 μm screen using a cutting mill (FRITSCH universal cutting mill, Netherlands). Milled seed coat was stored in sealed bags at 4°C away from direct light.

3.4.3 Preparation of seed coat extracts

Phenolic compounds were extracted following a slightly modified version of the method described by Aguilera et al. (2010). Samples of seed coat (2 g) were weighed and mixed with 20 mL of extraction solvent (70% v/v ethanol or deionized water) in 50 mL screw-capped plastic tubes and extracted using a shaking water bath for 15 h at 23°C while mixing at 100 rpm. The mixture was then centrifuged at 3000 g at 4°C for 10 min. The supernatant was separated and brought to 20 mL volume with the extraction solvent and filtered through Whatman No 1 filter paper. The residue was re-extracted four more times with the same solvent conditions.

The water was evaporated by freeze drying (Labconco, MO, USA) at -40°C under reduced pressure (0.01 mBar). The supernatant obtained from 70% v/v aqueous ethanol extraction was evaporated under vacuum at 40°C in a rotary evaporator followed by lyophilization. The prepared extracts were stored at -20°C until further use. The dried samples were reconstituted with the same extraction solvent to prepare the test solutions to obtain 100, 200, 300, 400, 500 and 600 ppm concentration of total phenolics for the antioxidant assays.

3.4.4 Determination of phenolic compounds

3.4.4.1 Total phenolic content (TPC)

The content of total phenolics in extracts was determined using the Folin-Ciocalteu method as described by Djordjevic et al. (2011). The extract (0.5 mL) was shaken for 1 min with 0.5 mL of Folin-Ciocalteu reagent and 5 mL deionized water. After 5 min, 5 mL of 7% Na_2CO_3 was added and the volume was made to 25 mL using deionized water. After 2 h of incubation at room temperature, the absorbance of the solution was read at 765 nm ($25^{\circ}C$) with a UV/Visible spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) against a blank. The blank was prepared following the same procedure by adding 0.5 mL of deionized water instead of sample. The total phenolic content was determined from a gallic acid standard curve (0-1.6 mg/mL) and expressed as mg of gallic acid equivalents (GAE) per gram of seed coat.

3.4.4.2 Total flavonoid content (TFC)

Total flavonoid content was determined using to the method described by Zhishen et al. (1999). The assay mixture prepared by adding 1 mL of sample extract, 4 mL of deionized water and 0.3 mL of 5% (w/v) sodium nitrite in a 10 mL volumetric flask. After 5 min, 0.3 mL of 10% (w/v) aluminum chloride was added and another 2 mL of 1 mol/L sodium hydroxide was added to the mixture after 6 min. Assay mixture was adjusted to 10 mL with deionized water and the absorbance of the flavonoid-aluminum stable complex of flavonoids was read at 510 nm. The total flavonoid content was expressed as mg catechin equivalent per gram of sample using a standard curve prepared with (+)-catechin (0.0 - 0.5 mg/mL) in 70% (v/v) ethanol.

3.4.4.3 Tartaric esters and flavonols contents

The tartaric esters and flavonols contents were determined as described by Oomah et al. (2011). A 100 μL aliquot of phenolic extract was added to 150 μL of 2% HCl in 80% ethanol in a 96-well ultraviolet flat-bottom plate and mixed for 2 min. The absorbance of the mixture was monitored by Microplate Spectrophotometer (Bio-Rad Laboratories Ltd, Japan, Model xMark) at 320 nm, and 360 nm using caffeic acid (0-20 μg/mL) and quercetin (0-30 μg/mL) as the standards, respectively.

3.4.5 Determination of antioxidant activity

Antioxidant activity of phenolic extracts of seed coat was evaluated via four different antioxidant assays that tested the ability of radical scavenging, ferrous ion chelation and inhibition of phospholipid peroxidation. Antioxidant activity was evaluated at concentrations of TPC from 100 - 600 ppm. The antioxidant activity of seed coat extracts was also compared with four commercial antioxidants: Herbalox®, sodium ascorbate, (+/-)-α-tocopherol, and (+)-catechin, which are commonly used in meat products.

3.4.5.1 DPPH free radical scavenging activity

The free-radical scavenging activity of extracts against diphenylpicrylhydrazyl (DPPH) free radical was measured using the method of Brand-Williams et al. (1995) with modifications. Antioxidant solution (0.1 mL) was mixed with 4.9 mL of 125 μmol DPPH in ethanol. After incubation for 30 min, the absorbance was read at 515 nm using a spectrophotometer. Ethanol (70%) was mixed with DPPH instead of the antioxidant solution and this served as the control. The DPPH radical scavenging activity was calculated as follows.

$$\text{Radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of sample at 515 nm}}{\text{Absorbance of control at 515 nm}}\right) \text{X } 100 \tag{3.1}$$

3.4.5.2 ABTS free radical scavenging activity

The ABTS (2,2-azinobis-3-ethyl-benzthiazoline-6-sulfonic acid) assay was employed following the method of Han and Baik (2008) with modifications. ABTS was dissolved in water to a 7 mmol concentration. ABTS radical cation (ABTS$^+$) was produced by reacting ABTS stock solution with 2.45 mmol potassium persulphate and allowing the mixture to stand in dark for 16 h at room temperature. The reaction was started by adding 2.7 mL of ABTS reagent into 0.1 mL of antioxidant solution. The mixture was held at room temperature for 6 min to complete the reaction, and the absorbance was determined at 734 nm. The control sample was prepared by adding 0.1 mL of water instead of antioxidant solution. ABTS radical scavenging activity of antioxidant solutions was calculated as:

$$\text{Radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of sample at 734 nm}}{\text{Absorbance of control at 734 nm}}\right) \text{X } 100 \tag{3.2}$$

3.4.5.3 Ferrous ion chelating activity

The chelating activity of extracts on Fe^{2+} ion was measured using the method of Tang et al. (2002) with modifications. Antioxidant solution (0.1 mL) was mixed with 3.7 mL of 10% ammonium acetate and then the mixture was reacted with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1, 2, 4-triazine (ferrozine) for 20 min. The absorbance of the titration mixture was determined at 562 nm using a UV–Vis Spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan). Control was prepared with 0.1 mL of deionized water instead of the antioxidant solution. The percentage Fe^{2+} ion chelating activity was calculated as follows:

$$\text{Chelating activity (\%)} = \left(1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}}\right) \text{X } 100 \tag{3.3}$$

3.4.5.4 Inhibition of phospholipid oxidation

The phospholipid peroxidation assay followed a previously described method (Tan et al., 2014) with modifications. The phospholipid from fresh egg yolk was dissolved in chloroform: methanol (2:1, v/v) to obtain phospholipid content of 30 mg/mL. Aliquots (100 µL) were transferred to tubes and dried under vacuum. After the removal of solvent, the phospholipid was resuspended in 2 mL of PBS buffer (pH 7.4) and 0.1 mL of the antioxidant solution, mixed for 20 min in a mechanical shaker followed by ultrasonic dispersion for 3 min in an ice water bath. The lipid peroxidation was initiated by mixing with 0.1 mL of $FeCl_3$ (0.04 mol/L) and 0.1 mL of ascorbic acid (0.04 mol/L) and lipid peroxidation was quantitated by colorimetric determination of malonaldehyde after complexing with thiobarbituric acid. After incubation at 37°C for 2 h, the assay mixture was mixed with 0.1 mL of 0.2% (w/v) butylated hydroxytoluene and 1 mL each of 1% (w/v) thiobarbituric acid (TBA) and 2.8% (w/v) trichloroacetic acid and heated in a water bath at 95°C for 30 min to promote the formation of pink pigment resulting from the reaction with malonaldehyde. Afterwards, the mixture was cooled rapidly in an ice water bath and then centrifuged for 5 min at 3000 *g*. Control sample was prepared by adding 0.1 mL of water instead of antioxidant solution. The absorbance of the supernatant was measured using a spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) at 532 nm. The inhibition of phospholipid peroxidation was calculated by following equation:

43

$$\text{Inhibition of phospholipid oxidation (\%)} = \left(1 - \frac{\text{Absorbance of sample at 532 nm}}{\text{Absorbance of control at 532 nm}}\right) \text{X } 100 \qquad (3.4)$$

3.4.6 Preparation of meat model systems

This experiment was designed to evaluate the effects of lentil seed coat components (ground seed coat, water extract and aqueous (70%) ethanol extract) on oxidative stability of color and lipids of MSC. The seed coat of cultivar CDC Greenland was used in this experiment. The MSC stored at 1°C was mixed by passing through 1/8 mm hole plate followed by tumbling (Glass VSM 150 vacuum mixing machine, Rostfrei, Germany) at 80% vacuum for 3 min. Samples of ground meat were mixed with seed coat components separately to final concentration of 500 mg/kg meat. For comparison Herbalox®, sodium ascorbate, (+/-)-α-tocopherol, and (+)-catechin was added to samples of meat at same concentration (500 mg/kg meat). The control sample was prepared by adding deionized water instead of the antioxidant compound. The meat system was thoroughly mixed in a mixer (KitchenAid® with flat paddle) for 3 min. The average temperature of the meat homogenate after mixing was 10°C.

3.4.6.1 Preparation of uncooked samples

The samples of meat batter from each treatment were divided into smaller portions (160 g) and were spread uniformly (1 cm thickness) on Styrofoam trays (15 cm x 15 cm). These samples were covered with gas permeable film (3387 cc/cm^2) and stored in the dark at 4°C for 7 days. Sampling was conducted at days 1, 3, 5, and 7 for analysis of lipid oxidation.

Samples for the frozen storage study were shaped into patties (10 cm diameter and 1.5 cm thick), placed in polyethylene bags and stored at -18°C for 7 months. Sampling for analysis was conducted at the end of each month.

3.4.6.2 Preparation of cooked samples

Samples of meat batter from each treatment were filled and spread as a thin layer (4-5 mm) in polyethylene bags (18 cm x 28 cm) and vacuum sealed. The bagged samples were cooked in a water bath at 80°C for 20 min followed by rapid cooling in cold water. The cooked samples were ground separately for 1 min in a food processer (Cuisinart® Mini-Prep® Plus Processor) and spread uniformly on Styrofoam trays (15 cm x 15 cm), covered with gas permeable film (525 cc/in^2) and

stored in the same way as the uncooked samples. Sampling was conducted at days 1, 3, 5 and 7 for analysis of lipid oxidation. For the frozen storage study, sample preparation, storage and sampling were conducted following the same procedures applied for raw samples.

3.4.6.3 Preparation of samples for the assessment of color stability

A sample of around 65 g from each meat treatment was weighed into a petri dish, spread uniformly (1 cm thick) and covered with the gas permeable film. These samples were kept in a display case at temperature $2 \pm 4°C$ and 1228 ± 56 lx intensity of light for seven days and color was measured at 1, 3, 5 and 7 days of storage. The color change in the raw samples stored under dark condition at 4°C was also measured.

3.4.6.4 Sampling

On each sampling day each sample was ground for 30 sec in a food grinder (Cuisinart® Mini-Prep® Plus Processor). Samples of 20 g for the analysis of lipid oxidation products were filled into separate polythene bags and they were vacuum sealed. These samples were then stored at -80°C until analysis. A 20 g sample from each treatment was used for the determination of pH and pH was measured on the same day of sampling.

3.4.7 Measurement of CIE color

The color was measured using a HunderLab MiniScan XE (Hunter Association Laboratory, Reston, VA) with a 25 mm aperture size based on L^*, a^* and b^* dimensions with illuminant A and 10 ° observer. For color measurement, samples were covered with gas permeable film (525 cc/in²) and placed to the center of the probe. The instrument was standardized with black and white tiles before the measurement of color. Color difference (ΔE) was calculated based on the difference of L*, a* and b* values on days 1, 3, 5 and 7 using the equation as shown below (AMSA, 2012):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \tag{3.5}$$

3.4.8 pH determination

The pH value of each treatment was determined according to AOAC 943.02 (1990). An aliquot (20 g) of sample weighed into a filter bag (Whirl-Pak® Filter Bags, Sterile, Nasco®) was homogenized using a Stomacher Lab Blender (Stomacher® 400 Lab Blender Series, Seward, US) with 80 mL of deionized water for 3 min. The pH of the filtrate was measured using a pH meter.

3.4.8 Analysis of thiobarbituric acid reactive substances (TBARS)

The extent of lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) values according to the method of Bedinghaus and Ockerman (1995). A sample of 2.5 g was transferred to a Stomacher filter bag (Whirl-Pak® Filter Bags, Sterile, Nasco®). An aliquot (25 mL) of TCA solution (20% TCA containing 1.6% phosphoric acid) was added to the sample bag and homogenized for 2 min in a Stomacher Lab Blender (Stomacher® 400 Lab Blender Series, Seward, US). Then, 25 mL of cold deionized water was added to the bag and homogenized for another 1 min. The supernatant was then filtered through a Whatman No. 1 filter paper into a 50 mL volumetric flask and volume was brought up with TCA solution and deionized water (1:1). A 5 mL of filtrate was transferred to a centrifuge tube and mixed with 5 mL of 0.02 mol/L thiobarbituric acid reagent. The tubes were placed in a boiling water bath for 35 min followed by cooling in ice-water. After cooling for 10 min, the absorbance was read at 532 nm against a blank using a spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan).

Simultaneously a standard curve was created. A series of solutions of 1,1,3,3-tetramethoxypropane (TMP) was prepared by mixing 0.75, 1.5, and 2.25 mL of 2×10^{-7} mol/mL of TMP with TCA and deionized water (1:1). Five milliliters of this mixture were mixed with 5 mL of TBA reagent, and the samples handled following the same procedure described above. Recovery of TMP from the meat matrix were determined by spiking samples with 0.75, 1.5, or 2.25 mL of TMP solution. TBARS of the spiked samples were determined following the same procedure as above. The TBARS values of the samples were calculated using the following equations.

$$\% \text{ Recovery of TMP} = \frac{\text{Absorbance of spike on meat sample}}{\text{Abs of TMP standard after dilution}} \; X \; 100 \tag{3.6}$$

$$\text{K value} = \left(\frac{A \; x \; M_W \; x \; 10^8}{S \; x \; S_W \; x \; P}\right) \tag{3.7}$$

TBARS value (mg malonaldehyde per kg sample) $=$ Abs of meat sample x K value $\qquad$ (3.8)

Where,

A = standard concentration (moles/5 mL)

M_W = molecular weight of malonaldehyde (72.03 g/mol)

S = slope of standard curve

S_W = sample mass (g)

P = percentage of TMP recovery

3.4.9 Data analysis

All experiments were performed in three replicates and all analysis were conducted at least twice within each replicate. The effect of treatments was determined by the analysis of variance (ANOVA) using the PROC MIXED procedure and Tukey's mean separation was performed to compare differences between the treatments using statistical software, SAS version 9.4 (SAS Institute Inc. 2014). Data from storage studies were analysed using repeated measurements in ANOVA. Significant differences were defined at $p < 0.05$.

3.5 Results and Discussion

3.5.1. Proximate composition of seed coat

The proximate composition of seed coat of the two cultivars of lentil (green and gray seed coat) is shown in Table 3.1. The results revealed that the seed coat of both cultivars was comprised of approximately the similar composition of macronutrients. Protein, ash, and fat constituted around 12% of the dry matter of seed coat, hence, the carbohydrates, particularly the fiber would be the major constituent of the lentil seed coat irrespective of the seed coat color. The total carbohydrate content determined by the difference method was 80%.

3.5.2 Extractable phenolics of seed coat

The levels of phenolics in 2 g of seeds extracted in consecutive extractions are shown in Table 3.2. The highest percentage of TPC was found in the first extract, and this decreased gradually in the subsequent extracts. During the first three extractions, around 80% of the total

Table 3.1 Proximate composition of seed coat of two lentil cultivars (n = 3)

Cultivar	Moisture (%)	Ash (%)	Protein (%)[NS]	Fat (%)[NS]
CDC Greenland (green seed coat)	8.80[a]	2.71[a]	7.40	0.25
CDC Maxim (gray seed coat)	9.89[b]	2.41[b]	7.36	0.25
SEM[1]	0.079	0.019	0.083	0.008

[a,b]Means with different superscripts within the same column are significantly different (p<0.05)
[NS]No significant difference
[1]Standard error of mean

phenolics were extracted, and significant differences were not observed for the extraction pattern of different phenolic classes between cultivars or solvents. Only slight variation (CV less than 10%) was observed in the phenolic composition of lentil seed coats obtained from three different seed lots (different fields) implying that different seed lots had a relatively similar phenolic composition. The levels of phenolic compounds in plants are usually affected by environmental factors such as soil composition, rainfall temperature and humidity (Rezende et al., 2015). Thus, the limited variations observed between different seed lots could be due to that all samples were obtained from the same area (Moose Jaw), although they were obtained from different plants at different maturity, and from different fields.

The extractable total phenolic content (TPC) varied within a narrow range between the cultivars and extraction solvents used. Extracts of 70% v/v aqueous ethanol showed slightly higher (p<0.01) TPC (4-12%) compared to the water extracts in both cultivars. These results are in agreement with those reported by Li (2017). Li (2017) investigated the extractable phenolic content of the seed coat of the same lentil cultivars and found that aqueous ethanol extracts had higher levels of total phenolics compared to the water extracts. Similarly, 70% v/v ethanolic extracts of edible plant parts (crown daisy leaf, pumpkin leaf, chamnamul, fatsia, leek leaf, bok choy, acanthopanax, butterbur leaf, soybean leaf, and broccoli) presented higher levels of total phenolics than in water extracts (Kim et al., 2013). In contrast, Oomah et al. (2011) noticed that water had extracted more phenolics than 80% v/v aqueous ethanol from lentil seed coat. However, different lentil cultivars (CDC Plato and CDC Redberry) were used in their study. Khokhar and Magnusdottir (2002) also found water to be the best solvent to extract tea phenolics compared to 70% ethanol and 80% methanol. Differences in the solubility of total phenolics of different plant extracts are most likely due to the presence of many different compounds and their distinct

Table 3.2 Extractable total phenolics, flavonoids, tartaric esters and flavonols of seed coat of two lentil cultivars in 70% v/v aqueous ethanol and water (n = 3)

Type of phenolics	Cultivar	Solvent	Extraction number 1	2	3	4	5	Total
Total	CDC	70% Ethanol	15.60[a]	17.08[a]	8.74[a]	5.38	3.66	50.46[a]
phenolic	Greenland	Water	14.31[a]	13.00[b]	8.09[a]	4.84	4.07	44.30[b]
content	CDC	70% Ethanol	13.01[ab]	14.82[ab]	7.99[ab]	4.48	3.65	43.96[b]
(TPC)	Maxim	Water	10.28[b]	15.78[a]	7.28[b]	4.59	3.70	41.63[c]
		SEM[1]	0.738	0.596	0.171	0.219	0.193	0.829
Flavonoid	CDC	70% Ethanol	0.57[a]	0.63[a]	2.04[b]	1.84[a]	1.20[ab]	6.27[a]
content	Greenland	Water	0.54[a]	0.56[a]	1.80[c]	1.59[b]	1.32[a]	5.81[b]
(TFC)	CDC	70% Ethanol	0.37[b]	0.61[a]	2.28[a]	1.47[bc]	1.13[b]	5.86[b]
	Maxim	Water	0.30[c]	0.39[b]	1.64[d]	1.33[c]	1.17[b]	4.83[c]
		SEM[1]	0.012	0.022	0.02	0.036	0.033	0.062
Tartaric	CDC	70% Ethanol	0.11[b]	0.14[c]	0.16[c]	0.17[b]	0.22	0.80[c]
esters	Greenland	Water	0.12[ab]	0.23[b]	0.22[b]	0.29[a]	0.26	1.12[b]
	CDC	70% Ethanol	0.12[ab]	0.13[c]	0.14[c]	0.12[b]	0.21	0.73[c]
	Maxim	Water	0.14[a]	0.30[a]	0.32[a]	0.30[a]	0.24	1.31[a]
		SEM[1]	0.005	0.004	0.003	0.019	0.021	0.031
Flavonols	CDC	70% Ethanol	0.21[c]	0.22[c]	0.27[c]	0.30[b]	0.43[b]	1.42[c]
	Greenland	Water	0.30[ab]	0.48[b]	0.47[b]	0.67[a]	0.61[a]	2.53[b]
	CDC	70% Ethanol	0.25[bc]	0.22[c]	0.25[c]	0.21[b]	0.24[c]	1.17[d]
	Maxim	Water	0.35[a]	0.72[a]	0.76[a]	0.72[a]	0.61[a]	3.15[a]
		SEM[1]	0.015	0.009	0.026	0.042	0.031	0.049

[a-c]Means with different superscripts within the column for each phenolic compound are significantly different (p<0.05)

[1]Standard error of mean

Concentrations of phenolic compounds are expressed as mg gallic acid, (+)-catechin, caffeic acid and quercetin as equivalents/g sample (dry basis) for total phenolics, total flavonoids, tartaric eaters and flavonols, respectively.

polarities. Therefore, the choice of solvent for phenolic compound extraction depends on properties of the phenolic compounds of the plants concerned. Comparison between two different cultivars used in this study showed that TPC was significantly higher ($p<0.05$) in both water and aqueous ethanol extracts of CDC Greenland than those from CDC Maxim. The water and 70% aqueous ethanol extractable TPC of CDC Greenland were 44.30 and 50.46 mg GAE/g, respectively, while the corresponding values for CDC Maxim were 41.63 and 43.96, respectively. No significant difference was found ($p>0.05$) in the TPC between the water extract of CDC Greenland and ethanol extract of CDC Maxim.

The extractable total flavonoid content of the seed coat of two cultivars was determined. The extraction pattern in the five consecutive extractions was different from the TPC. Although TPC of the extracts declined with the number of extractions, the amount of flavonoid increased until the third extraction. The highest amount of TFC was found in the third extract in both water and aqueous ethanol. The aqueous ethanol extracts showed TFC at 6.27 and 5.86 mg catechin equivalents/g for cultivars CDC Greenland and CDC Maxim, respectively, which were higher than water extracts. A 7% to 17% difference was observed between the total flavonoids soluble in ethanol and water of two cultivars. Irrespective of the solvent system, the seed coat of CDC Greenland had higher TFC than the seed coat of CDC Maxim (6% to 16% difference). Similar findings have previously been reported by Do et al. (2014) for other plant materials. They observed higher levels of TFC in ethanol extracts in comparison to water extracts of *Limnophila aromatica* plant.

The relative amounts of tartaric esters and flavonols differed significantly between cultivars, type of solvent, and the number of extractions. The total water extractable tartaric esters and flavonols were 1.12 mg caffeic acid equivalents/g and 2.53 mg quercetin equivalents/g, respectively in CDC Greenland, whereas the corresponding values for CDC Maxim were 1.31 mg caffeic acid equivalents/g and 3.15 mg quercetin equivalents/g, respectively. In contrast to the TPC and TFC, tartaric esters and flavonols were more soluble in water than in 70% aqueous ethanol and there was around 30%-40% and 40%-60% difference in the levels of tartaric esters and flavonols between water and ethanol extracts. These results were in agreement with the findings reported by Oomah et al. (2011) for lentil and pea seed coats. They demonstrated that water extracts had higher level of tartaric eaters and flavonols than in the corresponding acetone extracts. They further

reported that the TPC, tartaric esters and flavonols contents of lentil seed coat were higher than those reported for yellow pea seed coat (Oomah et al., 2011).

Overall, the results of the present work revealed that TPC and TFC were higher in CDC Greenland (green seed coat), while tartaric esters and flavonols were higher in CDC Maxim (gray seed coat). These differences might be due to variations in the phenolic composition in seed coat between cultivars. Mirali (2017) also reported slight variations in levels of different phenolic compounds between green and gray seed coat. For example (+)-catechin, (-)-gallocatechin and catechin-3-glucoside were higher in green seed coat compared to the gray seed coat while procyanidin B1, quercetin-3-rhamnoside and myricetin-3-O-rhamnoside were similar between seed coats. Further, some differences in the phenolic contents were observed between water and 70% ethanol extracts in the present study, indicating that the solubility of different phenolic classes may differ depending on the solvent. It was reported that the yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample (Do et al., 2014). In the present work, temperature, extraction time, and pH were similar between the two solvent systems used; thus, the differences observed would mainly be influenced by the seed coat phenolic composition and their polarity. The extraction yield of different phenolic classes changed with subsequent extractions, especially the flavonoids, tartaric esters and flavanol levels were higher in third extract than in the first two extracts. This may suggest that the studies in which only one extraction is used might miss some of the phenolics in their extracts. Phenolic compounds generally exist in free, esterified or glycosylated form in plants (Czemplik et al., 2017). The free and bound forms are therefore extracted successively in solvents (Harukaze et al., 1999). This phenomenon may explain the differences found in the extraction yields of different phenolic classes during the successive extractions

3.5.3 Antioxidant activities of water and aqueous (70% v/v) ethanol extracts of seed coat
3.5.3.1 DPPH free radical scavenging activity

Free radicals play a major role in numerous chronic pathologies such as cancer and cardiovascular disease through involvement in the process of lipid peroxidation (Arabshahi-De louis, 2007). The radical DPPH has been accepted as a model compound for lipid-derived free radicals and was widely used to assess the free radical scavenging capacity of various natural products (Da Porto et al., 2000). In the present study, DPPH radical scavenging ability of water
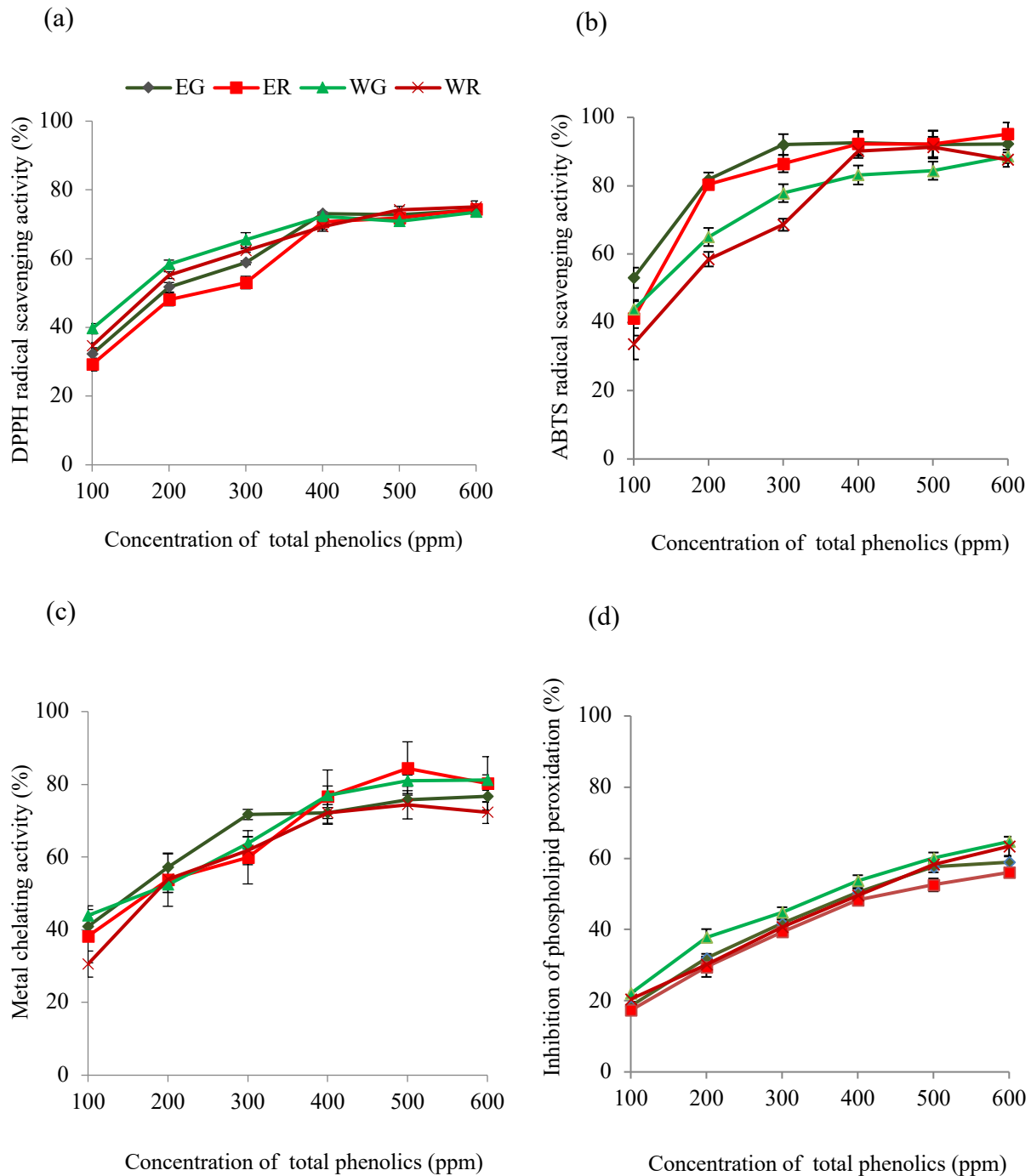
Figure 3.1 Antioxidant activity of lentil seed coat extracts at different concentrations of total phenolics (a) DPPH scavenging activity (b) ABTS scavenging activity, (c) Fe^{2+} ion chelation activity, (d) Inhibition of phospholipid peroxidation

(EG: 70% aqueous ethanol extract of CDC Greenland, ER: 70% aqueous ethanol extract of CDC Maxim, WG: water extract of CDC Greenland, WR: water extract of CDC Maxim). Values are the means and standard deviation of duplicate assays from three replicates.

and aqueous ethanol extracts of two cultivars of lentil were evaluated at different concentrations (Figure 3.1a). All extracts showed some degree of radical scavenging capacity in a concentration dependent manner. Significant differences were observed in the DPPH radical scavenging ability among different extracts at lower concentrations; however, at concentrations above 400 ppm, all extracts tend to have almost the same scavenging activity. When comparing the antiradical activity between the two cultivars and two solvent systems, CDC Greenland and water showed greater activity (p<0.001) than CDC Maxim and aqueous ethanol, respectively (Table 3.3). Han and Baik (2008) compared DPPH radical scavenging ability of 80% ethanol extracts of two cultivars of lentil (Pardina with gray color seed coat and Crimson with brown color seed coat) and found no significant difference between the two cultivars. The DPPH free radical scavenging activity of seed coat extracts ranged between 69% to 75% at concentrations above 400 ppm showing significant antiradical activity of seed coat extracts. Xu and Chang (2008) showed that lentil had the highest antiradical capacity when measured as DPPH radical scavenging activity in comparison with yellow pea, chickpea, and green pea. However, Zhang et al. (2015) found that the free radical scavenging ability differed among 20 Canadian lentil cultivars. Such variability may be due to the genetic diversity among these different lentil cultivars. However, in these studies, the free radical scavenging activity has been measured in whole lentil seeds. Dueñas et al. (2006) and Li (2017) reported that seed coat exhibited higher DPPH radical scavenging ability than cotyledon. The radical scavenging activity of seed coat phenolics were found to be more than 10 times higher than that of whole seed (Li, 2017).

3.5.3.2 ABTS free radical scavenging activity

The antioxidant potency of plant extracts has also been measured using the ABTS assay. An additional advantage offered by the ABTS assay is that ABTS is soluble in aqueous and organic solvents thus useful in assessing antioxidant activity of samples in different media (Shalaby and Shanab, 2013). As shown in Table 3.3, significant cultivar, solvent and phenolic concentration as well as solvent x phenolic concentration and cultivar x phenolic concentration interaction (p<0.001) effects were observed for ABTS radical scavenging activity indicating different behavior of extracts with the increasing concentrations. CDC Greenland's water extract showed a gradual increase in scavenging activity from 100 ppm to 600 ppm concentration, whereas ethanol extract displayed a rapid increase of up to 300 ppm and then plateaued. For CDC Maxim, there was a rapid

Table 3.3 Effect of lentil cultivar, extraction solvent and phenolic concentration on antioxidant activity of seed coat

Source of variance	Factor	DPPH	ABTS	MC	LP
Cultivar	CDC Greenland	62[a]	78[a]	66[a]	45[a]
	CDC Maxim	59[b]	75[b]	63[b]	41[b]
Solvent	70% ethanol	59[b]	82[a]	65[a]	41[b]
	Water	63[a]	71[b]	63[b]	45[a]
Concentration	100	34[e]	42[d]	38[a]	20[f]
	200	53[d]	71[c]	54[a]	31[e]
	300	60[c]	81[b]	64[b]	42[d]
	400	72[b]	89[a]	74[c]	50[c]
	500	72[b]	87[a]	78[d]	57[b]
	600	74[a]	90[a]	77[e]	60[a]
Cultivar x Solvent		NS	NS	***	NS
Solvent x Concentration		***	***	NS	NS
Cultivar x Concentration		***	***	***	NS
Cultivar x Solvent x Concentration		NS	NS	***	NS

[a-f]Means with different superscripts within the column for each component are significantly different ($p < 0.05$)
***interaction effect significant at $p < 0.001$
NS: Not significant
DPPH: DPPH free radical scavenging activity, ABTS: ABTS free radical scavenging activity, MC: Fe^{+2} ion chelation activity, LP: Inhibition of phospholipid peroxidation

rise from 100 to 200 ppm and almost plateaued afterward. For the water extract, there was nearly a steady increase in activity hitting the peak activity at 400 ppm.

Overall, the ABTS scavenging activity of the four extracts increased with the increasing concentration of total phenolics in the extract, and the trend was almost the same as those of the DPPH assay (Figure 3.1b). However, the percent ABTS radical scavenging activity was greater at higher phenolic levels (> 400 ppm) than the respective samples determined by the DPPH assay. Antioxidant compounds scavenging ABTS radical at a higher level compared to DPPH radical were also reported by Sachindra et al. (2007) and Zou et al. (2011) for marine carotenoid fucoxanthin and lentil, respectively.

Generally, both water and aqueous ethanol extracts of lentil seed coat were found to be effective in scavenging DPPH and ABTS free radicals. A similar trend was observed for water and aqueous methanol extracts of *Spirulina platensis* (Shalaby and Shanab, 2013). These authors observed that water extract of *S. platensis*, a blue -green algae, was effective in scavenging DPPH radicals while 50% methanol extracts was effective in scavenging ABTS radicals. Kim et al. (2013) reported that the DPPH scavenging activities of 70% ethanol extracts of crown daisy, pumpkin, soybean, leek, and butterbur leaves were relatively higher (p≤0.05) than their water extracts whereas water extracts of bok choy and broccoli exhibited stronger activity than 70% ethanol extracts. However, none of these studies have measured the antioxidant activity at same concentrations of the phenolics and antioxidant activities were relatively proportional to the phenolic content. In the current study, CDC Greenland showed significantly higher activity in scavenging both DPPH and ABTS radicals than CDC Maxim. The antiradical activity of the lentil seed coat extracts exhibited against the DPPH and ABTS radicals suggest that these extracts may also be capable of scavenging peroxyl radicals generated in meat products by lipid oxidation preventing the propagation of oxidation process.

3.5.3.3 Ferrous ion chelating activity

The Fe^{2+} ion chelating activity of seed coat extracts are shown in Figure 3.1c. Analysis of variance (ANOVA) of the Fe^{2+} ion chelation data showed significant effects of cultivar, solvent system, and concentration as well as significant interactions (p<0.001) between the three factors (Table 3.3). The water and aqueous ethanol extracts of CDC Maxim and water extract of CDC Greenland displayed nearly a steady increase in activity with the increasing phenolic concentration.

CDC Greenland's water extract activity increased to 300 ppm and then almost plateaued. Similar to the DPPH and ABTS antiradical effects of the seed coat extracts, the overall Fe^{2+} ion chelating ability was found to be higher for the cultivar CDC Greenland and solvent 70% ethanol. However, Li (2017) compared the Fe^{2+} ion chelating ability of water-soluble components of the lentil cultivars CDC Greenland and CDC Maxim and found no difference between the two cultivars. This difference could be due to the differences in the composition of antioxidant compounds in the extracts used between the two studies. Rate of Fe^{2+} ion chelation by seed coat extracts was dose-dependent. The Fe^{2+} ion chelation increased with the increasing concentration of the total phenolics up to 500 ppm and there was no difference between the concentrations 500 and 600 ppm. The extracts showed 76%, 81%, 84% and 74%, for GE, GW, RE and RW, respectively, Fe^{2+} chelating ability at a concentration of 500 ppm.

Transition metals such as Fe^{2+} are typically and predominantly bound in tissues, thus their removal is important in the control of lipid oxidation in meat and meat products. The metal ions participate in redox reactions and cause oxidative stress through the formation of free radicals (van Lith and Ameer, 2016). The ferrous ion is also capable of catalyzing hydrogen peroxide conversion to hydroxyl radical via Fenton reaction, initiating lipid peroxidation (Stohs & Bagchi 1995). Chelation is the formation of multiple coordination bonds between organic molecules and a transition metal ion that leads to metal sequestration, making these metal ions unavailable for oxidation reactions. The findings of this study revealed that water and 70% aqueous ethanol extracts of lentil seed coat has a marked iron-chelation capacity indicating that their action as a peroxidation protector which would be useful in controlling oxidative deterioration in meat systems.

3.5.3.4 Inhibition of phospholipid peroxidation

Phospholipids are usually thought to be the major lipid fraction responsible for the oxidative deterioration of food owing to its higher degree of unsaturation (Wu and Sheldon, 1998). Phospholipids can, therefore, be considered a more relevant substrate for assessing antioxidant activity in the food systems. Figure 3.1d shows the effect of different lentil seed coat extracts on the inhibition of phospholipid peroxidation. The inhibitory effect varied between cultivars, extraction solvent and different phenolic concentrations. However, no interaction effects were noted among the factors: cultivar, solvent, and phenolic concentration (Table 3.3) and all extracts

exhibited similar behaviour with the increasing TPC. Inhibitory activity increased with increasing concentration of the total phenolics as same as in other assays. However, the antioxidant activity of the seed coat extracts as determined by the capacity to inhibit phospholipid peroxidation was relatively lower than that of the corresponding concentrations determined in other three assays.

Overall, the cultivar CDC Greenland displayed higher inhibitory activity than that of CDC Maxim. Between the two solvents, water extracts exhibited a greater effect on the inhibition of phospholipid peroxidation. Each extract at the phenolic concentration of 600 ppm showed 59, 64, 55 and 61% inhibition of phospholipid peroxidation for GE, GW, RE and RW, respectively. The results of this study suggest that lentil seed coat extracts can play a significant role in protecting meat products against oxidative damage.

3.5.3.5 Comparison of antioxidant properties between seed coat extracts and other antioxidants

The antioxidant properties of seed coat extracts were compared with commercial antioxidant compounds and results are shown in Figure 3.2. Figure 3.2a illustrates the DPPH radical scavenging activity. At 300 ppm concentration, water extract of lentil seed coat of both cultivars presented the highest DPPH scavenging activity and it was comparable to the activity of (+)-catechin (C). Catechin is a naturally occurring polyphenol which belongs to the group of flavonoids. Catechin is present in many plants, fruits, red wine, beer, cocoa, etc, and are the major polyphenols in tea (Esselen and Barth, 2014). Aguilera et al. (2010) reported that catechins are among the predominant phenolics in lentil seeds. The antioxidant action of catechin is well established by different *in vitro* and *in vivo* methods, thus, used as a reference compound in the present study. Li (2017) reported that the DPPH radical scavenging potential of lentil seed coat water-soluble compounds was 6-fold higher than that of catechin even at a concentration of around 200 ppm. This can support the superior antiradical role of seed coat antioxidant compounds on DPPH compared to pure catechin.
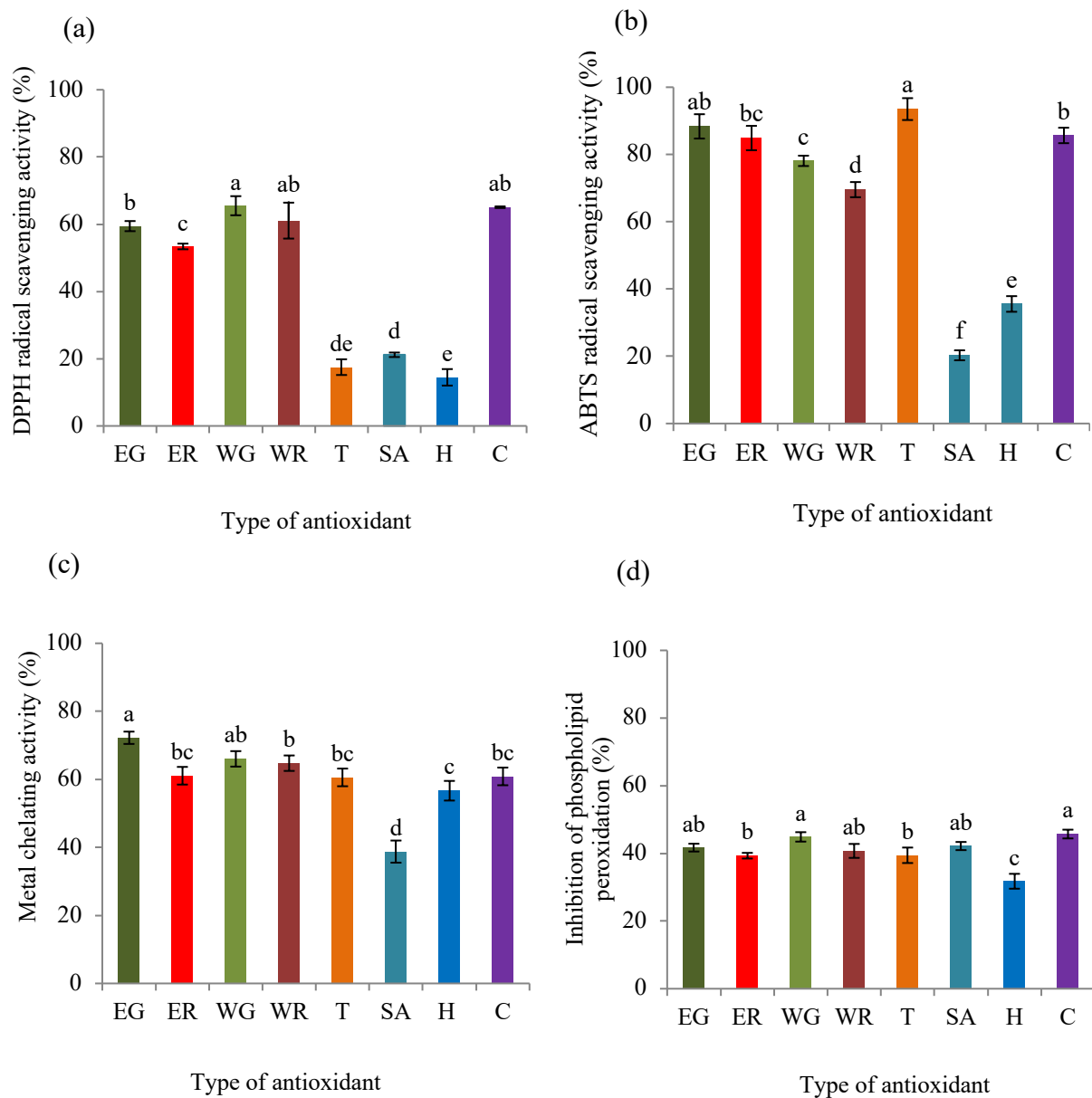
Figure 3.2 Antioxidant activity seed coat extracts compared with other antioxidants at 300 ppm total phenolic concentration (a) DPPH scavenging activity (b) ABTS scavenging activity, (c) Fe^{2+} ion chelation activity, (d) Inhibition of phospholipid peroxidation (EG: 70% aqueous ethanol extract of CDC Greenland, ER: 70% aqueous ethanol extract of CDC Maxim, WG: water extract of CDC Greenland, WR: water extract of CDC Maxim, T: (+/-)-α-tocopherol, SA: sodium ascorbate, H: Herbalox®, C:(+)-catechin). Error bars are the standard deviations of duplicate assays of three replicates. [a-d]Means with different letters are significantly different ($p < 0.05$)

It is noteworthy that all seed coat extracts had higher DPPH scavenging activity than sodium ascorbate (SA), Herbalox® rosemary extract (H), and (+ /-) α- tocopherol (T). Sodium ascorbate (SA) is used as an antioxidant in many foods, including meat products. It is a chelating agent that binds metal ions, as well as scavenging free radicals and acting as a reducing agent (Amaral et al., 2018). It might also act as a prooxidant depending on the concentration, the presence of metal ions, and the amount of tocopherol (Mielche and Bertelsen, 1993; Schaefer et al., 1995; Decker and Xu, 1998). Tocopherols are effective natural fat-soluble antioxidants, widely used as a natural alternative for BHA and BHT (Mielnik et al., 2003). By competing with the substrate over peroxyl radicals, α-tocopherol can act as an electron donor. Besides, its antioxidant activity can also be correlated with delaying hydroperoxide decomposition (Georgantelis et al., 2007). Rosemary (*Rosmarinus officinalis* L.) showed significant antioxidant potential mainly because of its phenolic compounds. Phenolic acids (caffeic, ferulic, and rosmarinic acid) and phenolic diterpenes (carnosic acid and carnosol) were the predominant phenolic compounds responsible for its antioxidant activity (Amaral et al., 2018). It was suggested that the antioxidant activity of rosemary was due to chelation of metal ions and elimination of superoxide radicals (Amaral et al., 2018). The present study demonstrated that the lentil seed coat extracts had around 40% higher DPPH scavenging activity than sodium ascorbate, Herbalox® rosemary extract, and (+ /-) α-tocopherol. In contrast, Zou et al. (2011) exhibited significantly higher DPPH radical scavenging activity by ascorbic acid than the phenolic extracts from lentil (variety Morton). This contrasting observation with the present study might be attributed to the differences in the phenolic concentration in the extracts. However, similar to our observations, they also observed that the scavenging activity of lentil phenolics were superior to α-tocopherol. These results suggest that lentil seed coat would be superior to other antioxidant compounds used in controlling the progression of lipid oxidation in foods by eliminating free radicals generated in foods.

In terms of the ABTS radical scavenging activity (Figure 3.2b), the trend was almost same as those of DPPH assay except for T. The ABTS radical scavenging effect decreased in the order of T, EG, C, ER, WG, WR, H and SA. α- tocopherol (T), which is a strong antioxidant, exhibited the highest ABTS radical scavenging capacity, however, its activity was similar to ethanol extract of CDC Greenland. Radical scavenging activity of all seed coat extracts surpassed those of sodium ascorbate and Herbalox® rosemary extract. Differences between DPPH and ABTS scavenging behavior showed by α- tocopherol (T) can be interpreted as being due to the different kinetics of

the two radicals and different affinity to different free radicals (Vamanu and Nita, 2013). Other antioxidant compounds did not show different behavior on these two different radicals.

The Fe^{2+} ion chelation activity of lentil seed coat extracts in comparison to other antioxidants compounds are illustrated in Figure 3.2c. Ethanol extract of CDC Greenland (EG) had the highest activity (72%), whereas sodium ascorbate exhibited the lowest activity (38%). No significant difference was observed among ER, WG, WR, H, and C, showing that lentil extracts at least as good as three common antioxidants; α- tocopherol, Herbalox®, and catechin and better than sodium ascorbate with respect to the iron chelation ability.

With regard to the inhibition of phospholipid peroxidation (Figure 3.4d), drastic differences were not observed between the antioxidant compounds. The water extract of CDC Greenland (WG), which showed the highest inhibitory activity, was comparable to all other seed coat extracts and other commercial antioxidant compounds except Herbalox®, and their activity ranged between 39% to 45%. The lowest inhibitory activity was noted for Herbalox® (31%).

3.5.4 Correlation between total phenolic content and antioxidant activity of seed coat extracts

Pearson correlation analysis was performed to analyze the linear relationships between TPC, and antioxidant activity and the findings are shown in Table 3.4. Strong positive correlations were found between TPC and all antioxidant assays suggesting that phenolic content contributes a lot to the antioxidant activity of seed coat extracts. Among the antioxidant assays, ABTS assay had the lowest correlation with the TPC (r = 0.79). For the TPA the highest correlation was found with inhibition of liposome peroxidation (r = 0.96), followed by DPPH radical scavenging activity (r = 0.91) and Fe^{2+} chelation activity (r = 0.90). Strong positive correlations between TPC and antioxidant activity were also reported in lentils (Amarowicz et al., 2003; Han and Baik, 2008; Zou et al., 2011), chickpeas and soybean (Xu and Chang, 2007; Han and Baik, 2008). All antioxidant assays were significantly correlated with each other, suggesting that all assays were reliable and interchangeable.

Table 3.4 Pearson correlation coefficients among total phenolic content and antioxidant activity of seed coat (n=36)

	DPPH	ABTS	MC	LP
TPC	0.91***	0.79***	0.90***	0.96***
DPPH		0.83***	0.93***	0.95***
ABTS			0.88***	0.80***
MC				0.93***

TPC: total phenolic content, LP: Inhibition of phospholipid peroxidation, ABTS: ABTS free radical scavenging activity, DPPH: DPPH free radical scavenging activity, MC: Fe^{+2} ion chelation activity, LP: Inhibition of phospholipid peroxidation

*, **, *** Significant at $p < 0.05$, 0.01 and 0.001, respectively.

3.5.5 Antioxidant potential of phenolic extracts from lentil seed coat in raw and cooked MSC stored under refrigerated and frozen conditions

Natural and synthetic antioxidants have been commonly used in the meat industry in order to control the development of oxidative changes in meat products. However, the demand for natural antioxidants is growing worldwide due to the increasing awareness among consumers regarding health benefits of natural antioxidants. The present study demonstrated that lentil seed coat had significant antioxidant activity in *in vitro* assays. However, it is also necessary to understand the activity of these compounds in food matrices which are complex mixtures of different types of compounds. Further, no information has been reported on the application of lentil phenolics in mechanically separated chicken meat (MSC). Therefore, this experiment investigated the efficacy of seed coat and water and aqueous ethanol extracts of seed coat of cultivar CDC Greenland on controlling oxidative deterioration of color and lipids of MSC. The antioxidant capacity of these seed coat components was also compared with the four commercial antioxidants used in previous experiments.

3.5.5.1 CIE color of meat

The changes in color during storage of meat in the retail display case under florescent light and in dark condition are shown in Tables 3.5 and 3.6, respectively. Comparison of the color data between two storage conditions revealed that the samples stored under dark conditions followed similar trends to the samples stored under fluorescent light. The color of meat forms the first impression that greatly affects the consumer selection of fresh meat. The meat pigment myoglobin,

Table 3.5 Color changes (L*, a* and b* values) of mechanically separated chicken stored in display case (1200 lx) at 4°C for seven days (n = 3)

Storage time	CON	ESC	WSC	SC	C	H	T	SA	SEM[1]
L* value									
Day 1	63.11[ab]	62.00[ab]	62.79[ab]	60.23[b]	62.85[ab]	62.62[ab]	62.65[ab]	63.96[a]	0.754
Day 3	57.54[ab]	58.09[ab]	57.93[ab]	55.56[b]	58.26[ab]	57.97[ab]	58.60[a]	58.83[a]	1.939
Day 5	54.13[ab]	54.96[a]	55.23[a]	53.46[b]	54.90[a]	54.94[a]	55.09[a]	54.74[a]	1.398
Day 7	54.51[ab]	54.49[ab]	54.66[ab]	53.68[b]	54.42[ab]	54.85[ab]	54.94[ab]	55.14[a]	0.364
a* value									
Day 1	26.42[a]	25.75[a]	25.60[a]	22.77[b]	27.25[a]	26.69[a]	26.59[a]	27.73[a]	0.584
Day 3	23.66[ab]	22.22[b]	22.19[b]	19.40[c]	23.07[ab]	22.04[b]	23.10[ab]	24.54[a]	0.777
Day 5	22.65[a]	21.49[a]	21.75[a]	18.98[b]	22.43[a]	21.65[a]	22.24[a]	23.10[a]	1.239
Day 7	21.65[a]	20.52[a]	20.57[a]	17.80[b]	21.26[a]	20.03[a]	21.89[a]	21.70[a]	0.504
b* value									
Day 1[NS]	25.62	24.97	24.97	23.78	22.2	26.94	25.49	26.20	1.462
Day 3[NS]	21.41	20.80	20.93	19.82	21.37	22.70	21.12	22.00	1.592
Day 5	19.25[b]	19.16[b]	18.97[b]	19.06[a]	19.20[b]	22.08[b]	18.94[b]	19.00[b]	2.176
Day 7	17.45[a]	17.47[a]	17.51[a]	17.67[a]	17.42[a]	20.28[b]	17.25[a]	16.70[a]	1.532

CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate,
[ab]Means with different superscripts within the same row are significantly different (P<0.05)
[1] Standard error of mean
[NS]No significant difference within the row

Table 3.6 Color changes (L*, a* and b* values) of mechanically separated chicken treated with different antioxidants stored in dark conditions at 4ºC for seven days (n = 3)

Storage time	Type of antioxidant								
	CON	ESC	WSC	SC	C	H	T	SA	SEM[1]
L* value									
Day 1	64.65[ab]	62.79[bc]	64.18[ab]	61.75[c]	64.05[ab]	64.21[ab]	64.65[ab]	64.89[a]	0.415
Day 3[NS]	60.51	59.47	60.32	58.84	59.54	60.26	61.06	60.85	1.539
Day 5	57.63[ab]	57.28[ab]	57.97[a]	56.04[b]	57.54[ab]	58.31[a]	58.33[a]	58.02[a]	1.540
Day 7	56.69[a]	56.09[ab]	56.59[ab]	55.06[b]	56.07[ab]	57.13[a]	56.00[ab]	56.99[a]	0.699
a* value									
Day 1	26.55[bc]	25.84[c]	25.78[c]	22.68[d]	26.98[ab]	26.88[ab]	26.16[bc]	27.55[a]	0.879
Day 3	21.96[ab]	20.27[bc]	20.76[ab]	17.79[c]	23.46[a]	21.47[ab]	21.37[ab]	23.00[ab]	1.378
Day 5	23.52[ab]	21.63[bc]	22.38[ab]	19.95[c]	23.33[ab]	23.10[ab]	23.34[ab]	23.84[ab]	0.657
Day 7	21.36[a]	20.65[a]	20.88[a]	18.42[b]	20.52[a]	20.60[a]	21.49[a]	21.46[a]	0.847
b* value									
Day 1	25.43[bc]	24.88[c]	24.90[bc]	23.53[d]	25.68[bc]	26.98[a]	25.05[bc]	25.88[b]	0.511
Day 3[NS]	22.13	21.69	22.06	21.52	22.06	23.23	21.26	22.05	1.154
Day 5[NS]	19.32	19.41	19.44	19.44	19.34	21.30	19.38	21.23	1.493
Day 7[NS]	17.66	19.79	18.28	17.88	17.37	19.39	17.45	17.51	1.899

CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate,
[a-d]Means with different superscripts within the same row are significantly different (P<0.05)
[1] Standard error of mean
[NS] No significant difference within the row

which determines the color of fresh meat, undergoes various chemical reactions which are influenced by oxygen, light and heat (Kropf, 2002). Therefore, the chemical state of myoglobin plays a vital role in consumer acceptability. Oxymyoglobin is the oxygenated form of myoglobin which gives a bright red color to the meat. The myoglobin converts to oxymyoglobin as a result of oxygen binding to the iron atom in the molecule. However, after several days of exposure to air, the iron atom of myoglobin becomes oxidized and loses its ability to bind oxygen, turning meat to a brown color (Aberle et al., 2012). This reduces the consumer acceptability, even though this meat is still safe for consumption.

In the present experiment lentil seed coat and its extracts were added to fresh MSC to study their effectiveness in preserving the color of meat stored in light and in dark. The color of MSC is generally redder than the regular chicken meat due to the inclusion of hemoglobin separated from the bone marrow during the manufacturing process (Song et al., 2014). In addition, the relatively higher pH value of mechanically separated meat also contributed to the dark red color. In the present study the fresh MSC had an attractive red color (a* ~ 26) at the time of purchase.

Samples stored in both conditions showed similar trends regarding color stability. The a* values, which indicates the redness of meat, was higher at day one and these values gradually declined over storage in all treatments. The a* value of all samples on day seven was significantly lower ($p<0.05$) than that on day one. The type of antioxidant and storage time had a strong significant effect ($p<0.001$) on a* value whereas the interaction effect (antioxidant x storage time) was not significant. The a* value of all treatments was similar at day one except the samples treated with ground seed coat (SC) which were darker. By day three, a* value of the samples treated with seed coat components (ethanol extract, water extract and ground seed coat) and α-tocopherol and Herbalox® was significantly lower than that of the sodium ascorbate treated sample. Similar trend in a* value have also been noted by Li (2017) for raw beef burgers. He reported that the beef burgers with 500 ppm of sodium erythrobate displayed higher a* value during the first three days of storage at 4°C compared to the products with 6% whole lentil flour, and 0.6% lentil seed coat. However, the levels of antioxidant compounds (level of TPC from lentil and sodium erythorbate) added to the products were not similar in his study, and therefore the antioxidant effect on color may have been affected mainly by compound concentration and not by the type of antioxidant compound. On the contrary, in the present study all antioxidant compounds were added at similar levels in all treatments; thus, the different antioxidant effects exhibited between treatments may primarily reflect the impact of the type of antioxidant compound rather than the compound concentration.

Although significant differences were found among the treatments during the first five days of storage, the a* values of all samples were similar after seven days of storage, indicating that treatments have undergone color loss at different rates but ended up with similar redness. It was important to note that, although a color loss occurred during storage at 4°C, a* values remained above 20 after seven days of storage in all samples except the samples with seed coat. CIE a* value of 20 is considered as the cut-off point which indicates the loss of red color in meat. Pathiratne

(2014) reported lower a* values (~17) in beef burgers treated with 6% of IR treated lentil flour at day 4 of storage in retail case at 4°C. Li (2017) also reported that the redness of the beef burgers with 6% whole lentil flour or 0.6% lentil seed coat had lost their red color following just three days of storage. Nevertheless, compared to the present study, the beef burgers contained relatively lower amounts of total phenolics from the added seed coat. The poor color stability found in beef burgers may also be related to the higher fat, and iron content, and lower pH of the beef relative to the MSC used in the current work. The fat content of the beef burgers was about 4% greater than MSC. Fat content in the product can influence the rate of color change since, lipid oxidation products such as 4-HNE promote oxidation of oxymyoglobin (Lynch and Faustman, 2000). Other than the level of fat, iron content might make differences on oxidation between different meat types. Iron promotes lipid oxidation by generating reactive oxygen species capable of abstracting a proton from unsaturated fatty acids via the Fenton reaction (Buzala et al., 2016) and the accelerated lipid oxidation results in a reduced color stability. Beef generally has higher level of iron compared to chicken. According to Cross et al. (2012) the iron content in beef were between 4.63 and 5.97 mg/100 g whereas the iron content of MSC in the present work was 1.25 ± 0.34 mg/100 g meat. Also, the oxidation of myoglobin in meat is accelerated at a low pH leading to a decrease in color stability (Neethling et al., 2017). It was further reported that pH influences the metmyoglobin reduction ability (MRA), with a rise in pH contributing to increased MRA (Ledward, 1971). In addition, the findings of previous studies showed that beef with pH < 6.1 had an earlier onset of metmyoglobin formation relative to the beef at pH ≥ 6.1 (Neethling et al., 2017). The MSC used in the current work was at pH of 6.4 and the pH of beef burgers was 5.5 (Li, 2017). This high pH of MSC might have influenced the greater color stability observed in the present work.

The addition of seed coat resulted in a reduction in the redness value of MSC. These samples showed the lowest redness value throughout the storage period as compared to other treatments, and finally reached a redness value lower than 20 suggesting that products containing ground seed coat may have reduced consumer acceptability. This observation could be ascribed to the greenish color of the seed coat which has masked the red color of meat. This kind of color transferring effect from non-meat ingredients have also been reported in previous studies. The incorporation of green leaf extracts in meat products resulted in reduced red color (Kim et al., 2013). Further, Li (2017) reported that the addition of lentil flour and seed coat resulted in lower a* values in beef burgers than the control product with no added binders. Ahn et al. (2007) found

that a* value decreased in cooked beef patties treated with 1% of rosemary oleoresin (Herbalox®) compared with the control. However, several other studies showed that the addition of extracts as natural antioxidants from fruits such as plum, grape and cranberries increased the a* value in various meat and poultry products (Lee and Ahn, 2005; Carpenter et al., 2007; De Gonzalez et al., 2008; De Gonzalez et al., 2009). This increase in a* value was also suggested to be decreasing the consumer acceptability, since the products could be perceived as undercooked (Karre et al., 2013).

For the lightness (L* value), samples incorporated with seed coat showed lower L* values compared to the other treatments including the control samples (Table 3.6). During the seven days of storage, L* value reduced gradually in all samples, which could be due to the oxidation of myoglobin. The type of antioxidant had no significant effect ($p > 0.05$) on the change of L* value during storage, however, the storage time had a significant effect ($p < 0.05$). A significant reduction in L* was observed until day five of storage under florescent light and there was no significant change afterwards. Overall, the samples with seed coat were darker compared to the control and other treatments which might be due to the same effect which was observed for redness; transferring of color from the seed coat. This observation is consistent with the findings of other authors, even with darker meat than poultry. Li (2017) observed the darkening effect of beef burgers due to the addition of lentil seed coat. Biswas et al. (2012) noticed the same effect when the extract of curry and mint leaf were added in ground pork.

Regarding the yellowness (b* value), the trend was very similar to the changes observed for lightness (L*). Significant reduction ($p < 0.001$) in the b* values occurred over the seven days of storage. The type of antioxidant also showed significant influence on the b* value however, a significant interaction effect (type of antioxidant x storage time) was not found. Samples treated with Herbalox® showed comparatively larger b* values throughout the stored period in the display case. At day seven, these samples had significantly higher b* value than all other treatments, which agreed with results reported by Ahn et al. (2007) that b* value increased in the samples of cooked beef patties treated with oleoresin rosemary Herbalox®.

Display lighting can have an effect on the appearance or the discoloration of meat, resulting from the temperature elevation at the meat surface and the photochemical effects (Kropf, 2002). In order to determine the protective effects of lentil seed coat components on light induced oxidation of meat pigments in MSC, the total color changes between the samples stored under fluorescent light in the display case was compared with the samples stored in the dark and results are illustrated

in Figure 3.3. The total color changes during the first three days of storage was not affected (p>0.05) by the storage condition or the type of antioxidant. However, with the extended storage up to five days, significant differences were noticed between the two storage conditions and type of added antioxidant ingredient. The control sample and the samples added with sodium ascorbate and (+)-catechin showed significantly higher color change than the samples with ground seed coat. Further extension of the storage time up to seven days resulted in the same effects. Samples treated with seed coat showed the lowest color change during the seven days of storage irrespective of the storage condition. Seed coat and the ethanol extracts of seed coat exhibited significant color protective effects compared to controls and to samples treated with sodium ascorbate and (+)-catechin. In contrast, Li (2017) reported that sodium ascorbate had better antioxidant properties against color changes in beef burgers than lentil seed coat. These results revealed that the seed coat components have significant protective effects against the color oxidation of uncooked mechanically separated chicken stored at 4°C. However, the lower a* values observed in the samples treated with seed coat indicated that the application of ground seed coat in chicken meat could be limited, as they impart their color to the meat, although they do have better antioxidant properties as shown later.
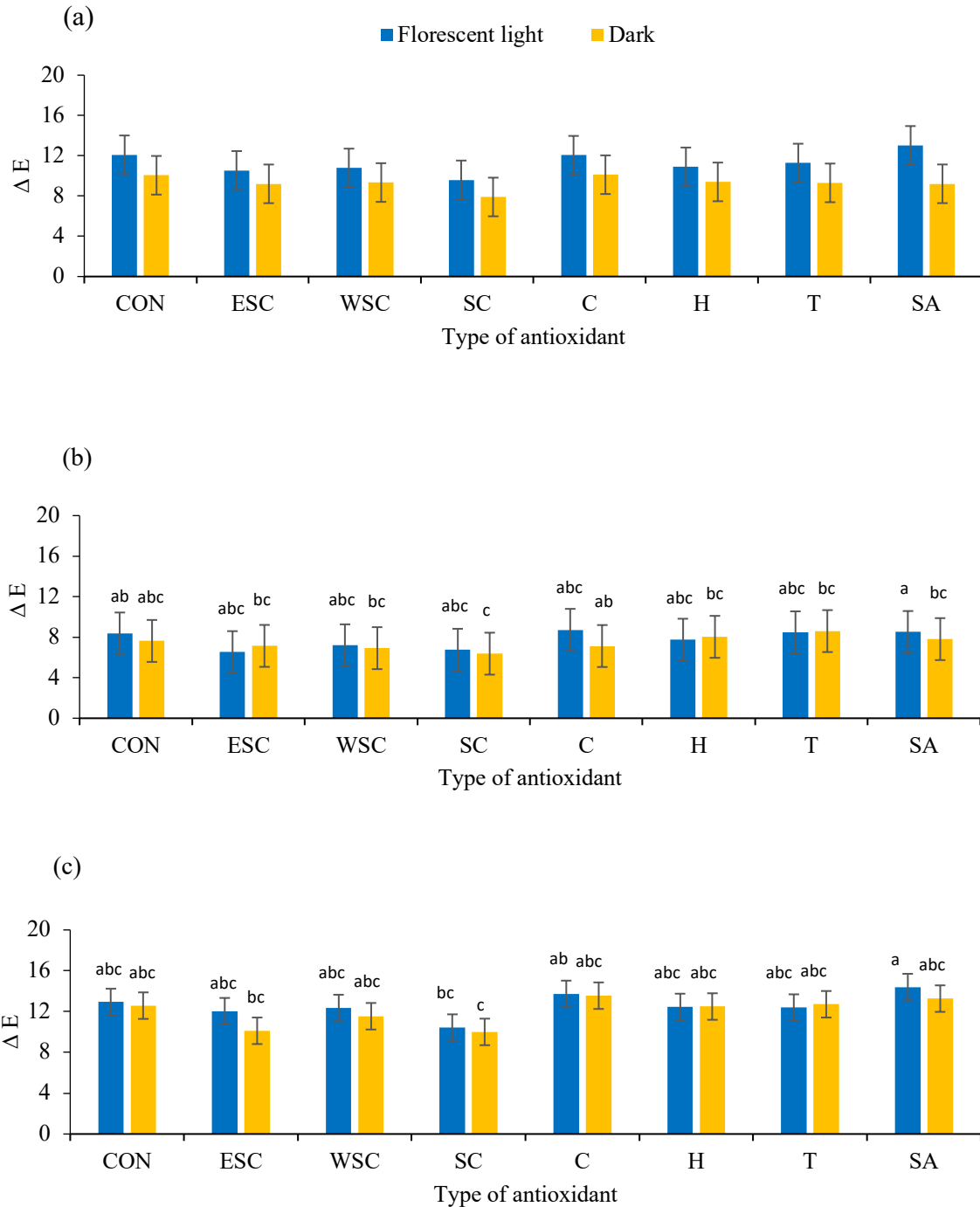
Figure 3.3 Color difference (ΔE) of mechanically separated chicken treated with different antioxidants stored under florescent light and dark conditions at 4° C (a) color difference between day 1 and 3, (b) color difference between day 1 and 5, (c) color difference between day 1 and 7

(CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C :(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate. Error bars are the standard deviations of duplicate measurements of three replicates. [a-c]Means with different letters are significantly different ($p < 0.05$)

3.5.5.2 pH of meat

The pH of meat and meat products indicates their freshness and possible level of bacteriological activity. Generally, as a result of the incorporation of bone marrow, mechanically separated meat presents higher pH than manually deboned meat (Trindade et al., 2004). In general, the pH of MSC ranges from 6.2 to 7.0 (Trindade et al., 2004). Although, these high pH values promote water holding capacity of meat, they favor bacterial growth accelerating the process of spoilage (Trindade et al., 2004). The pH of the raw and cooked chicken meat measured in this study are shown in Table 3.7. The pH of both raw and cooked meat varied within a narrow range (6.37 to 6.57) and did not differ significantly among treatments (p>0.05). Also, the pH of all samples was within the range (6.2 to 7.0) shown by Trindade et al. (2004) for MSC. The meat pH remained nearly unchanged throughout the storage at 4°C, indicating there was no spoilage.

3.5.5.3 Fatty acid composition and iron content of MSC

The fatty acid composition of MSC is given in Table 3.8. The rate and extent of lipid oxidation mainly depend on the level of polyunsaturated fatty acids in a particular muscle system (Allen and Foegeding, 1981). Poultry meat generally exhibits a higher degree of unsaturation compared to red meat, and is therefore more prone to oxidation. Particularly, the membrane lipids which are high in polyunsaturated fatty acids are responsible for the initial development of oxidation in raw and cooked meat products during storage (Gray and Pearson, 1987). The mean total fat content of the meat used in this study was 11.84%. The total fat content of this MSC was higher (8.94%) and lower (14.7%) than those reported by Samaranayake (2003) and Pussa et al. (2009) respectively. Table 3.8 shows the fatty acid composition of MSC. Similar fatty acid compositions have also been presented for MSC in other work (Jantawat and Dawson, 1980).

Several authors have compared the fatty acid profiles of mechanically separated meat with hand-deboned meat. Mott et al. (1982) found higher concentrations of unsaturated fatty acid in mechanically separated chicken. In contrast, Jantawat and Dawson (1980) found very close fatty acid profiles in hand deboned and mechanically separated meat. The grinding and chopping of meat disrupt the membranes and expose the membrane lipids to oxygen, heme pigments and metal ions which can cause a rapid development of oxidation in meat (Pearson et al., 1977). The susceptibility of phospholipid to oxidation is due in part of their high content of

Table 3.7 Change of pH of raw and cooked mechanically separated chicken treated with different antioxidants stored at 4°C for seven days (n = 3)

Treatment	pH			
	Day 1 [NS]	Day 3 [NS]	Day 5 [NS]	Day 7 [NS]
pH of raw meat				
CON	6.40	6.41	6.49	6.56
ESC	6.41	6.41	6.51	6.55
WSC	6.41	6.42	6.50	6.57
SC	6.41	6.42	6.50	6.53
H	6.37	6.37	6.47	6.56
SA	6.43	6.43	6.51	6.56
C	6.40	6.40	6.51	6.58
T	6.42	6.43	6.52	6.58
SEM[1]	0.088	0.075	0.060	0.074
pH of cooked meat				
CON	6.54	6.54	6.56	6.56
ESC	6.57	6.56	6.57	6.57
WSC	6.55	6.56	6.57	6.54
SC	6.57	6.57	6.58	6.57
H	6.54	6.55	6.54	6.56
SA	6.55	6.55	6.57	6.55
C	6.57	6.56	6.57	6.56
T	6.58	6.56	6.58	6.56
SEM[1]	0.076	0.065	0.052	0.078

Means with different superscripts within the same column are significantly different (P<0.05)
[1] Standard error of mean
[NS] No significant difference within the column ((P<0.05)
CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, SA: sodium ascorbate, T: (+/-) - α- tocopherol

polyunsaturated fatty acids (Igene et al., 1980) and the close association of these membrane lipids with the catalysts of the oxidation.

Free iron ions may bind to negatively charged cell membrane phospholipids (such as phosphatidylcholine) and catalyze pre-formed lipid hydroperoxide breakdown (Buzala et al., 2016). The average iron content of the MSC used in this experiment was 1.25 ± 0.34 mg/100 g meat. This level is within the range of iron content previously been reported for MSC. EFSA (2013) reported iron content ranging from 1.0 to 1.8 mg/100 g for MSC obtained by applying different deboner head pressure (40-150 lb/in^2). The iron content of hand deboned meat reported in the same study was 0.6 mg per 100 g meat, which was half of the iron content noted for MSC in the current work.

Table 3.8 Fatty acid composition of raw mechanically separated chicken (n = 3)

Fatty acid	g fatty acid/ 100 g total lipids		
	Mean	Minimum	Maximum
C14:0	0.33	0.30	0.37
C16:0	16.03	14.66	17.66
C16:1	3.81	3.43	4.18
C18:0	4.57	4.02	5.34
C18:1	32.17	30.83	36.00
C18:2	13.91	11.96	17.97
C18:3/n-3	1.90	1.59	2.22
C18:3/n-6	0.11	0.08	0.14
C20:1	2.31	2.06	2.57
C20:4	0.36	0.26	0.44

3.5.5.4 Effect of antioxidant compounds on lipid oxidation of raw MSC stored at 4ºC

The mean TBARS values of uncooked meat stored at 4ºC for seven days are given in Figure 3.4. The TBARS in raw samples showed very low oxidation which did not change significantly over the storage period of seven days. The TBARS values of the samples added with antioxidant compounds except seed coat were similar to the TBARS values of the control samples and no interaction was found between the treatments and storage time. These results indicated that neither
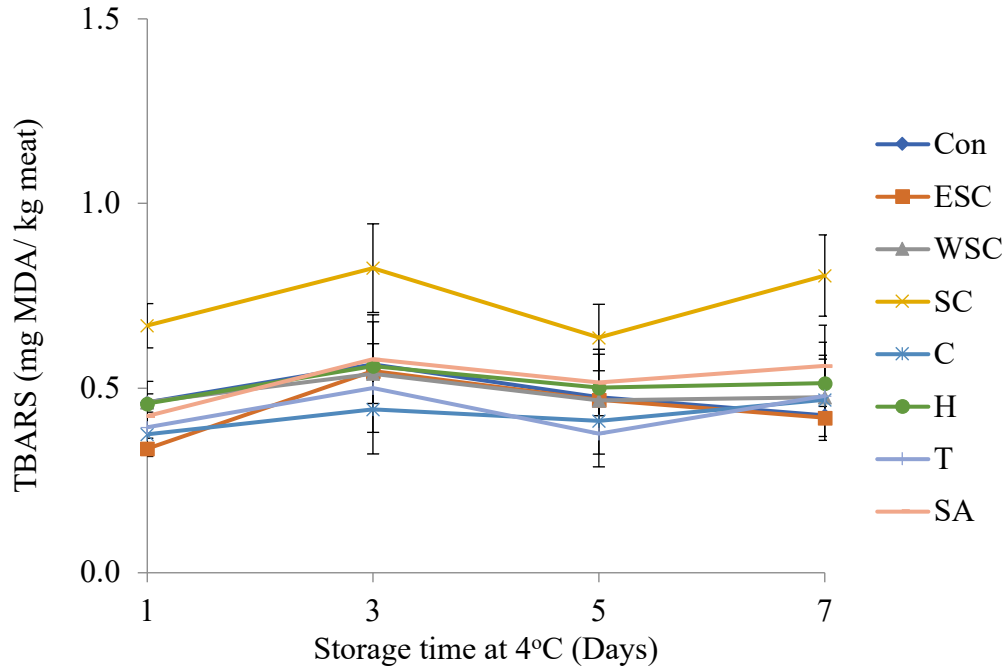
Figure 3.4 Effect of antioxidant compounds (500 ppm) on lipid oxidation of raw mechanically separated chicken stored at 4°C for seven days.
CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate. Error bars are the standard deviations of duplicate assays of three replicates.

the antioxidant treatments nor the storage time affected the oxidation of MSC. The fresh MSC was fairly stable for oxidation during refrigerated storage of seven days. The oxidative stability exhibited by the fresh MSC including the control could possibly be due to the intrinsic antioxidant capacity of meat. Many studies have shown that the dietary antioxidants such as α-tocopherol can influence the stability of lipids in meats during storage. Monahan et al. (1990) and Ashgar et al. (1991) reported that dietary vitamin E supplementation resulted in elevated concentrations of α-tocopherol in the cell membranes resulting in a significantly improved oxidative stability of both raw and cooked pork muscle during storage at 4°C up to 8 days, and stabilized the membrane bound lipids against metmyoglobin and hydrogen peroxide induced oxidation. Avila-Ramos et al. (2013) reported that dietary supplementation of vitamin E and oregano oil resulted in higher antioxidant effects on chicken meat. The MSC used in the present study was processed the same day as the slaughtering of the birds and the study was carried out starting the following day, and therefore, the meat was very fresh. The lower TBARS values observed in all treatments including the control

during the seven days of refrigerated storage might therefore be due to the strong intrinsic antioxidant capacity of the fresh meat. However, this study did not track the feeding practices of the birds used for the production of MSC.

The samples with seed coat had significantly higher overall TBARS values compared to the control samples ($p<0.05$). This observation could possibly be due to some prooxidant properties of the seed coat. Li (2017) demonstrated that lentil seed coat has some lipoxygenase activity of up to 0.02×10^3 units/mg of its water extracts. Lipoxygenase was found to initiate lipid oxidation in food products by catalyzing the unsaturated fatty acids containing *cis,cis*-1,4-pentadiene causing more rapid lipid oxidation as a result (Eriksson, 1982). Li (2017) compared the oxidative stability of beef burgers with incorporated raw and infra-red heat-treated lentil flour including cotyledon and seed coat and observed higher level of TBARS in samples treated with raw lentil flour in comparison to the samples with heat treated lentil flour. He concluded that the low TBARS values resulted from the addition of heat-treated lentil flour were due to the inactivation of lipoxygenase during the infra-red heat treatment. Also, an elevated TBARS was observed for fish mince with the presence of lipoxygenase compared to the control (Fu et al., 2009). Even though the ground seed coat treated samples displayed a slightly higher level of oxidation in the present study, all TBARS values varied below 1 mg MDA/kg of meat over the 7- day refrigerated storage, which was well below the detection levels of oxidized flavor (Green and Cumuze, 1981). However, it is worth mentioning that, the accuracy of the estimated TBARS values can sometimes be affected by the color interfering substances. Indeed, several other compounds present in fresh and processed meat such as water-soluble proteins and peptides, aldehydes, sugars, nitrites and nitrates, metal chelators, pigments, amino acids, fatty acids and phenolic antioxidants were suggested to be reacting with the 2-thiobarbituric acid (TBA) reagent (Díaz et al., 2014) use in TBARS assay. These compounds form yellow or orange complex with TBA reagent and leads to overestimation of absorbance at 532 nm which corresponds to the pink complex (Díaz et al., 2014). Thus, there was a possibility that the color of seed coat components added in meat interfered with the TBARS values observed.

3.5.5.5 Effect of antioxidant compounds on lipid oxidation of raw MSC stored at -18ºC

The results of the TBARS analysis in the samples of uncooked meat stored under frozen conditions for seven months are shown in Figure 3.5. The antioxidant treatments significantly (P<0.05) influenced TBARS values. During the first four months of the storage, all treatments showed fairly stable TBARS values. The TBARS levels of the control samples started to rise from the third month of storage indicating higher level of lipid oxidation progression than other treatments. The meat incorporated with ground seed coat also showed higher lipid oxidation than the other treatments which was similar to the raw samples stored at 4ºC. The higher TBARS values observed in samples with seed coat might be attributed to the activity of the oxidative enzymes associated with raw lentil seed coat, such as lipoxygenase, which could be active even under frozen conditions. The progression of lipid oxidation remained significantly higher in the control and the samples with seed coat from the 4th month until the end of the seven months of study period. The samples with the ethanol extract of seed coat showed stable TBARS values during the whole storage period, and TBARS values did not rise above 1.36 mg MDA/kg meat. According to Green and Cumuze (1981), the threshold for consumers to detect oxidized flavors in meat was approximately 2 mg MDA/kg meat. Although some fluctuations were noted in TBARS values in the samples treated with water extract and sodium ascorbate, generally, no significant variations were found during the storage among treatments. All samples with antioxidant compounds except ground seed coat could keep TBARS values below 2 mg MDA/kg of meat throughout the storage period. These findings revealed that ethanol and water extracts from the seed coat and other commercial antioxidant compounds provide protective effects against lipid oxidation in raw MSC for seven months of storage at -18ºC. Lentil seed coat extracts could be used as a source of antioxidant to inhibit lipid oxidation in uncooked MSC. The antioxidant activity of lentil seed coat extracts was most likely related to the phenolic compounds in the extracts.
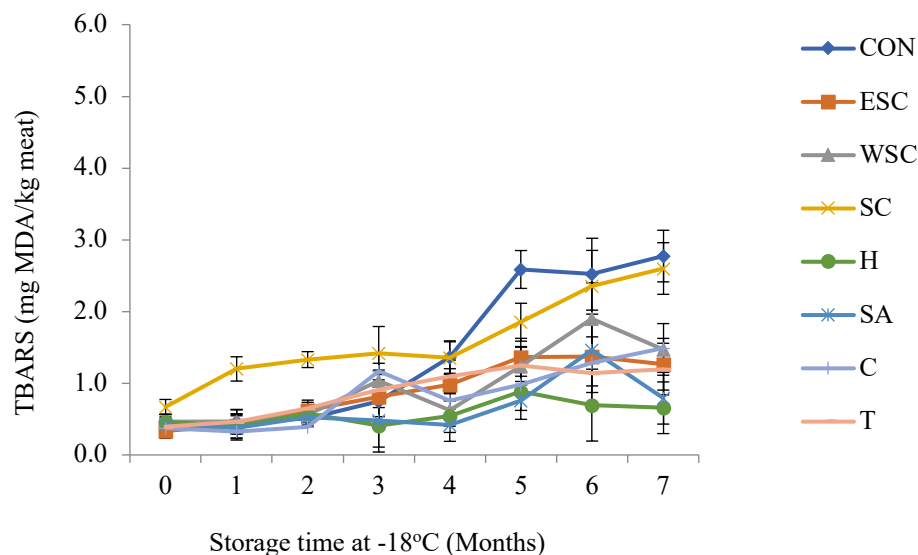
Figure 3.5 Effect of antioxidant compounds (500 ppm) on lipid oxidation of raw mechanically separated chicken stored at -18°C for seven months.
CON: control, ESC: ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate. Error bars are the standard deviations of duplicate measurements of three replicates.

Results of previous experiments showed a positive relationship between the total phenolic content and the antioxidant activity of seed coat extracts. Several other studies agree with the findings of our study that shows strong positive correlations between phenolic content and antioxidant capacity. Phenolics can act in a number of ways as antioxidants. Phenolic compounds are good donors of electrons/hydrogen that can react with reactive oxygen species in the termination reaction by interrupting the cycle of the generation of new radicals (Heim et al., 2002; Valentão et al., 2002; Allen and Cornforth, 2010). The data from DPPH and ABTS assays suggested that the lentil seed coat extracts were able to donate electron/ hydrogen to reactive radicals, converting them into more stable and unreactive species. The most potent antioxidant capacity of both water and ethanol extracts of green lentil seed coat determined using DPPH and ABTS assays were noted at phenolic concentrations between 400-600 ppm and 300-600 ppm, respectively.

The antioxidant capacity of phenolic compounds is also attributable to their ability to chelate metal ions (Pereira et al., 2009). Transition metals serve as catalysts to generate the first few free radicals that initiate oxidative chain reactions (Min and Ahn, 2005). Iron is the most likely

catalyst for facilitating most *in vivo* or *in vitro* $^\bullet OH$ generation through the Fenton reaction. Although iron has different oxidation states (from -2 to +6), the forms of Fe^{2+} and Fe^{3+} dominate in biological systems (Min and Ahn, 2005). Moreover, MSC was found to have comparatively high iron content due to the incorporation of heme pigments from the bone marrow. In agreement, the iron content of MSC (1.25 mg/100 g meat) detected in the current study was twice that found for hand deboned chicken (0.6 mg/100 g meat) reported by EFSA (2013). The significant activity displayed in the Fe^{2+} ion chelation assay in our previous experiments (72%-76%) indicated the potential ability of seed coat extracts to chelate metal ions present in MSC. The seed coat phenolics could either chelate metal ions or suppress their reactivity by occupying the all coordinating sites of the metal ion (Mahoney and Graf, 1986). Other than the antioxidant effects displayed in the present study, Pellegrini et al. (2006) and Li (2017) reported that lentil possesses significant ferric ion reducing capacity. In another study, lentil was found to have high oxygen radical absorption capacity that is even greater than that of chickpea, yellow pea and green pea (Xu and Chang, 2007). The demonstrated radical scavenging activity and metal ion chelation capacity, as well as the inhibition of peroxidation in the phospholipid model system, may explain the protective mechanisms of lentil seed coat extracts against lipid oxidation in MSC.

3.5.5.6 Effect of antioxidant compounds on lipid oxidation of MSC during cooking

The effects of cooking on the TBARS values of MSC treated with different antioxidant compounds are illustrated in Figure 3.6. The cooking process significantly influenced (p<0.001) the lipid oxidation in control samples resulting a 4-fold increase in TBARS values. These values were consistent with those reported by Gomez and Lorenzo (2012) and Dominguez et al. (2014). Dominguez et al. (2014) observed an increment in TBARS values from around 0.03 to 1.7 mg MDA/kg during microwave cooking of foal meat. Grilling pork patties at 175°C also resulted in a 4-fold increase in TBARS (McCarthy et al., 2001). Moreover, several researchers have studied the effect of convection oven cooking on the malonaldehyde content of chicken meat and found that cooking increases TBARS values by as much as 5 to 20-fold compared to raw meat samples (Igene et al., 1979b; Newburg and Concon, 1980; Pikul et al., 1984b). Since heat and oxygen are
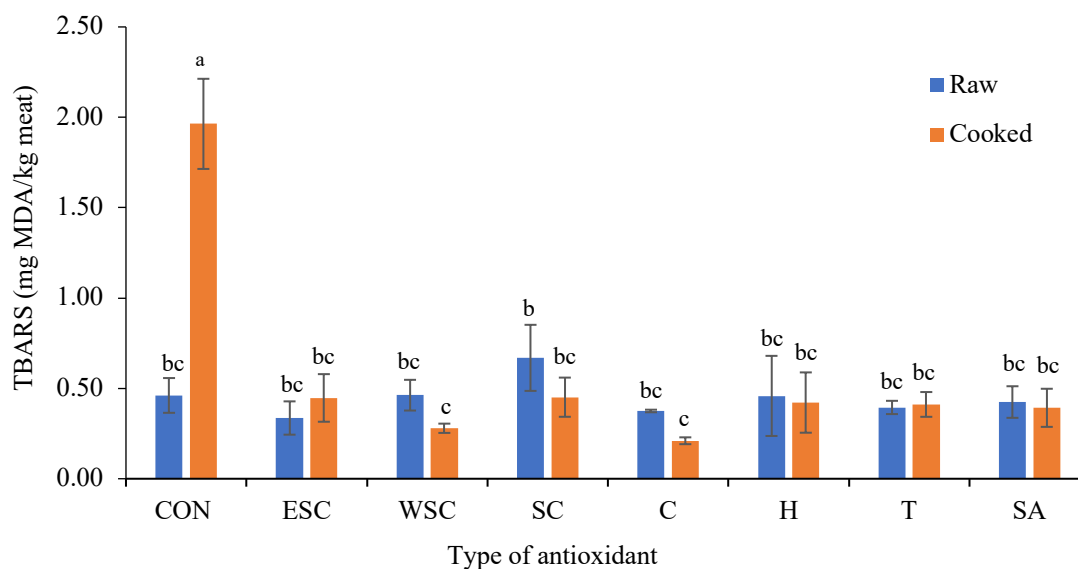
Figure 3.6 Effect of cooking and antioxidant compounds (500 ppm) on lipid oxidation of mechanically separated chicken.

CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate. Error bars are the standard deviations of duplicate measurements of three replicates. [a-c]Means with different letters are significantly different ($p < 0.05$)

factors that promote lipid oxidation, cooking processes are thought to increase the oxidized fat content of food (Pikul et al., 1984b). It was shown that membrane lipids which are high in polyunsaturated fatty acids are responsible for the initial development of oxidation in cooked meat (Gray and Pearson, 1987). Pikul et al. (1984a) suggested that, the phospholipid fraction contributed nearly 90% of the malonaldehyde measured in chicken meat, and the polyunsaturated fatty acid content of phospholipids was positively associated with rancidity development (Igene et al., 1980). Iron is also one of the main catalysts for oxidative rancidity in meat (Min and Ahn, 2005). Non-heme iron is known to be the major catalyst in cooked meats, and heat destroys the heme iron and increases the level of non-heme iron in cooked meats. The release of heme iron is the resulted of the oxidative cleavage of the porphyrin ring during heating (Schricker and Miller, 1983). It should be emphasized that the cooked MSC treated with seed coat components and the other antioxidant compounds had significantly lower TBARS values compared to the control samples indicating their strong antioxidant capacity. The TBARS values ranged from 0.21 to 0.45 mg MDA/kg meat for the samples treated with antioxidant compounds, while the control sample was recorded with

1.96 mg MDA/kg meat (Figure 3.6). These results clearly show that the application of antioxidant compounds had inhibited the lipid oxidation in MSC, which was a good source of phospholipids and iron, during cooking. Furthermore, no significant differences were noted in the TBARS values among the samples treated with antioxidant compounds showing that the antioxidant capacity of seed coat components was similar to commercial antioxidants; α- tocopherol, sodium ascorbate, Herbalox® and (+)-catechin.

3.5.5.7 Effect of antioxidant compounds on lipid oxidation of cooked MSC stored at 4ºC

A steady increase in TBARS values was found in control samples during 7 days of storage at 4ºC (Figure 3.7). However, the samples treated with the antioxidant compounds (500 ppm) had greater oxidative stability as shown by the consistently lower (p<0.001) TBARS throughout the storage period compared to the control. Among the treatments, the lowest TBARS values were observed in the samples treated with water extract of seed coat, about a 90% reduction compared to the control sample. At day one and seven of refrigerated storage, the TBARS values of the
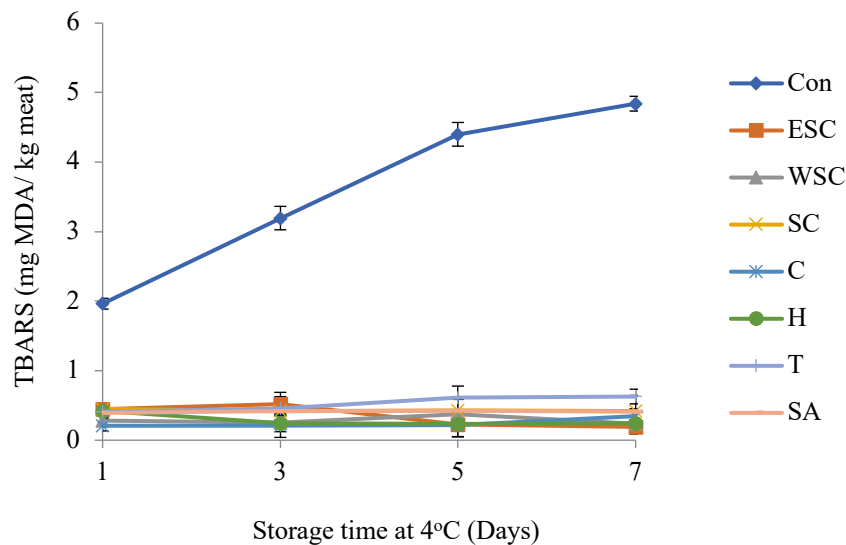
Figure 3.7 Effect of antioxidant compounds (500 ppm) on lipid oxidation of cooked mechanically separated chicken stored at 4ºC for seven days.
CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), H: Herbalox®, T: (+/-) - α- tocopherol, SA: sodium ascorbate, Error bars are the standard deviations of duplicate measurements of three replicates.

samples with antioxidant compounds ranged between 0.21 to 0.41 and 0.24 to 0.63 mg MDA/ kg meat, respectively. Statistical analysis revealed there were no significant variations in the TBARS values between the samples treated with antioxidant compounds, as well as no significant increases in the TBARS values over the storage in any of the treated samples. This reveals that seed coat and seed coat extracts can impart antioxidant effects in cooked MSC at levels equivalent to α- tocopherol, sodium ascorbate, Herbalox® and (+)-catechin. In several studies, plant extracts were successfully introduced as inhibitors of the oxidative deterioration in meat products (Wong et al., 1995; McCarthy et al., 2001; Tang et al., 2001; Fernández-López et al., 2005). Nissen et al. (2004) investigated the uses of rosemary as a natural source of antioxidant in cooked pork patties. The pork patties treated with 200 ppm rosemary extract were cooked at 80°C and packaged in high-barrier vacuum bags. They reported a significant decrease in the TBARS values in the rosemary treated patties stored at 4.5°C for 10 days compared to the control with no added antioxidants. McCarthy et al. (2001) reported the high efficacy of natural source antioxidants against oxidative reactions showing comparable activity to synthetic antioxidants such as BHT. They found superior antioxidant activities of rosemary, tea catechins, and α-tocopherol compared to the synthetic antioxidants BHT/BHA against the lipid oxidation in raw and cooked pork patties. The results from the present study showed that the antioxidant efficacy of lentil seed coat and extracts were similar to the rosemary, catechin, and α-tocopherol. Thus, the possibility of replacing synthetic antioxidants such as BHT and BHA with lentil seed components could be speculated based on these findings.

The antioxidant properties of the seed coat components displayed in the present study were consistent with those reported by Li (2017). He noticed that the beef burgers formulated with 0.6% lentil seed coat had higher oxidative stability than the control samples with no antioxidants added, and were similar to samples with 500 ppm of sodium ascorbate. The TBARS values observed were closer to the TBARS values obtained in MSC in the present study, though the total fat content was higher in the beef burgers (16.3%) than in MSC (11.9%). Part of the explanation for this outcome is likely due to the differences in the fatty acid composition of fat in chicken and beef. No literature could be found comparing the antioxidant ability of lentil phenolics in chicken meat or MSC. However, oleoresin rosemary, one of the reference antioxidant compounds used in the current study, had significant antioxidant effects in chicken nuggets (Teruel et al., 2015).

Unlike in the raw meat, the cooked samples with ground seed coat exhibited antioxidant effects similar to seed coat extracts (water and ethanol) and the other antioxidants. This outcome could be due to the inactivation of lipoxygenase or other oxidative enzymes in lentil seed coat and therefore, they had reduced or inhibited prooxidant characters observed in the raw meat system. The oxidative stability exhibited by MSC treated with lentil seed coat components could be attributed to the antioxidant properties of phenolic compounds. The antioxidant mechanisms of phenolic compounds in biological systems are thought to be due to quenching free radicals to terminate the radical chain reactions, chelating transition metal ions, acting as reducing agents, or stimulating the antioxidative enzyme activities (Trojakova et al., 2001; Kong et al., 2010). As described above, the antioxidant assays in this study revealed that lentil seed coat phenolics are effective free radical scavengers and Fe^{2+} ion chelators, therefore, these mechanisms could be contributing to the oxidative stability of cooked MSC during refrigerated storage.

3.5.5.8 Effect of antioxidant compounds on lipid oxidation of cooked MSC stored at -18ºC

The results for TBARS measurements of cooked MSC stored under frozen conditions are shown in Figure 3.8. There were significant changes in TBARS over the seven months of storage, and antioxidant treatment x time interactions (P<0.05) were also detected. The TBARS values of the control samples remained high during the seven months of frozen storage starting from the beginning. During the first four months, these values were significantly higher than those from the other treatments. After 3 or 4 months of storage, the TBARS values increased in all samples, however, TBARS values declined during the latter part of the storage period in all treatments, including control samples. Apparent loss of TBARS after several months of storage has also been reported previously (Igene et al., 1979b; Gokalp et al., 1983; Lai et al., 1991) and is not unusual. The interaction of malonaldehyde and amino groups or the decomposition of malonaldehyde may cause decreases in TBARS values during long term storage (Lai et al., 1991). This may explain the reason for the decline in the TBARS values observed in the latter part of the storage. Results of ANOVA revealed that the antioxidant effects of lentil seed coat and its extracts at 500 ppm were comparable to the other commercial antioxidants and overall TBARS values of these samples were significantly lower than that of the control samples. This showed that shelf-life of MSC would be
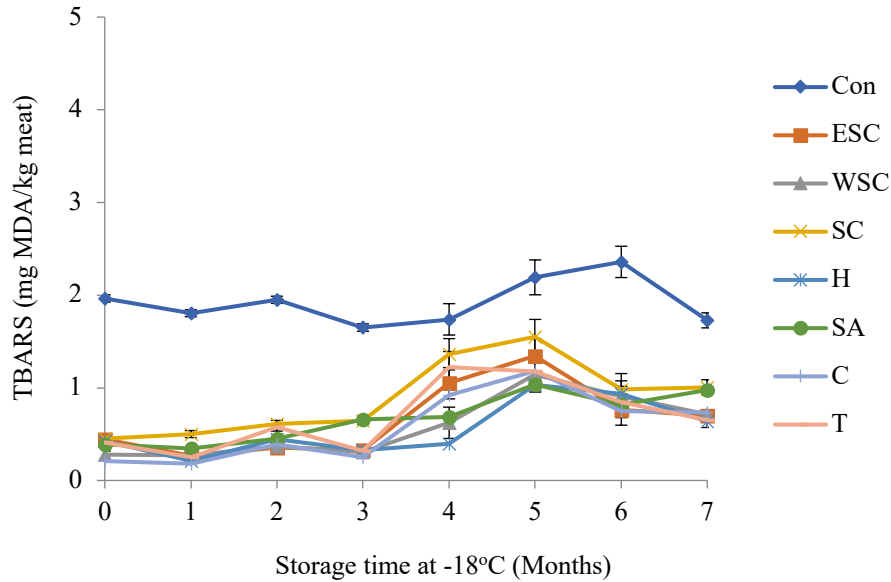
Figure 3.8 Effect of antioxidant compounds (500 ppm) on lipid oxidation of cooked mechanically separated chicken stored at -18°C for seven months.

CON: control, ESC: 70% aqueous ethanol extract of seed coat, WSC: water extract of seed coat, SC: seed coat, C:(+)-catechin), SA: sodium ascorbate, H: Herbalox®, T: (+/)-α-tocopherol. Error bars are the standard deviations of duplicate measurements of three replicates.

at optimum for at least three or four months with the antioxidants. Samples treated with seed coat water extract, Herbalox, and sodium ascorbate were relatively stable for four months, while the TBARS of other treatments started to rise from the third month. Comparable results were also reported for the beef burgers formulated with lentil seed coat (Li, 2017). It was found that beef burgers containing 0.6% of lentil seed coat delays lipid oxidation compared to the control, and TBARS values in the control had continuous increase during the frozen storage (Li, 2017). TBARS values of control (cooked MSC) did not reach above 2.36 mg MDA/kg meat in present study under frozen conditions which was lower than that observed during refrigerated storage (4.84 mg MDA/kg). Therefore, it appeared that there was an additive effect between the low storage temperatures and antioxidant activity on MSC on delaying lipid oxidation.

Although the literature data were not available for the application of lentil phenolics in poultry for comparison, several studies have been conducted to study the antioxidant effectiveness of rosemary products in various meat and poultry products (Chen et al., 1999; Mielnik et al., 2003; Lee et al., 2005; Sebranek et al, 2005). Mielnik et al. (2003) have examined the effect of commercial rosemary (Herbalox®) and ascorbic acid in mechanically separated turkey meat.

Turkey meat was treated with 800 ppm, 1600 ppm and 2400 ppm Herbalox® and ascorbic acid and oxidative stability was investigated in the stored meat at −25°C for seven months. TBARS values for all samples treated with antioxidants showed lower TBARS than the values of the control samples, and the TBARS values of the samples treated with 800 ppm Herbalox® and ascorbic acid were 0.11 and 0.17 mg MDA/kg, respectively, after seven months of storage, which was lower than the TBARS observed in the present study. They also reported that the antioxidant effects were dependent on the concentration. Lee et al. (2005) and Chen et al. (1999) reported that oleoresin rosemary used at 200 ppm did not prevent lipid oxidation in ready-to-eat hamburgers or pork patties. In the present study the Herbalox® and other antioxidants were used at 500 ppm concentration and all showed almost similar antioxidant properties in MSC. At the end of the seven months of storage, the control sample had TBARS values of 1.73 mg MDA/kg meat, while samples with the added seed coat compounds were noted with significantly lower TBARS values between 0.65 and 0.97 mg MDA/kg meat, indicating their ability to control lipid oxidation in MSC. The inhibitory effects of lentil seed coat and its water and ethanol extracts on lipid oxidation are likely due to the antioxidant potential of its phenolics. Different phenolic compounds that have antioxidant properties have been identified from lentil seed coat. In fact, lentil seed coat is very rich in catechins and procyanidins (Dueñas et al., 2002) which are described as effective antioxidants due to their multiple hydroxyl groups which can donate hydrogen, chelate metals and quench $^•O_2^-$ (Fukumoto and Mazza 2000). As described, the inhibition of lipid oxidation could be multifunctional including blocking radical chain reaction and binding prooxidant metal ions.

3.6 Conclusions

The results of the present study showed that seed coat of lentil has considerable amounts of phenolic compounds which are extractable in water and aqueous (70% v/v) ethanol. These phenolic extracts had significant antioxidant activity, which depends on cultivar, concentration and extraction solvent. CDC Greenland (green seed coat) contained relatively higher levels of total phenolics. Seed coat extracts exhibited a concentration dependent antioxidant activity irrespective of the cultivar and extraction solvent. The ethanol extracts showed higher activity in scavenging ABTS, chelating Fe^{2+} and inhibiting liposome peroxidation whereas water extracts had greater DPPH scavenging ability. Further, the antioxidant activity of seed coat extracts was comparable with (+)-catechin and the ability of scavenging free radicals was higher than that of Herbalox® and

sodium ascorbate at 300 ppm concentration. The potent free radical scavenging activity and metal chelating effects of phenolic compounds present in seed coat of lentil may be mechanisms for lentil phenolics as potential antioxidants for meat products. The seed coat and seed coat extracts exhibited significant antioxidant activity against the lipid oxidation in cooked MSC stored at 4°C and -18°C and greater protective effect against oxidation was exhibited during cooking of meat. The antioxidant activity of lentil seed coat and its extracts were comparable to the antioxidant activity exhibited by the other antioxidant compounds (+/-) - α-tocopherol, (+)-catechin, sodium ascorbate and Herbalox®. These antioxidant effects exhibited by the seed coat constituents could possibly be attributed to the phenolic compounds available in the seed coat. The data in this study suggested that lentil has potential as a natural antioxidant source for meat product quality preservation. Further research is needed to study the successful means of incorporating lentil and its fractions in real life meat products.

3.7 Connection to next study

The overall objective of the current study was to evaluate the phenolic composition and the antioxidant potential of lentil seed coat of two lentil cultivars with green and gray seed coat. Results showed that variation exists between the two cultivars with overall antioxidant capacity, as measured by *in vitro* assays, and the total phenolic content relatively higher in CDC Greenland (green seed coat). There is wide variation in lentil seed coat color and patterns and this variability indicates that there are differences in phenolic profiles between different genotypes (Mirali, 2017). There is limited information reported on how phenolic profiles and their antioxidant potential are linked to different lentil cultivars with different seed coat colors and patterns. Moreover, this study showed that lentil seed coat is a potential source of phenolic compounds with potent antioxidant capacity. Both water and 70% aqueous ethanol were viable solvents for extracting antioxidant compounds from the lentil seed coat. However, water extraction is preferred as it is closely related to the actual phenomenon taking place in many food systems including meat where the primary solvent is water. Therefore, the following study was designed to investigate the profile and antioxidant potential of water-soluble phenolic compounds of seed coat from seven lentil cultivars with different seed coat colors, including both tannin and zero tannin types.

4. STUDY II: PHENOLIC COMPOSITION AND ANTIOXIDANT POTENTIAL OF WATER EXTRACTS OF NORMAL AND ZERO TANNIN LENTIL SEED COAT

4.1 Abstract

Lentil seed coat can be a valuable source of different phenolic compounds which can provide antioxidant benefits. Water extracts from seed coat of seven lentil cultivars with normal tannin and zero tannin seed coat were evaluated for phenolic composition and antioxidant activity. Results showed that normal tannin seed coat has a significantly higher content of total phenolics (TPC), flavonoid (TFC) and condensed tannin (CTC) compared to seed coat from two zero tannin cultivars. TPC varied significantly among cultivars and ranged between 5.01 and 39.72 mg GAE/g. CDC Greenland had the highest flavonoid content at 5.14 mg CE/g followed by CDC Greenstar. CTC of the regular-tannin seed coat ranged between 21.63 and 28.07 mg CE/g, indicating that condensed tannin are the main phenolic compounds in normal tannin seed coats. Condensed tannins were not found in zero tannin genotypes. In addition, 30 phenolic compounds were identified in the water extracts by LC-MS techniques. Kaempferol tetraglycoside, catechin-3-glucoside and procyanidins were the dominant phenolic compounds in normal tannin seed coats whereas kaempferol tetraglycoside was dominant in zero tannin seed coats. Water extracts of normal tannin seed coat showed significant antioxidant activity as revealed by DPPH and ABTS free radical scavenging activity, ferrous ion chelation activity and phospholipid peroxidation inhibition activity. The antioxidant activity was concentration dependent and cultivar CDC Greenland exhibited the greatest activity in all assays. The antioxidant capacity of water extracts from normal tannin seed coats were about 6 - 9 times higher than that of zero tannin seed coats. Correlation analysis revealed strong positive correlations ($r = 0.91$ to 0.98) between antioxidant activity and TPC, TFC, CTC, flavon-3-ols, and procyanidin content of seed coat. The hierarchical clustering analysis categorized seven cultivars into three clusters and cluster one, consisting of cultivars with green seed coat, had a relatively higher phenolic content and antioxidant capacity. These results suggested that the water extracts of normal tannin lentil seed coat would be a promising source of antioxidant phenolics.

4.2 Introduction

Phenolic compounds are one of largest groups of secondary metabolites in plants. They include benzene rings with one or more hydroxyl substitutes, ranging from simple phenolic molecules to highly polymerized compounds (Ajila et al., 2011). These compounds can occur in combination with mono- and polysaccharides, attached to one or more phenolic groups, or as derivatives such as esters or methyl esters (Minatel et al., 2017). Among the several classes of phenolic compounds, the main dietary phenolic compounds are the phenolic acids, flavonoids, and tannins (King and Yong, 1999). Dietary phenolic compounds are recognized as reducing the oxidative damage associated with many diseases including diabetes, cardiovascular disease, immune deficiency, cancer, and aging (Halliwel et al., 1992; Willet 1994; Halliwel, 1997). Epidemiological and interventional studies have also confirmed a positive correlation between consumption of phenolic-rich food and a decline in several chronic disease conditions (Kushi et al., 1999; Kris-Etherton et al., 2002) and such protection has been suggested to be due to the antioxidant properties of various phenolic compounds. Free radicals are known to cause oxidative deterioration of biomolecules, such as membrane lipids, proteins, and nucleic acids. Therefore, the phenolic compounds that quench free radicals could play an important role in the prevention of degenerative diseases (Kabera et al., 2014). In addition, the ability to chelate metal ions and the ability to quench singlet oxygen were known to contribute to the antioxidant potential of phenolic compounds (Hall and Cuppett, 1997). The antioxidant characteristics of phenolic compounds depend on molecular structures, especially the number and locations of hydroxyl groups, and the nature of substitutions on the aromatic rings (Pereira et al., 2009; Kabera et al., 2014; Minatel et al., 2017), consequently, the antioxidant potential may vary between single compounds and their combinations.

Phenolic compounds may also play a significant role as natural antioxidants in preventing undesirable changes in color, flavor and nutritional quality of food. Currently, antioxidants from natural sources are in high demand for clean label products. Different plant parts and their extracts are good sources of natural antioxidants. For example, extracts from rosemary, oregano, pomegranate peel, coca, and tea show positive effects on the inhibition of lipid oxidation in meat products (Tang et al., 2001; Mielnik et al., 2003; Nissen et al., 2004; Mitsumoto et al., 2005; Rojas and Brewer, 2007; Naveena et al., 2008a). A study comparing the phenolic compounds and antioxidant activity of legumes, including pea, lentil, chickpea, common beans, and soybean

revealed that lentil possessed the highest concentration of phenolics and antioxidant activity (Xu and Chang, 2007).

Lentil (*Lens culinaris*) is rich in proteins, carbohydrates and dietary fiber and constitutes an important source of food in many countries. The world annual production of lentil is reported to be about 4 Mt. Lentil has been gaining increasing attention among the other legumes due to their high phenolic content. Various phenolic compounds have been identified in lentil, including phenolic acids, stilbenes and several types of flavonoids (Milari et al., 2017). These compounds are located primarily in the seed coat; thus, it is possible to obtain a concentrated form by decorating the seeds. Commercial processing of lentil seed coat as a by-product that has limited use at the present, and therefore, creates enormous scope for the extraction of bioactive compounds and development if value-added products for food applications.

Lentil has great diversity in seed coat background colors and patterns. The four basic background colors (brown, gray, tan and green) are determined by two independent genes; Ggc and Tgc (Vandenberg and Slinkard, 1990).This genotypic diversity is likely the cause of variability in phenolic composition (Mirali et al., 2017) and in the associated antioxidant characteristics of the seed coats. However, little information is available regarding the relationship between different genotypes that influence phenolic profiles, and their antioxidant properties.

Phenolic extracts are generally prepared from plant materials using various solvents and extraction techniques (Schwarz et al., 2001; Brewer, 2011; Kim et al., 2013). The solubility of phenolic compounds in a particular solvent is affected by its chemical nature and polarity (Turkmen et al., 2006; Shah et al., 2014). Though organic solvents are known to be effective in extracting phenolic compounds (Shan et al., 2009; Do et al., 2013; Kim et al., 2013), they are less relevant with respect to the application in food systems with aqueous matrix. While a few studies have investigated the phenolic compounds in lentil seed coat, most research was focussed on the compounds extracted in organic solvents, and their solubility in water is less known.

In this study an attempt was made to determine (i) phenolic profiles, and (ii) free radical scavenging activity, metal ion chelation activity and phospholipid peroxidation inhibition activity of water extracts of seed coats of seven lentil cultivars.

Hypothesis:

Different cultivars of lentil with varying seed coat colors associated with specific genetic combinations that determine seed coat color, will have different phenolic profiles and antioxidant capacity.

4.3 Materials

Lentil: Certified seeds of two lentil cultivars/ genotypes (CDC Greenland and CDC Maxim-C from the 2014 harvest) were obtained from a certified seed grower in Saskatchewan, Canada. Seeds of 6 other cultivars/ genotypes (CDC Greenstar, CDC Maxim, CDC Robin, CDC Spanish Brown (SB-3), ZT-4 and 6502-ZT) were provided by the Crop Development Centre (University of Saskatchewan), Canada from the 2017 harvest. The seed coat and cotyledon color of lentil seeds are summarized in Table 4.1. Three batches of seeds of each cultivar were from different plots. The samples of CDC Maxim grown in Moose Jaw (in 2014) and Saskatoon areas (in 2017) were included in the study to compare the effect of storage. Three replicate batches of seeds of each cultivar were from different plots. All samples were received and stored at ambient temperature.

Chemicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (FerroZineTM), ferrous chloride, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridy-s-triazine (TPTZ), gallic acid, Folin-Ciocalteu reagent, potassium persulfate, butanol, ferric ammonium sulfate, aluminum chloride, sodium hydroxide, boric acid, sulfuric acid, ethanol, trichloro acetic acid, thiobarbituric acid, caffeic acid, quercetin and ascorbic acid used were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

Kaempferol-3-O-rhamnoside, kaempferol 3-O- robinoside-7-O-rhamnoside (Robinin), ±-catechin-2,3,4-$^{13}C_3$, dihydromyricetin, resveratrol-(4-hydroxyphenyl-$^{13}C_6$), (-)-gallocatechin, Taxifolin (dihydroquercetin), 3,4-dihydroxybenzoic acid, vanillin-(ring-$^{13}C_6$), 4-hydroxybenzoic acid - $^{13}C_7$, vanillic acid, vanillic acid-4-β-D-glucoside, caffeic acid, procyanidin B1, procyanidin C1, 4-hydroxybenzoic acid, gallic acid, ferulic acid (trans), p-coumaric acid (trans) were purchased from Sigma-Aldrich (St. Louis, MO, US). Kaempferol-3-O-glucoside, kaempferol-3-O-rutinoside, myricetin-3-O-rhamnoside, quercetin-3,4'-di-O-glucoside, quercetin-3-O-rhamnoside (quercitrin), quercetin-3-O-rutinoside (Rutin), luteolin, luteolin-4'-O-glucoside, Luteolin-7-O-glucoside, (+)-Catechin, (-)-epicatechin were obtained from Extrasynthese (Genay Cedex, France). Resveratrol-

Table 4.1 Cotyledon and seed coat color of lentils used in the study

Cultivar	Area grown	Year of harvest	Cotyledon color	Seed coat color	Remarks
CDC Greenland	Moose Jaw	2014	Yellow	Green (Gn)	Tannin
CDC Greenstar	Saskatoon	2017	Yellow	Green (Gn)	Tannin
CDC Maxim	Saskatoon	2017	Red	Gray (Gy)	Tannin
CDC Maxim-C	Moose Jaw	2014	Red	Gray (Gy)	Tannin
CDC SB-3	Saskatoon	2017	Yellow	Gray dotted (GyD)	Tannin
CDC Robin	Saskatoon	2017	Red	Brown (Bn)	Tannin
ZT-4	Saskatoon	2017	Yellow	Transparent (T)	Zero tannin
6502-ZT	Saskatoon	2017	Red	Gray translucent (GyT)	Zero tannin

3-β-mono-D-glucoside (Polydatin), Procyanidin B3 were from Santa Cruz Biotech, US and AdooQ, US, respectively.

4.4 Methods

4.4.1 Preparation of seed coats

Seed coat tissue was obtained by decorticating the whole seeds using a bench top mill (Satake, Japan) and separating them using a column blower (Crop Development Centre, University of Saskatchewan). Seed coat samples were ground using an Ultra-Centrifugal Mill (Retsch, PA, USA, Model ZM 200) equipped with a screen (250 µm aperture size). Milled seed coat was stored in sealed bags at 4°C away from direct light.

4.4.2 Measurement of CIE color

Color of lentil seed coat was measured using a Hunterlab ColorFlex colorimeter with D65 optical sensor (Hunter Associations Laboratory Inc., Reston, VA, USA) based of L*, a* and b* values. A sample filled into a transparent glass petri dish (~5 mm thickness bed) was placed above the light source, covered with a black lid and color values were recorded. The instrument (45°/0° geometry, 10° observer) was calibrated against standard black and white colored reference tiles.

| CDC Greenstar | CDC Greenstar | CDC Maxim | CDC Maxim-C |
| CDC SB-3 | CDC Robin | ZT-4 | 6502-ZT |
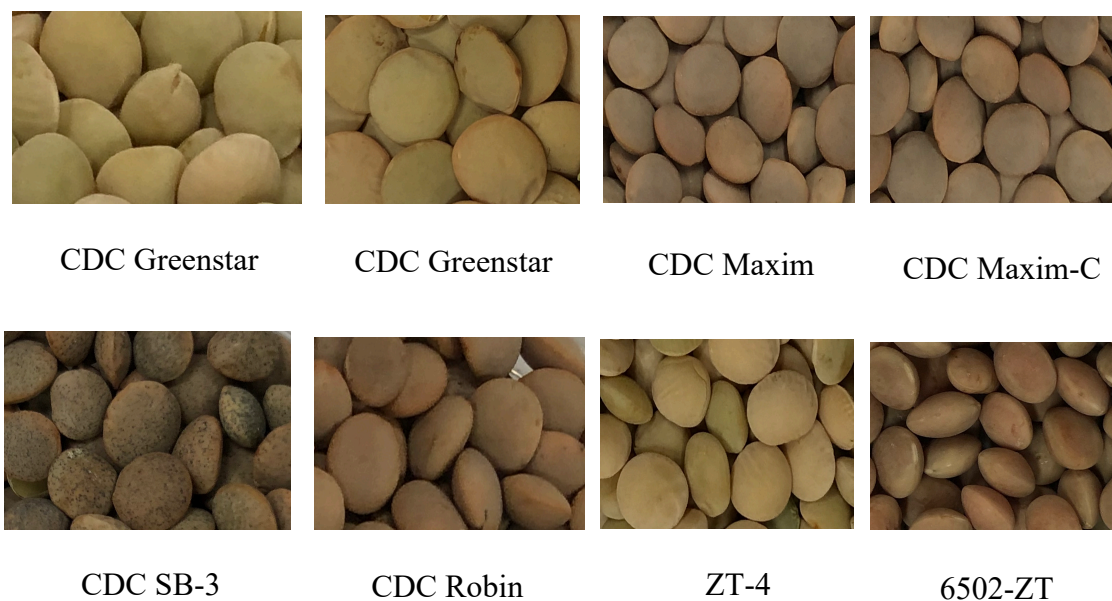
Figure 4.1 Images of lentil seeds used in the study

4.4.3 Preparation of water extracts

Water extracts of seed coat were prepared, and compounds were extracted following a slightly modified version of the method described by Aguilera et al. (2010). Briefly, 2 g of sample was weighed into a 50 mL screw-capped plastic tube and extracted with 20 mL of deionized water using a shaking water bath at 100 rpm for 15 h at 23°C. Then mixture was then centrifuged at 3000 g for 10 min. The supernatants were collected, and residue was re-extracted under the same conditions. The supernatants were filtered through a Whatman No 1 filter paper. The solvent (water) from the supernatants was removed by freeze drying (Labconco, MO, USA) at -40°C under reduced pressure (0.01 mBar).

4.4.4 Determination of phenolic compounds
4.4.4.1 Total phenolic content (TPC)

The content of total phenolics was determined according to the procedure described in section 3.4.4.1.

4.4.4.2 Total flavonoid content (TFC)

Total flavonoid content of each sample was conducted according to the procedure described in section 3.4.4.2.

4.4.4.3 Condensed tannin content (CTC)

Condensed tannin content of extracts was determined according to a previously reported procedure of Porter et al. (1985) with modifications. In brief, 1 mL of acidified butanol (5% concentrated HCl in *n*-butanol, v/v) was mixed with 0.1 mL of sample extract, 0.2 mL of 2% (w/v) ferric ammonium sulfate in 2 mol/L HCl. The tube containing the assay mixture was incubated in a boiling water bath for 50 min. The absorbance of the hydrolyzed tannins and ferric complex was measured at 550 nm after cooling down to ambient temperature. A sample that did not contain extract was used as the blank. (+)-catechin was used as standard compound for the quantification of condensed tannin. The condensed tannin content was expressed as milligram catechin equivalents (mg CE) per gram of sample.

4.4.4.4 Liquid chromatography-mass spectrophotometry (LC-DAD-MS) analysis of phenolic compounds

To analyze the seed coat phenolic profile, extracts were prepared as described by Mirali et al. (2014). In brief, ~100 mg of each sample was placed into separate micro centrifuge tubes. The tubes were covered, put in a −80°C freezer for 1 h, and then freeze-dried overnight. Two 6 mm ceramic sphere beads were added to each tube and the seed coats pulverized using a Fast PrepFP 120 (Qbiogene, Inc., Canada). One mL of the extraction solvent (100% MilliQ-water containing the internal standards) was added to the pulverized seed coats and then mixed for 1 h on a Thermomixer C at a speed of 1400 rpm and 23°C. The samples were then centrifuged at a speed of 1900 *g* for 10 min, and the supernatant were transferred again under same conditions into new labelled tubes. The supernatants were centrifuged a second time to ensure removal of all of the seed coat pellets. Two hundred μL of each extract were transferred to new Eppendorf tubes, dried down in the vacuum concentrator and reconstituted in 200 μL of MilliQ-water: MeOH (90:10 v/v). The phenolic profile was evaluated using Liquid Chromatography (LC) coupled to a Diode Array Detector (DAD) and a Mass Spectrometer MS; LC-DAD-MS. Following reversed-phase LC separation, eluent passed through an in-line Agilent G4212 DAD detector (250-680 nm), which was coupled to the heated electrospray ionization (HESI) source of a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher) for MS detection. Selective reaction monitoring (SRM) mode employed authentic standards and was used for quantification. Full scan mode was

used in combination with the DAD to investigate compounds present in the sample, but not in the SRM mode method.

Chromatographic separation was achieved using an Agilent poroshell 120 PFP (2.1x100 mm, 2.7 μm) column. The mobile phases; solvent A consisted of H_2O: formic acid (99.9:0.1, v:v) and solvent B was H_2O: acetonitrile: formic acid (9.9:90:0.1, v:v:v). The column compartment temperature was set to 25°C and the injection volume was 5 μL. The mobile phase flow rate was 0.35 mL/min and the gradient used is shown in Table 4.2.

Table 4.2 Mobile phase flow gradient used in LC-DAD

Time (min)	0	1	21	24	24.1	26	26.1	30
Solvent A%	99	99	59	40	20	20	99	99
Solvent B%	1	1	41	60	80	80	1	1

4.4.5 Determination of antioxidant activity

The DPPH and ABTS free radical scavenging activity, ferrous ions chelation activity and Inhibition of phospholipid peroxidation were determined according to the procedures described in section 3.4.5.1, 3.4.5.2, 3.4.5.3 and 3.4.5.4, respectively.

4.4.6 Statistical analysis

All analysis was performed in three replicates and data were statistically analyzed using the Proc Mixed Procedure of SAS 9.4 (SAS, Inst. Inc., Cary, NC). Analysis of variance (ANOVA) and Tukey's method was used to compare the means. Significant difference was defined at $p < 0.05$. Associations between antioxidant activity and phenolic concentrations were assessed by Pearson's correlation analysis and FactoMineR in the R statistical system was used to perform the principal component analysis and hierarchical cluster analysis.

4.5 Results and Discussion

4.5.1 CIE color of lentil seed coats

The CIE color parameters L*, a*, and b* of lentil seed coats are summarized in Table 4.3. Color parameters varied significantly (p<0.05) among cultivars. The lightness (L* value) of lentil seed coats ranged from 50.12 to 69.23 in normal tannin cultivars. ZT-4 had the highest lightness (69.23) which could be due to its transparent nature. Cultivar 6502-ZT with a gray translucent seed coat showed a L* value of 58.33 which was significantly different from ZT-4. The lightness of green seed coats (CDC Greenland and CDC Greenstar) was higher than that of gray (CDC Maxim and CDC SB-3) and brown (CDC Robin) seed coats. CDC SB-3 is a specialty market class of lentil that has a gray dotted seed coat; thus, the L* was found to be similar to CDC Maxim.

With respect to a* value, which indicates redness/greenness, no distinct differences were observed between regular and zero tannin seed coat types, and the values ranged between 3.08 and 6.02. The CDC Robin (brown seed coat) had the highest value, reflective of its brown color followed by CDC Maxim (gray seed coat). As anticipated, the a* value was lower for green seed

Table 4.3 CIE color values of lentil seed coat (n = 3)

Cultivar[1]	CIE Color		
	L	a*	b*
CDC Greenland (Gn)	59.38[bc]	3.27[e]	15.82[abc]
CDC Greenstar (Gn)	62.25[b]	3.83[cde]	17.00[ab]
CDC Maxim-C (Gy)	50.66[de]	5.51[ab]	11.40[c]
CDC Maxim (Gy)	52.17[de]	4.83[bc]	11.84[bc]
CDC SB-3 (GnD)	53.85[d]	3.59[de]	12.05[bc]
CDC Robin (Bn)	50.12[e]	6.02[a]	12.71[abc]
ZT-4 (T)	69.23[a]	3.08[e]	17.83[a]
6502-ZT (GyT)	58.33[c]	4.63[bcd]	14.42[abc]
SEM[2]	0.729	0.229	1.126

[1]Colour of seed coat is indicated in parenthesis and refer to Table 4.1
[2] Standard error of mean
[a-d]Means with different superscripts within the same column are significantly different (P<0.05)

coat (CDC Greenland and CDC Greenstar) than that of brown and gray seed coat except CDC SB-3. It was noted that the a* value of CDC SB-3 with a gray background color seed coat was similar (p>0.05) to that of the green seed coat. The two zero tannin cultivars differ from each other based on a* values. ZT-4 with a greenish transparent seed coat was noted with a* values comparable to normal tannin green seed coat while 6502-ZT with gray translucent seed coat had a* values relatively similar to gray normal tannin seed coat.

The b* value, the indicator of yellowness, also varied significantly among the cultivars. Lentils with gray seed coat (CDC Maxim and CDC SB-3) had a relatively lower b* value than those with green seed coat. Zero tannin ZT-4 (green transparent) showed higher b* value compared to 6502-ZT and no distinct differences were noted between the two categories; normal tannin and zero tannin.

Seed coat color is not stable and may change over time depending on storage conditions and duration (Mirali et al., 2017). However, the seed coat color of the CDC Maxim-C which has been stored for 3 years did not differ from the seed coat from the fresh CDC Maxim sample. This could possibly be due to that, storage of seeds in the dark may have not caused any light-induced color change. Overall, no distinct differences were found between normal tannin and zero tannin seed coats for the color parameters. Among the normal tannin seed coats, gray and brown seed coat showed relatively similar color parameters and were different from the green seed coat.

4.5.2 Total phenolic content and composition of water-soluble fraction of lentil seed coat

TPC, TFC and CTC of seed coats are presented in Table 4.4. Phenolic content varied significantly between cultivars, and as anticipated, the greatest differences were observed between normal tannin and zero tannin cultivars. TPC of normal tannin cultivars varied within a narrow range. CDC Robin topped the list (39.72 mg GAE/g) followed by CDC Maxim (38.18 mg GAE/g), CDC Greenstar (37.14 GAE/g), CDC Greenland (36.46 mg GAE/g), and CDC SB-3 (35.88 mg GAE/g). The TPC of zero tannin cultivars were found to be 6-fold lower than that of normal tannin seed coat. Oomah et al. (2011) reported a relatively higher content of total phenolics in lentil seed coat than the present study and the range was from 52.75 to 57.07 mg CE/g sample. However, due to the differences in cultivars, extraction procedures, and analytical methods employed, it is difficult to compare results of the present study with the values reported in previous studies.

Table 4.4 Total phenolic content, flavonoid content and condensed tannin content of lentil seed coat (n = 3)

Cultivar[1]	TPC[4] (mg GAE/g)	TFC[5] (mg CE/g)	CTC[6] (mg CE/g)
CDC Greenland (Gn)	36.46[d]	5.14[a]	28.07[a]
CDC Greenstar (Gn)	37.14[c]	4.09[b]	26.65[ab]
CDC Maxim-C (Gy)	37.92[b]	4.23[b]	25.37[b]
CDC Maxim (Gy)	38.18[b]	4.19[b]	25.75[b]
CDC SB-3 (GnD)	35.88[d]	3.70[c]	22.12[c]
CDC Robin (Bn)	39.72[a]	3.50[c]	21.63[c]
ZT-4 (GnT)	5.92[e]	0.07[d]	Nd[3]
6502-ZT (GyT)	5.01[f]	0.08[d]	Nd[3]
SEM[2]	0.124	0.069	0.299

[1]Color of seed coat is indicated in parenthesis and refer to Table 4.1.

[2] Standard error of mean

[a-f]Means with different superscripts within the same column are significantly different (P<0.05

[3] not detected

[4] TPC: total phenolic content expressed as mg gallic acid equivalents/ g sample (dry basis), [5]TFC: Total flavonoid content expressed as mg (+)-catechin equivalents/ g sample (dry basis), [6]CTC: Total condensed tannin content expressed as mg (+)-catechin equivalents/g sample (dry basis)

They also reported that the TPC content of lentil seed coat was about five times higher than that of yellow pea hulls. TPC in seed coat of 60 Chinese black soybean varieties ranged between 5.2 to 60.57 mg GAE/g (Zhang et al., 2011). The highest TPC observed in their study were around 1.5 times higher than the highest TPC found in the normal tannin cultivars analyzed in the present study. Differences of this magnitude could be expected between studies due to the variation in plant materials, and extraction conditions used.

TFC of normal tannin cultivars ranged from 5.14 mg CE/g to 3.50 mg CE/g and these levels were more than 50 times higher than the TFC found in zero tannin cultivars. CDC Greenland (green seed coat) had the highest TFC and the second highest level was detected in CDC Greenstar (green seed coat) and CDC Maxim (gray seed coat). Lowest level of TFC (3.50 mg CE/g) observed among normal tannin cultivars was in CDC Robin (brown seed coat) and CDC SB-3 (gray seed coat). According to these observations, no clear relationship between seed coat color and TFC was noted. TFC of zero tannin ZT-4 and 6502-ZT were 0.07 mg CE/g and 0.08 mg CE/g, respectively.

Condensed tannins are formed by phenolic acid condensation and have a variety of molecular structures (Xu et al., 2007). The condensed tannin content (CTC) of the normal tannin seed coat water extract ranged between 21.63 and 28.07 mg CE/g, indicating condensed tannin are the main phenolic compounds (53% to 77%) of the water-solubles of these seed coats. These results were similar to the Canadian lentil samples reported by Zhang et al. (2015). They also observed that a large proportion of phenolic compounds in whole lentil that were soluble in methanol were condensed tannins. In the present study, relatively higher level of CTC was found in green seed coat at 26.65 mg CE/g and 28.07 mg CE/g in CDC Greenland and in CDC Greenstar, respectively with the greatest proportion of TPC (70% - 77% of TPC) among the water extracts of all lentil seed types studied. The corresponding values for gray seed coats (CDC Maxim and CDC SB-3) was 65%-67% of TPC and the lowest proportion was observed in brown seed coat of CDC Robin. Not surprisingly, no condensed tannin was not detected in the water extract of zero tannin genotypes (ZT-4 and 6502-ZT).

Based on these findings, it was evident that there was no marked variation in the water-soluble phenolic content among normal tannin lentil cultivars. Therefore, the water-solubles of all normal tannin seed coats presently studied could be expected to have similar antioxidant potential. According to Amarowicz et al. (2000), condensed tannins have strong antioxidant activity, and proportionately green seed coat had the highest level of CTC. Hence, green seed coat may exhibit relatively stronger antioxidant capacity among lentil types. On the other hand, there was much lower total phenolic content in the water extracts of zero tannin seed coat compared to the normal tannin seed coat and no clear relationship can be observed between lentil seed coat color and water-soluble total phenolic contents.

4.5.3 Identification and quantification of water-soluble phenolic compounds

In this study, LC-MS was employed for further investigation of the water-soluble phenolic profile of lentil seed coat, which might help in understanding which individual compounds are available and have potency to contribute antioxidant properties of the seed coat water extracts. Figures 4.2 and 4.3 shows the LC-DAD chromatograms of the normal tannin seed coat (green, gray and brown background colors) and zero tannin seed coat, respectively, recorded at 250-600 nm in the UV spectra. Using the targeted method, 30 phenolic compounds which belong to the subclasses of hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, flavonols, flavones, flavan-3-ols, and

procyanidins were detected and quantified in the water extracts. Table 4.5 gives the retention times (R_t), internal standards (IS) used and MS/MS parameters including, molecular ion, product ion, collision energy (CE) and S-lens voltage of the compounds identified.

Phenolic composition was affected by cultivar/ genotype, and the greatest differences observed were between the normal tannin and zero tannin cultivars (Table 4.6). Concentration of most compounds varied within a narrow range between normal tannin cultivars, showing that all normal tannin seed coats have close phenolic composition. Differences in the concentration of phenolic compounds were also observed between the replicates of each cultivar which may be due to the variations in the seeds received from different seed lots. These variations observed were relatively higher as compared to the total phenolic determinations (TPC, TFC and CTC) using colorimetric methods. Although, relatively greater variability was observed between replicates in LC-MS method, the coefficients of variation were within an acceptable range (<20%) except for few compounds. The coefficient of variance calculated for TPC, TFC and CTC determinations were below 10%. In comparison to the colorimetric methods, the higher variability observed in LC-MS determination might be due to the higher sensitivity or specificity of the method to detect the differences between seed lots.

Phenolic acids with a single carboxylic acid functionality contain two distinguishing carbon frameworks: hydroxycinnamic acid and hydroxybenzoic acid (Ajila et al., 2011). The hydroxybenzoic acids identified in lentil seed coat were 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, gallic acid, vanillic acid, and vanillic acid-4-β-D-glucoside. Vanillic acid-4-β-D-glucoside was the most abundant phenolic acid and was observed for all cultivars. This compound was eluted at R_t of 5.53 min (Table 4.5) and showed a molecular ion [M-H]$^-$ at *m/z* 329.
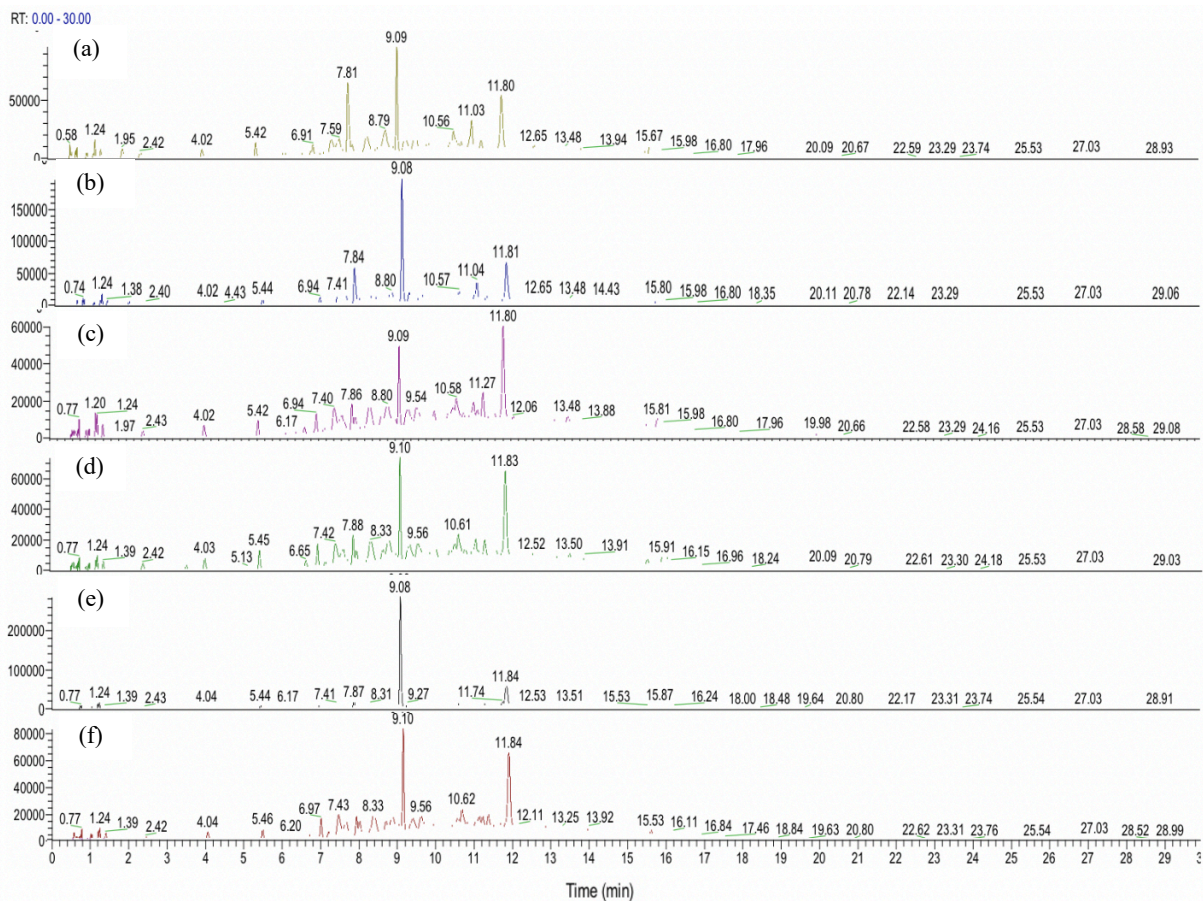
Figure 4.2 LC-DAD chromatograms for phenolic compounds in normal tannin lentil seed coat (a) CDC Greenland (b) CDC Greenstar (c) CDC Maxim-C (d) CDC Maxim (e) CDC SB-3 (f) CDC Robin
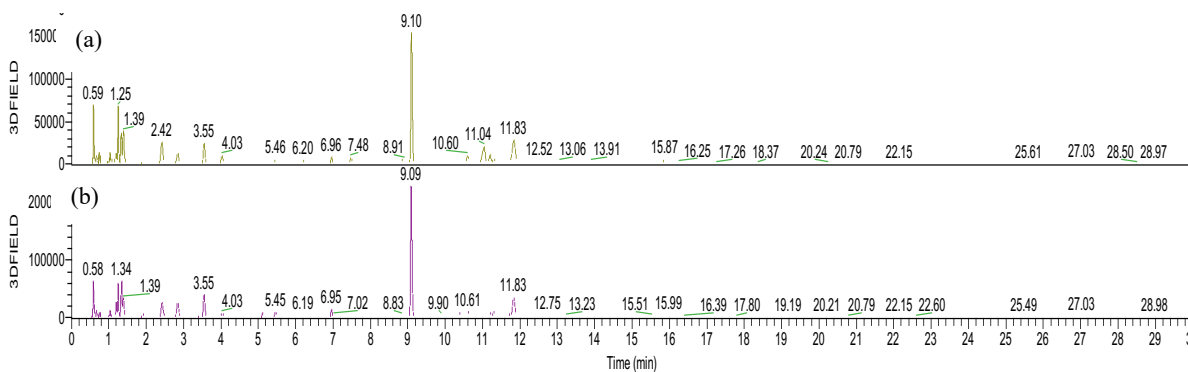
Figure 4.3 LC-DAD chromatograms for phenolic compounds in zero tannin lentil seed coat (a) ZT-4 (b) 6205-ZT

97

Table 4.5 Types of phenolic compounds detected in water extracts of seed coat of different lentil cultivars and their MS/MS parameters

Compound name	Precursor ion (*m/z*)	Product ion (*m/z*)	CE[1] (eV)	S-lens voltage (V)	R_t[2] (min)	IS[3] used
Hydroxybenzoic acids						
3,4-Dihydroxybenzoic acid	153	109	17	46	6.02	H
4-hydroxybenzoic acid	137	93	17	42	7.56	H
Gallic acid	169	125	17	54	4.14	H
Vanillic acid	167	108	20	45	8.66	V
Vanillic acid-4-β-D-glucoside	329	167	15	70	5.53	V
Hydroxycinnamic acids						
Caffeic acid	179	135	18	50	9.43	H
Ferulic acid (trans)	193	134	17	55	11.94	F
p-Coumaric acid (trans)	163	119	17	45	11.11	H
Stilbenes						
Resveratrol-3-β-mono-D-glucoside (Polydatin)	389	227	20	90	12.72	R
Flavonols						
Kaempferol tetraglycoside	901	755	30	150	9.15	C
Kaempferol-3-*O*-rhamnoside	431	285	23	100	15.41	C
Kaempferol 3-*O*-robinoside-7-*O*-rhamnoside (Robinin)	739	593	33	130	11.06	C
Kaempferol-3-*O*-glucoside	447	285	24	120	13.89	C
Kaempferol-3-*O*-rutinoside	593	285	38	140	13.42	C
Myricetin-3-*O*-rhamnoside	463	316	30	120	13.12	C
Quercetin-3,4'-di-*O*-glucoside	625	463	21	125	11.32	Q
Quercetin-3-*O*-rhamnoside (Quercitrin)	447	300	30	120	14.35	Q
Quercetin-3-*O*-rutinoside (Rutin)	609	300	42	140	12.58	Q

[1]Collision energy

[2] Retention times

[3]Internal standard time segment (Q: Quercetin-d3, C: ±-Catechin-2,3,4-[13]C3, R: Resveratrol-(4-hydroxyphenyl-[13]C6), V: Vanillin-(ring-[13]C6), F: Ferulic acid-d3

Table 4.5 Types of phenolic compounds detected in water extracts of seed coat of different lentil cultivars and their MS/MS parameters Cont.

Compound name	Precursor ion (*m/z*)	Product ion (*m/z*)	CE[1] (eV)	S-lens voltage (V)	R_t[2] (min)	IS[3] used
Flavones						
Luteolin	285	133	38	100	18.96	C
Luteolin-4'-*O*-glucoside	447	285	25	110	15.90	C
Luteolin-7-*O*-glucoside	447	285	25	110	13.59	C
Flavan-3-ols						
(+)-Catechin	289	203	21	75	8.93	C
Catechin-3-O-glucoside	451	137	21	95	7.96	C
(-)-Epicatechin	289	203	21	75	9.98	C
(-)-Gallocatechin	305	125	24	80	6.97	C
Dihydroflavonols						
Dihydromyricetin	319	193	14	65	11.24	C
Taxifolin (dihydroquercetin)	303	125	24	70	13.25	C
Procyanidins						
Procyanidin B1	577	289	28	100	8.04	C
Procyanidin B3	577	289	29	110	8.91	C
Procyanidin C1	865	407	45	140	10.97	C

[1]Collision energy

[2] Retention times

[3]Internal standard time segment (Q: Quercetin-d3, C: ±Catechin-2,3,4-$^{13}C_3$, R: Resveratrol-(4-hydroxyphenyl-$^{13}C_6$), V: Vanillin-(ring-$^{13}C_6$), F: Ferulic acid-d3

Table 4.6 Concentration (µg/g DW) of water-soluble phenolic compounds in lentil seed coat (n = 3)

Compound name	CDC Greenland	CDC Greenstar	CDC Maxim-C	CDC Maxim	CDC SB-3	CDC Robin	ZT-4	6502-ZT	SEM[1]
Hydroxybenzoic acids									
3,4-Dihydroxybenzoic acid	3.20[b]	1.68[de]	6.54[a]	2.25[cd]	2.83[bc]	2.00[cd]	0.53[f]	1.14[ef]	0.170
4-hydroxybenzoic acid	3.12[c]	2.78[c]	1.83[c]	1.48[c]	2.66[c]	1.95[c]	32.80[a]	12.23[b]	0.857
Gallic acid	11.08[b]	5.30[c]	19.55[a]	4.99[c]	5.94[c]	4.82[c]	0.00	0.00	0.400
Vanillic acid	6.96[c]	12.35[bc]	12.61[b]	8.91[bc]	12.08[bc]	7.78[bc]	18.09[a]	20.51[a]	1.603
Vanillic acid-4-β-D-glucoside	152.97[ab]	121.61[c]	140.71[abc]	159.16[a]	127.50[c]	132.83[bc]	50.82[e]	97.04[d]	4.508
Total	177.38[a]	140.60[c]	175.64[a]	178.39[ab]	157.04[bc]	147.89[c]	107.50[d]	133.5[c]	5.340
Hydroxycinnamic acids									
Caffeic acid	0.36[b]	0.66[b]	3.15[ab]	1.83[b]	1.49[b]	1.15[b]	5.57[a]	1.84[b]	0.611
Ferulic acid (trans)	1.45	1.50	4.20	3.70	3.77	3.00	2.24	4.83	0.400
p-Coumaric acid (trans)	8.90[b]	8.02[b]	2.38[c]	1.21[c]	1.77[c]	1.05[c]	66.67[a]	1.49[c]	0.751
Total	10.72[b]	10.29[bc]	9.35[bc]	6.43[cd]	6.74[cd]	5.11[d]	74.85[a]	7.86[bcd]	0.733
Stilbenes									
Resveratrol-3-β-mono-D-glucoside (Polydatin)	10.85[a]	7.45[b]	4.09[c]	2.87[d]	1.73[e]	3.17[cd]	0.00	0.00	0.046
Flavones									
Luteolin	1.66[a]	1.12[ab]	1.16[ab]	0.54[bc]	1.50[a]	0.54[bc]	0.00	0.00	0.153
Luteolin-4'-O-glucoside	1.72[bc]	1.64[c]	5.31[a]	4.05[ab]	5.67[a]	5.58[a]	0.00	0.00	0.490
Luteolin-7-O-glucoside	2.17[a]	1.28[b]	1.02[c]	0.41[de]	0.63[d]	0.24[ef]	0.00	0.00	0.049
Total	5.58[ab]	4.10[b]	7.54[a]	4.99[ab]	6.61[a]	6.28[ab]	0.00	0.00	0.644

[a-f]Means with different superscripts within the same row are significantly different

[1] Standard error of mean

Table 4.6 Concentration (µg/ g DW) of water-soluble phenolic compounds in lentil seed coat (n = 3) Cont.

Compound name	CDC Greenland	CDC Greenstar	CDC Maxim-C	CDC Maxim	CDC SB-3	CDC Robin	ZT-4	6502-ZT	SEM[1]
Flavonols									
Kaempferol tetraglycoside	868.75^{bc}	1198.59^{ab}	566.93^{c}	946.56^{abc}	1362.47^{ab}	772.00 ^{bc}	1215.19^{ab}	1448.2^{a}	115.29
Kaempferol-3-O-rhamnoside	0.18^{f}	0.33^{def}	0.55^{cd}	0.63^{c}	1.01^{b}	1.28^{a}	0.2^{ef}	0.47^{cde}	0.058
Kaempferol 3-O- robinoside-7-O-rhamnoside (robinin)	26.64^{c}	59.36^{b}	9.49^{c}	21.03^{c}	34.96^{bc}	15.28^{c}	103.84^{ab}	34.11^{bc}	5.897
Kaempferol-3-O-glucoside	0.56^{b}	0.34^{bc}	0.91^{a}	0.32^{bc}	0.53^{b}	0.25^{cd}	0.39^{bc}	0.00	0.055
Kaempferol-3-O-rutinoside	0.00	0.00	0.00	0.00	0.00	0.00	4.30^{a}	1.13^{b}	0.215
Myricetin-3-O-rhamnoside	3.08^{bc}	4.24^{a}	2.81^{bc}	2.61^{bc}	3.39^{ab}	2.35^{c}	0.00	0.00	0.205
Quercetin-3,4'-di-O-glucoside	2.11^{b}	2.34 ^{ab}	2.54^{a}	1.71^{c}	1.15^{d}	1.03^{d}	1.28^{d}	0.50^{e}	0.072
Quercetin-3-O-rhamnoside (Quercitrin)	4.67^{b}	7.84^{a}	5.28^{ab}	5.03^{ab}	7.78^{a}	5.63^{ab}	0.00	0.00	0.626
Quercetin-3-O-rutinoside (Rutin)	0.70^{a}	0.54^{ab}	0.49^{ab}	0.41^{abc}	0.24^{abc}	0.64^{a}	0.23^{bc}	0.00	0.094
Total	924.99^{bcd}	1290.02^{abc}	567.82^{d}	985.65^{abcd}	1228.08 ^{ab}	840.86^{cd}	1277.98^{abc}	1611.69^{a}	115.76
Flavan-3-ols									
(+)-Catechin	62.45^{a}	45.92^{b}	31.21^{c}	21.55^{e}	25.97^{d}	20.98^{e}	0.27^{f}	0.00	0.799
Catechin-3-glucoside	3048.53^{a}	2858.49^{a}	1760.47^{b}	1716.27^{b}	1814.06 ^{b}	1591.11^{b}	16.68^{b}	0.00	52.188
(-)-Epicatechin	3.90^{a}	2.37^{ab}	2.11^{b}	1.45^{bc}	1.39^{bc}	0.92^{bc}	0.00	0.00	0.314
(−)-Gallocatechin	179.26^{a}	88.61^{b}	24.66^{c}	18.52^{c}	23.22^{c}	15.73^{c}	0.00	0.00	2.841
Total	3292.12 ^{a}	2952.47 ^{b}	1786.25 ^{c}	1766.72 ^{c}	1938.01 ^{c}	1626.76^{c}	25.34^{d}	0.00	55.04

[a-f]Means with different superscripts within the same row are significantly different
[1] Standard error of mean

Table 4.6 Concentration (μg/ g DW) of water-soluble phenolic compounds in lentil seed coat (n = 3) Cont.

Compound name	CDC Greenland	CDC Greenstar	CDC Maxim-C	CDC Maxim	CDC SB-3	CDC Robin	ZT-4	6502-ZT	SEM[1]
Dihydroflavonols									
Dihydromyricetin	2.11[a]	1.72[a]	1.05[b]	0.75[bc]	0.92[bc]	0.49[c]	0.00	0.00	0.106
Taxifolin (dihydroquercetin)	1.20[a]	1.07[a]	0.88[b]	0.59[c]	0.89[b]	0.78[b]	0.19[d]	0.00	0.029
Total	3.29[a]	2.67[a]	1.88[b]	1.37[bc]	2.05[bc]	1.25[c]	0.19[d]	0.00	0.128
Procyanidins									
Procyanidin B1	183.17[a]	115.14[c]	145.62[b]	113.45[c]	91.60[d]	95.57[d]	0.00	0.00	2.963
Procyanidin B3	1278.15[a]	1146.77[b]	1056.02[c]	1010.11[c]	847.98[d]	864.04[d]	0.00	0.00	18.511
Procyanidin C1	632.13[c]	664.88[bc]	756.20[a]	742.36[ab]	621.42[c]	694.88[bc]	0.00	0.00	18.080
Total	2092.12[a]	1906.96[ab]	1919.74[ab]	1870.78[b]	1627.31[c]	1633.25[c]	0.00	0.00	37.76
Total phenolic content	6516.95[a]	6314.44[a]	4472.39[b]	4817.12[bc]	4967.94[b]	4264.58[c]	1485.87[d]	1753.06[d]	103.23

[a-f]Means with different superscripts within the same row are significantly different

[1] Standard error of mean

The product (fragment) ion at *m/z* 167 was related to the loss of a glycosyl moiety (Mirali et al., 2017). Of the water-soluble phenolic compounds, the content of vanillic acid-4-*β-D*-glucoside differed among different cultivars and the highest concentration was observed for CDC Maxim (159.16 µg/g) followed by CDC Greenland (152.97 µg/g) and CDC Robin (132.83 µg/g).

The zero tannin seed coat showed nearly 2-3 times lower concentration of vanillic acid-4-*β-D*-glucoside compared to the normal tannin cultivars. All other phenolic acids in the water-solubles were found at lower concentrations (around 6 times lower in normal tannin cultivars) in comparison to vanillic acid-4-*β-D*-glucoside. Among these, gallic acid was the most abundant in normal tannin seed coats (4.82 – 19.55 µg/g) whereas it was not detected in zero tannin cultivars. Dueñas et al. (2002) noted that the gallic acid content of lentil seed coat ranged from 1.22 µg/g to 4.94 µg/g, which were close to the gallic acid levels observed in the present work. However, hydroxybenzoic acids found in the present study was about 5 times higher than that reported by Dueñas et al. (2002) and were in the range of 107.50 µg/g to 178.64 µg/g. The differences observed between the two studies may be due to many factors including the differences in genotypes and extraction solvents.

Para-coumaric acid (trans), caffeic acid and ferulic acid (trans) were the phenolic acids detected from the subclass of hydroxycinnamic acids and they were detected in all seed coat types including those from the zero tannin cultivars. CDC Maxim-C and ZT-4 had the highest caffeic acid (3.15 µg/g) and p-coumaric acid (66.67 µg/g), respectively among the water-soluble phenolics. There was around 7-fold lower concentration of ρ-coumaric acid present in other cultivars compared to ZT-4. The range of total hydroxycinnamic acids in the water extract of normal tannin lentil was 5.11 µg/g to 10.72 µg/g and was more than 20 times lower in comparison to hydroxybenzoic acids found in lentil by other workers who used different solvent systems for extraction. According to Dueñas et al. (2002) the total cinnamic acid content in lentil seed coat of cultivars Pardina and Castellana was between 11.78 µg/g and 29.75 µg/g.

Stilbenes are reported to have an extraordinary potential for cancer prevention due to their antioxidant, cell death activation, and anti-inflammatory properties which are associated with low toxicity under *in vivo* conditions (Sirerol et al., 2016). Resveratrol-3-β-mono-D-glucoside which belongs to stilbene subclass was eluted at 12.72 min. The LC–MS showed a molecular ion [M-H]⁻ at an *m/z* 389 and a fragment ion [F-H]⁻ at *m/z* 227; the difference was loss of a glycosyl moiety. Resveratrol-3-β-mono-D-glucoside was only found at lower concentrations in the water extract of

normal tannin cultivars (1.73 μg/g - 10.85 μg/g) and its concentration in CDC Greenland and CDC Greenstar (green seed coat) was twice as high as in gray and brown seed coat.

Flavonoids constitute the largest group of plant phenolics in seed coat with a C6-C3-C6 skeleton that consists of two aromatic rings joined by a three-carbon link (Ajila et al., 2011). Variations in the pattern of replacement with heterocyclic rings result in the major classes of flavonoids, i.e., flavonols, flavanones, flavonols, isoflavones, flavanon-3-ols and anthocyanidins (Kabera et al., 2014; Brodowska, 2017). Several compounds belonging to the flavonols subclass were identified in all regular and zero tannin cultivars. A predominant peak in the UV spectra (250-600 nm) at the retention time of 9.09 min (Figure 4.2 and 4.3) was previously identified as kaempferol tetraglycoside (Taylor et al., 2007) and semi-quantified using the targeted method (concentrations shown in Table 4.5). Kaempferol tetraglycoside was the most abundant among the flavonols found in lentil seed coat and it was worth noting that the kaempferol tetraglycoside was the major type of phenolic compound in the water extract of zero tannin seed coat. The level of kaempferol tetraglycoside in seed coat water extract decreased in the order 6502-ZT > CDC SB-3 > ZT-4 > CDC Greenstar> CDC Greenland > CDC Maxim > CDC Maxim-C. The other compounds identified in this subclass included kaempferol-3-O-rhamnoside, kaempferol 3-O-robinoside-7-O-rhamnoside (robinin), kaempferol-3-O-glucoside, kaempferol-3-O-rutinoside, myricetin-3-O-rhamnoside, quercetin-3-O-rhamnoside (quercitrin) and quercetin-3-O-rutinoside (rutin), however, their concentrations were much lower than the concentration of kaempferol tetraglycoside (>10-fold). Several preclinical studies have demonstrated that kaempferol and certain kaempferol glycosides have a range of pharmacological functions, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, antidiabetic and anti-allergic activities (Kushi et al., 1999; Yochum et al., 1999; Kris-Atherton et al., 2002; Baliga et al., 2014). Results of this study suggest that lentil seed coat would be a beneficial source of kaempferol compounds that can be integrated into the diet or used for the extraction of these beneficial phytochemicals.

Flavan-3-ols were found in large quantities in lentil seeds (Dueñas et al., 2002; Escarpa et al., 2002; Dueñas et al., 2003; Amarowicz et al., 2009; Amarowicz et al., 2010; Zhang et al., 2015). They have been reported to possess several beneficial effects on health through functions as antioxidant, anticarcinogenic, cardiopreventive, anti-microbial, anti-viral and neuro-protective agents. Moreover, the ability of flavan-3-ols to support food functionality has also been established

in terms of microbial stability, oxidative stability, and heat stability (Aron and Kennedy, 2008). The compound that eluted at R_t of 7.96 had a molecular ion [M-H]$^-$ of m/z 451, and fragment ion [F-H]$^-$ at m/z 137 and was identified as catechin-3-O-glucoside of the flavon-3-ols subclass. Peaks observed at R_t 8.93, 9.98, and 6.97 corresponded to (+)-catechin, (-)-epicatechin, and (-)-gallocatechin, respectively, which belongs to the same flavon-3-ols subclass. Of the analyzed flavon-3-ols, the most abundant in lentil seed coat was catechin-3-O-glucoside and was found at 3-150 times higher concentrations compared to the other flavon-3-ols detected in normal tannin seed coat. Water extracts of CDC Greenland and CDC Greenstar (green seed coat) had the highest concentration of catechin-3-O-glucoside at 3048.53 µg/g and 2858.49 µg/g, respectively. The second highest concentration of catechin-3-O-glucoside was noted in cultivars with gray and brown seed coats. The levels found was nearly half that of the green seed coat and ranged between 1591.11 µg/g and 1814.06 µg/g. Between the two zero tannin cultivars, catechin-3-O-glucoside was detected only in ZT-4 at very low level (16.68 µg/g) in comparison to normal tannin seed coat (1591.11 - 3048.53 µg/g). In Dueñas et al. (2002) work, the total catechins in lentil seed coat (extracted in methanol) was reported to range from 917.55 µg/g and 1530.11 µg/g. Zhang et al. (2015) who studied the phenolic profiles of whole seeds from 20 Canadian lentil cultivars reported catechin glucoside levels between 101.97 and 131.07 µg/g, which were lower than (>12-fold) the levels observed in the water extracts of seed coat in the current study. (-)-epicatechin and (+)-catechin, which are the cis and trans configurations of catechin, were detected in the water extracts of seed coat but at lower concentrations. The levels of (-)-epicatechin was within 2.11 µg/g to 3.90 µg/g range while (+)-catechin were between 21.55 µg/g to 62.45 µg/g. Flavan-3-ols were absent in the zero tannin cultivar, 6502-ZT while small levels of (+)-catechin and catechin-3-glucoside were detected in ZT-4. The total water extractable flavan-3-ols was between 16.95 µg/g and 3292.12 µg/g and followed the order CDC Greenland > CDC Greenstar > CDC SB-3 = CDC Maxim-C = CDC Maxim = CDC Robin > ZT-4.

Luteolin, luteolin-4'-O-glucoside and luteolin-7-*O*-glucoside were the flavones identified in seed coat. In comparison to flavan-3-ols, their concentrations were far lower. None of the flavones were present in zero tannin cultivar. When comparing the concentrations of total flavones with the results reported by Dueñas et al. (2002), there was a 2 to 70-fold higher concentration observed in their study which may be due to the differences in the genotypes and extraction solvent.

The solvent used in their study was methanol-water (80:20 v/v) while water was used in the present study which is more polar.

Procyanidins are oligomeric compounds, formed from catechin and epicatechin molecules and are members of the proanthocyanidins (or condensed tannins) class of flavonoids. In relation to the major classes of procyanidins, procyanidin B1, B3 and C1 were detected in the water extracts of regular seed coat with concentrations varying within a narrow range. Procyanidins were absent in zero tannin cultivars. Procyanidin B3 eluted at R_t 8.91 and had a molecular ion [M-H]⁻ of m/z 577, and fragment ion [F-H]⁻ at m/z 289. It was the most abundant of the procyanidins identified in lentil seed coat (Table 4.5). This observation was in agreement with Dueñas et al. (2002). They also noted relatively higher levels of procyanidin B3 among the different procyanidins evaluated in lentil seed coat. CDC Greenland presented the highest concentration of procyanidin B3 at 1278.15 µg/g. CDC Greenstar had the second highest concentration (1146.77 µg/g) followed by CDC Maxim-C (1056.02 µg/g), CDC Maxim (1010.11 µg/g), CDC SB-3 (847.98 µg/g) and CDC Robin (864.04 µg/g).

The peak at R_t 10.97 min corresponded to procyanidin C1 with molecular ion [M-H]⁻ of m/z 865, and fragment ion [F-H]⁻ at m/z 407 and it was the second highest procyanidin found in seed coats. The concentrations of procyanidin C1 were nearly half as much as the procyanidin B3. Statistically, CDC Maxim had a significantly higher concentration of C1 compared to other cultivars though the concentrations among cultivars were changed within a narrow range. Total procyanidin values revealed that CDC Greenland, CDC Greenstar and CDC Maxim had relatively higher procyanidin than that of CDC SB-3 and CDC Robin. Procyanidins was the second highest group of phenolic compounds next to flavonol-3-ols in lentil seed coat.

The most obvious differences between water-solubles of normal tannin and zero tannin cultivars were the presence of resveratrol-3-β-mono-D-glucoside, quercetin-3-O-rhamnoside (quercitrin), flavones, (-)-epicatechin, dihydromyricetin and procyanidins in the normal tannin cultivars and the absence of these in zero tannin cultivars. These results are in accordance with the findings of Mirali (2016). The absence of these compounds reveals that the phenylpropanoid pathway is blocked in zero tannin cultivars (Mirali et al., 2016).

The comparison between CDC Maxim-C and CDC Maxim did not show large differences in the compounds analyzed except for a few. Across the 30 phenolic compounds analysed, the compounds which differed between these two samples with statistical significance were phenolic

acids (3,4-dihydroxybenzoic acid, gallic acid), resveratrol-3-β-mono-*D*-glucoside, kaempferol-3-O-glucoside, quercetin-3,4'-di-*O*-glucoside, (+)-catechin, taxifolin (dihydroquercetin) and procyanidin B1. Seed samples of CDC Maxim-C had been stored under dark and room temperature conditions for 3 years. Results indicated that those storage conditions had no larger impact on the water-soluble phenolic contents. In comparison, Mirali (2017), who studied the impact of long-term storage on lentil seed phenolic composition, documented changes in 27 flavan-3-ols and proanthocyanidin oligomers concentrations. The storage facility used in her study did not have temperature and humidity control and thus, was subject to seasonal fluctuations. In addition, seeds had been stored for long periods (7-14 years).

The LC-DAD spectra also contained several peaks that were from compounds not currently included in the targeted method. For example, the peak at 11.84 min showed a molecular ion [M – H]$^-$ at *m/z* 491 and was found in all seed coat extracts. In the normal tannin seed coat genotypes, there was a large unresolved peak (3-20 min) in the baseline that primarily consists of a group of high molecular weight polyphenols called proanthocyanidins. Unfortunately, quantification of this group using LC-MS is not feasible due to their numerous isomers and the lack of availability of commercial standards.

The sum of all phenolic compounds found in seed coat ranged from 1485.87 μg/g to 6516.95 μg/g and was much lower (3 to 5-fold) than the total phenolic content measured by the Folin–Ciocalteu method which may be due to the incomplete quantification of all peaks and possible interferences by other compounds in the Folin–Ciocalteu method (Zhang et al., 2015). Zhang et al. (2015) observed up to 10-fold lower concentration of TPC in HPLC determinations compared to Folin–Ciocalteu method. Conjugation between phenolic compounds with soluble components such as small peptides or oligosaccharides may also cause lower detection of TPC (Saulnier et al., 1999; Zhang et al., 2015).

Figure 4.4 illustrates the distribution (%) of phenolic compounds in the seed coat of different lentil cultivars. In general, kaempferol tetraglycoside, catechin-3-glucoside and procyanidin B3 were the dominant phenolic compounds found in the water extracts of normal tannin cultivars (green, gray and brown seed coat types) which contributes around 73% - 81% of the total phenolics. These results are in agreement with those reported by Amakowicz at al. (2010) and Zhang et al. (2015) who observed higher percentages of kaempferol tetraglycoside, catechin-3-glucoside and procyanidins in lentil seed coat and whole lentils, respectively. The
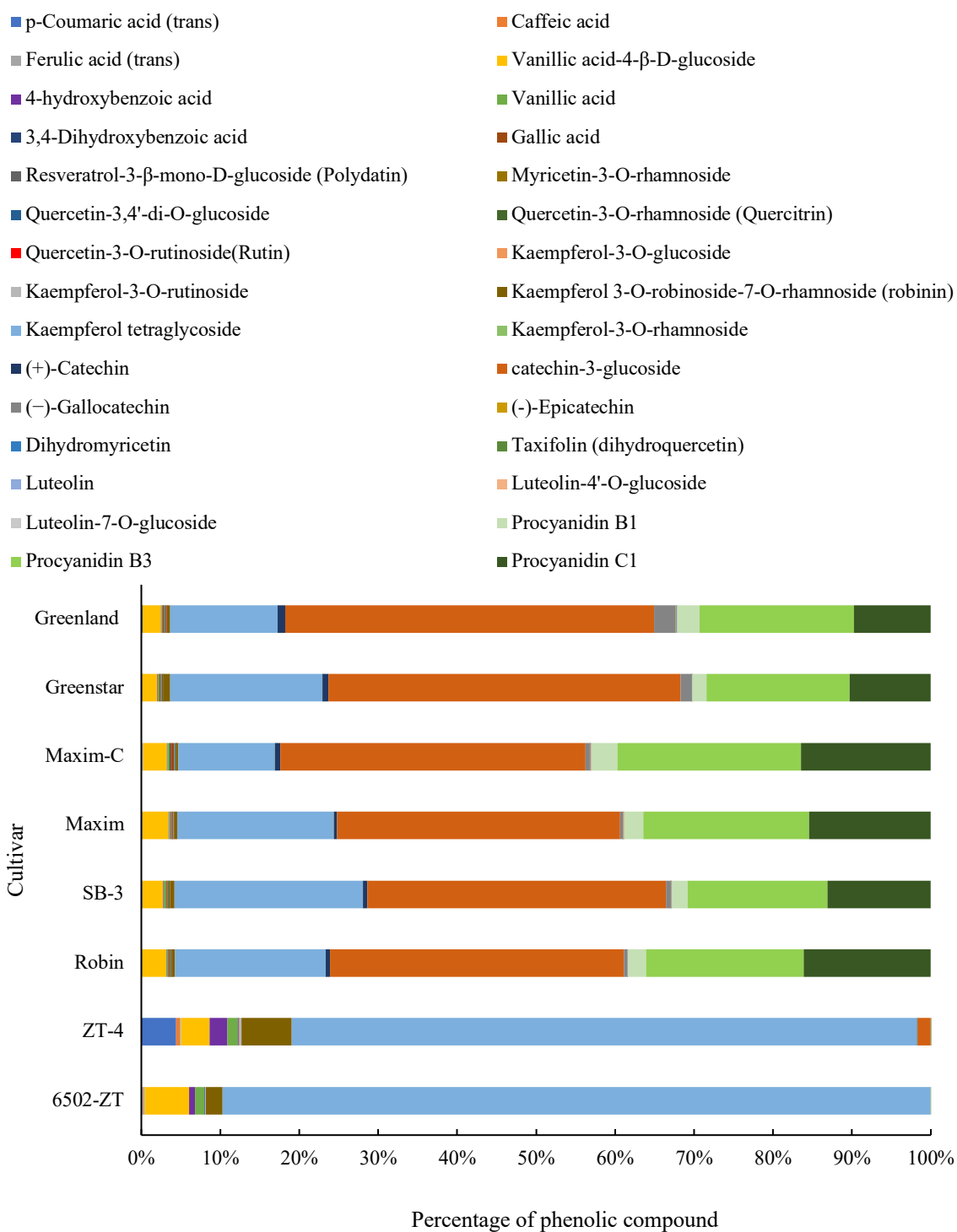
Figure 4.4 Distribution (%) of phenolic compounds in the seed coat water extracts of different lentil cultivars

proanthocyanidins are present in the largest amounts in normal tannin genotypes and would be a major component contributing to the antioxidant capacity (Hagerman et al., 1998). With respect to the zero tannin genotypes, 79%-89% of the total phenolics of water-solubles consisted of kaempferol tetraglycoside, while ~4%-6% consisted of phenolic acids.

4.5.4 Antioxidant activity of water extracts of lentil seed coat

Multiple assays are necessary to assess the antioxidant activity of phenolic compounds owing to the complex chemical nature of phenolic compounds and the determination of antioxidant activity depends on the reaction-mechanism. In the present study, DPPH and ABTS free radical scavenging assays, ferrous ion chelation and phospholipid peroxidation inhibition assays were performed to study the antioxidant capacity of lentil seed coat water extracts. Figure 4.5 illustrates the effect of TPC concentration on the antioxidant activity of water extracts from different lentil cultivars.

It is well established that free radicals are the primary factor causing lipid peroxidation in biological systems. In the present study, DPPH assay was conducted to elucidate the ability of seed coat extracts to scavenge free radicals. DPPH is a stable organic free radical. It can be reduced to a non-radical form of DPPH-H when an electron or hydrogen is accepted in the presence of a hydrogen-donating antioxidant (Zou et al., 2011). The effect of the concentration of total phenolics on DPPH scavenging activity of water extracts are shown in Figure 4.5a. Results indicated a concentration-dependent scavenging activity on DPPH radical. In general, the overall activity followed the order of; CDC Greenland > CDC Greenstar > CDC Maxim > CDC Robin > CDC SB-3 > 6502-ZT = ZT-4 and percent radical scavenging activity ranged between 65% and 76% at a total phenolic concentration of 500 ppm for the normal tannin seed coat indicating strong antioxidant activity. Generally, green seed coat showed slightly higher (~5%) activity compared to gray and brown seed coat. As illustrated, the DPPH radical scavenging activity of zero tannin seed coat was much lower (10 to 12-fold) compared to normal tannin seed coats. This could be due to the absence of condensed tannin in zero tannin seed coat. Amarowicz et al. (2010) and Amarowicz et al. (2009), observed a greater radical scavenging effect in phenolic extracts rich in condensed tannin compared to the extracts with low concentrations of tannin from red and green lentil, respectively.
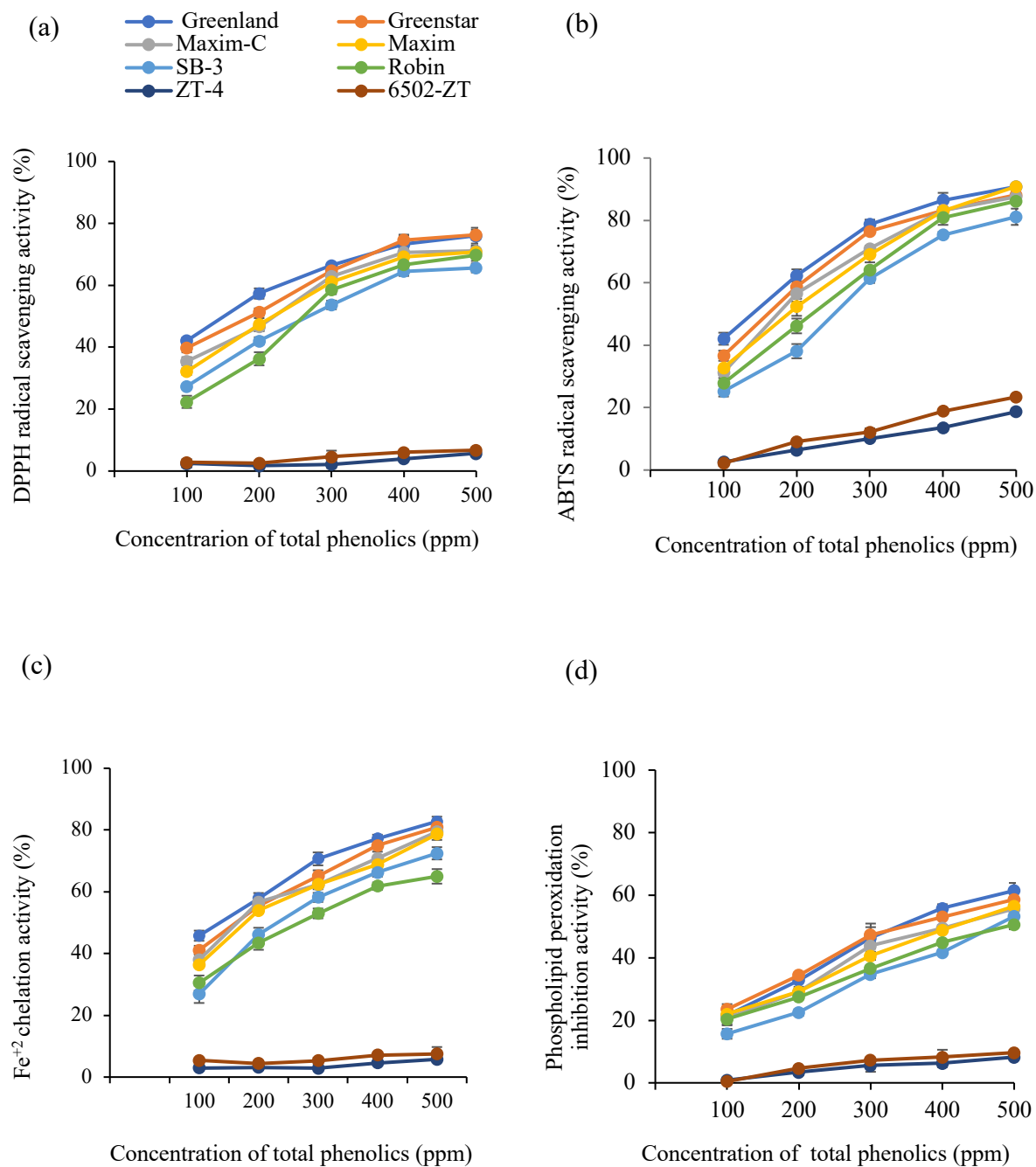
Figure 4.5 Effect of phenolic concentration on antioxidant activity of water extracts of lentil seed coat (a) DPPH scavenging activity (b) ABTS scavenging activity, (c) Fe^{2+} ion chelation activity, (d) Inhibition of phospholipid peroxidation. Values are the means and standard errors of duplicate assays from three replicates.

The ABTS radical scavenging activity of water extracts of different lentil cultivars are presented in Figure 4.5b. Similar to the DPPH assay, this assay also uses stable free radicals instead of radicals produced from oxidizing the substrate. ABTS free radical is soluble in both water and organic media, thus ABTS method was more sensitive than the DPPH assay when measuring the antioxidant activity of water-soluble compounds (Tang et al., 2001).

The trend of scavenging effects of water extracts on ABTS radical was very similar to DPPH radical in the present study. Water extracts showed a concentration dependent scavenging activity. The overall ABTS radical scavenging activity of seven cultivars decreased in the order of CDC Greenland > CDC Greenstar > CDC Maxim > CDC Robin > CDC SB-3 > 6502-ZT > ZT-4. It is noteworthy that, when free radical scavenging activity of the extracts were determined at a similar concentration of total phenolics, cultivar CDC Greenland found to have the highest activity. However, at higher concentrations (> 400 ppm) of total phenolics, free radical scavenging activity differs by less than 10 percent among normal tannin cultivars. Water extracts from normal tannin seed coat exhibited higher ABTS radical scavenging activity ($p<0.001$) compared to zero tannin seed coat types. It was noted that, compared to normal tannin cultivars, the ABTS radical scavenging activity of zero tannin seed coat was 4 to 6 times lower. Further, it was observed that, the ABTS scavenging activity exhibited at concentrations above ~400 ppm was slightly higher than that of the corresponding values displayed in DPPH assay. Such discrepancy may be due to differences in the affinity of different radicals to phenolic compounds present in the extracts.

The overall comparison of the results of free radical scavenging assays revealed that water extracts from all normal tannin seed coats have strong antiradical activity at high (>400 ppm) concentrations of TPC. The ability of a molecule to donate a hydrogen atom is one of the mechanisms involved in their antioxidant activity. Therefore, the DPPH and ABTS free radical scavenging activity of lentil seed coat extracts may primarily be related to their hydrogen donation ability (Hu et al., 2000). These results indicate that lentil seed coat extracts may possess a strong antioxidant activity against the free radical mediated lipid oxidation in foods. Moreover, they may also have the ability to control oxidative degeneration of biomolecules such as membrane lipids, proteins, and nucleic acid which is attributed to the ability of the molecules involved in scavenging free radicals (Cuvelier et al., 1992; Rice-Evans and Diplock, 1993), and therefore, lentil seed coat water extract would be a good source of natural antioxidants.

Transition metals such as iron and copper have an accelerating effect on the rate of lipid oxidation because the homolytic decomposition of lipid hydroperoxide is generally considered to be metal-catalyzed (Frankel, 2005). No food system can be regarded as free from metal ions. Therefore, the metal ion chelating capacity of water extracts of different cultivars was determined by assessing their ability to chelate Fe^{2+} ions. Figure 4.5c shows that ferrous ion chelating activity increased with the increasing concentration of total phenolics. With regard to the zero-tannin seed coat, the trend was almost the same as those observed in DPPH radical scavenging assays. It was noted that the ferrous chelating activity did not increase noticeably with the increasing concentration of total phenolics, in the zero tannin seed coat, irrespective of the cultivar. The ferrous chelating activity which ranged between 65% and 82% for the water extracts at 500 ppm concentration of total phenolics indicate strong chelating effect of the normal tannin seed coat types. At this concentration, seed coats of CDC Greenland, CDC Greenstar and CDC Maxim (78% to 82%) were found to have significantly stronger activity than that of CDC Robin (65%) and CDC SB-3 (72%). Considering the overall activity, green seed coat (CDC Greenland and CDC Greenstar) showed around 4% higher activity in comparison to gray seed coat.

Because of the higher degree of unsaturation, phospholipids are generally considered as the major fraction of lipids responsible for oxidative deterioration in foods. Phospholipids can therefore be a more appropriate substrate for assessing antioxidant activity in food systems. In the present study, phospholipids undergoing Fe^{3+} mediated peroxidation was determined by measuring the TBARS values. The effect of different concentrations of TPC from different lentil cultivars on TBARS formation is shown in Figure 4.5d. The inhibitory effect on the phospholipid peroxidation increased with increasing TPC in the extracts. The overall inhibition activity of different cultivars was in the following order: CDC Greenland = CDC Greenstar > CDC Maxim > CDC Robin > CDC SB-3 > 6502-ZT = ZT-4. All the samples at 500 ppm of TPC showed 61, 59, 56, 53, 50, 10 and 8% inhibition for CDC Greenland, CDC Greenstar, CDC Maxim, CDC SB-3, CDC Robin, 6502-ZT and ZT-4, respectively indicating that different seed coat extracts have different phospholipid peroxidation inhibition activity even at similar phenolic concentrations. It was further noticed that, the antioxidant activity of water extracts determined by the ability to inhibit phospholipid peroxidation was lower than the corresponding samples determined by free radical scavenging and ferrous ion chelation assays.

In general, all antioxidant assays showed similar trends and ANOVA showed that the cultivar and phenolic concentration have significant impact ($p < 0.001$) on the antioxidant activity of lentil seed coat. All cultivars exhibited significant antioxidant activity except the zero tannin cultivars. Zero tannin cultivars showed higher ABTS radical scavenging activity as concentration increased compared to other antioxidant assays showing its greater sensitivity. The antioxidant activity of standardized extracts of CDC Greenland found to be superior to the other cultivars in all assays. It was worth noting that there was no difference in antioxidant activity of the seed coat extracts obtained from the two seed lots of CDC Maxim indicating that three years of storage had no significant impact on their antioxidant capacity, and it could be attributed to the similar phenolic profiles observed. As shown by several other researchers (Shiriwardhana and Shahidi, 2002; Amarowicz et al., 2009; Amarowicz et al., 2010; Kong et al., 2010; Zhang et al., 2015), the antioxidant activity rate increased with the increasing concentration of phenolics in all cultivars, even though the rate of increase was different between cultivars. With increasing TPC concentrations from 100 ppm to 500 ppm, antioxidant activity increased by about 40-50% in normal tannin, whereas the corresponding values were 2 to 20 % for zero tannin cultivars. The water extracts of CDC Greenland and CDC Maxim-C were also evaluated in Study I for their antioxidant activity. The comparison of results obtained in both studied showed that the antioxidant capacity measured at varying concentrations of TPC did not differ markedly (<5% difference) between the studies although the sample preparation was slightly different between the studies.

It was noteworthy that, the antioxidant capacity of phenolic extracts of zero tannin seed coats were about 6 - 9 times lower than the antioxidant capacity of water extracts from other seed coats even at same concentrations of total phenolics. This highlights the fact that the type or the composition of phenolics determines the antioxidant potential of plant extracts. In normal tannin lentil with green, gray or brown seed coat, approximately 80% of the total phenolics were comprised of flavonoids; kaempferol tetraglycoside, catechin-*o*-glucoside and procyanidins. Flavonoids (flavones, flavonols, flavanols and flavanonens) with multiple hydroxyl groups are more effective antioxidants than those with one only (Brewer et al., 2011). The antioxidant effects of flavonoids are due to their ability to scavenge free radicals. In addition, flavonoids can dampen oxidation-enhancing transition metals by donating an H atom to them, making them less prooxidative (Brewer et al., 2011). Fernandez et al. (2002) reported that flavones and some flavanones can preferentially bind metal ions at the 5-hydroxyl and 4-oxo groups.

Among different flavonoids which includes rutin, dihydroquercetin, quercetin, epigallocatechin, and epicatechin gallate, catechin was found to be the most effective in inhibiting NADPH-dependant microsomal lipid peroxidation (Potapovich and Kostyuk, 2003). Moreover, Potapovich and Kostyuk (2003) reported that flavonoid compounds were able to chelate transition metal ions (Fe^{2+}, Fe^{3+}, and Cu^{2+}) and were effective scavengers of oxygen anion-radicals ($^{\bullet}O_2^-$) to varying degrees. They speculated that the relative antiradical activity to $^{\bullet}O_2^-$ may be responsible for the relative antioxidative difference among these compounds. Moreover, Zhang et al. (2015) observed that the antioxidant activity of pure catechin procyanidins indicating was 2 to 3-fold higher than that of kaempferol as measured by DPPH radical scavenging ability, ferric reducing antioxidant power and oxygen radical absorption capacity.

It was reported that the antioxidant potential of natural phenolics depends on the number of free -OH groups on the molecule (Brewer, 2011). Procyanidins are oligomeric compounds consisting of molecules of catechin and epicatechin (Dixon et al., 2005) and may possess many free -OH groups. Ursini et al. (2001) reported that these procyanidins were better antioxidants than the corresponding monomers; catechin and epicatechin. Procyanidins (condensed tannin) was one of the most abundant groups of polyphenols found in normal tannin seed coat which may explain the superior antioxidant capacity observed. In agreement, procyanidins found in fruits (apple, cherries), berries (raspberry, blackberry, strawberry) and leaves (tea, cocoa), seeds (grape, sorghum, soy, cocoa bean) have demonstrated strong antioxidant effects (Dixon et al., 2005; Buřičová, and Reblova, 2008; Bak et al., 2010). The phenolic profile of zero tannin seed coat water extracts were composed of approximately 80 - 90% of kaempferol tetraglycoside whereas the concentration of this compound in other seed coat types was only around 12% - 25% (Figure 4.4). Procyanidin was not found in both zero tannin seed coats and small quantity of catechin-3-glucoside was detected only in cultivar ZT-4. These observations suggest that the combination of kaempferol tetraglycoside, catechin-3-glucoside and procyanidin have better antioxidant properties than a system containing mostly the kaempferol tetraglycoside which could mainly be due to the differences in the composition and position of reactive OH groups in the system. As well there could be synergistic effects exist in the presence of different phenolic compounds which enhances the antioxidant efficacy of water extracts. Moreover, the activity of different phenolic compounds may vary depending on their affinity to the type of free radicals available (Minatel et al., 2017). Figure 4.6 shows the chemical structures of the three major phenolic compounds identified in water
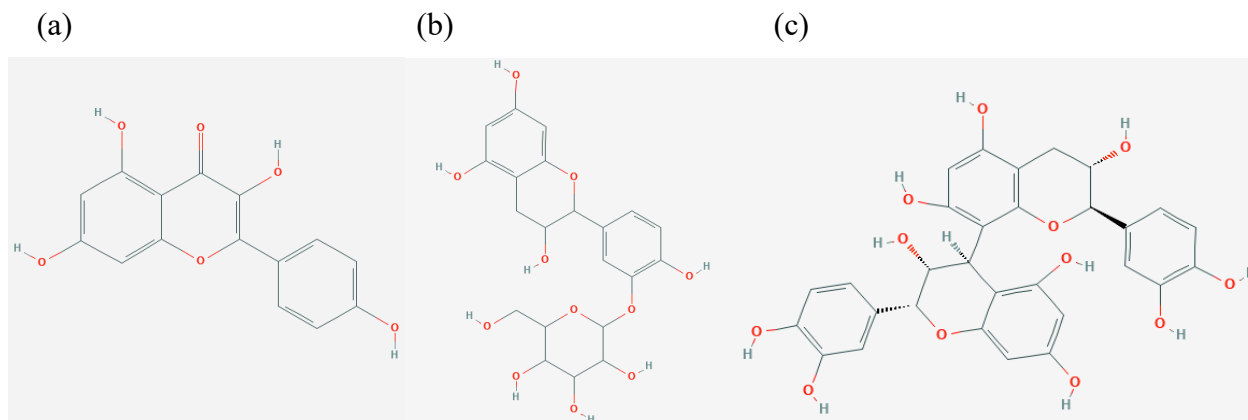
Figure 4.6 Chemical structures of (a) kaempferol (b) catechin 3-glucoside (c) procyanidin B1 (Source: PubChem, 2015)

extracts of normal tannin and zero tannin seed coat which may help explain the differences observed between lentil cultivars. extracts of normal tannin and zero tannin seed coat which may help explain the differences observed between lentil cultivars. In support of these findings, comparable results were reported by Hagerman et al. (1998) and Zou et al. (2011). Amarowicz et al. (2010) and Zou et al. (2011) observed that extracts of whole lentil which were rich in procyanidin exhibits higher antioxidant activity. In addition, condensed tannins have been shown to be effective antioxidants with greater activity than simple polyphenols, such as flavonoid monomers (Hagerman et al., 1998), which suggests even more importance of proanthocyanidins in total antioxidant capacity. A study carried out to characterize the polyphenol effect on inhibition and promotion of iron uptake by Caco-2 cells warranted that the phenolic composition is an important determinant of their activity (Hart, 2017). They reported that the antioxidant behavior of a compound is different when it was present individually or in mixture with other compounds which could be attributed to their synergistic activity.

4.5.5 Correlations among phenolic contents, antioxidant activities and color values

Pearson's correlation analysis was performed to investigate the linear relationships among the phenolics, antioxidant capacities and color values of seed coat and data are presented in Table 4.7. There were strong linear correlations between TPC and flavon-3-ols and procyanidins indicating that these compounds contribute significantly to the total phenolic content of lentil.

Table 4.7 Correlation coefficients among phenolic compounds, antioxidant capacity, and color values measured on lentil seed coat (n=24)

Variable	TPC	TFC	CTC	HC	HB	STB	FNOL	FL3OL	DF
TFC	0.95***								
CTC	0.97***	0.99***							
HC	-0.57**	-0.47	-0.49						
HB	0.68***	0.80**	0.78**	-0.69***					
STB	0.84***	0.73**	0.67*	-0.35	0.56**				
FNOL	-0.32	-0.53	-0.53	0.24	-0.63***	-0.39			
FL3OL	0.98***	0.93***	0.91***	-0.52**	0.66***	0.91***	-0.37		
DF	0.94***	0.88***	0.84***	-0.43*	0.64***	0.92***	-0.39	0.97***	
FLNES	0.68***	0.79**	0.80**	-0.57**	0.64***	0.36	-0.41*	0.61**	0.55**
PRC	0.93***	0.94***	0.96***	-0.62**	0.82***	0.73***	-0.58**	0.91***	0.85***
LP	0.93***	0.95***	0.97***	-0.60**	0.74***	0.69***	-0.54**	0.87***	0.79***
ABTS	0.95***	0.98***	0.98***	-0.58**	0.77***	0.83***	-0.53**	0.95***	0.89***
DPPH	0.96***	0.97***	0.97***	-0.56**	0.77***	0.82***	-0.48*	0.95***	0.92***
MC	0.94***	0.97***	0.98***	-0.60**	0.79***	0.82***	-0.55**	0.94***	0.89***
L*	-0.65***	-0.53**	-0.54**	-0.77***	-0.69***	-0.01	0.50*	-0.23	-0.15
a*	0.32	0.16	0.19	-0.49*	0.30	-0.18	-0.49*	-0.09	-0.18
b*	-0.45**	-0.39	-0.40	0.54**	-0.61**	0.05	0.46*	-0.13	-0.08

TPC: total phenolic content, TFC: total flavonoid content, CTC: total condensed tannin content, HC: hydroxybenzoic acids, HB: hydroxycinnamic acids, STB: stilbenes, FNOL: flavonols, FL3OL: flavon-3-ols, DF: dihydroflavonols, FLNES: flavones, PRC: procyanidins, , LP: inhibition of phospholipid peroxidation, ABTS: ABTS free radical scavenging activity, DPPH: DPPH free radical scavenging activity, MC: Fe^{2+} ion chelation activity, L*: CIE L* (lightness) parameter, , a*: CIE a* (redness) parameter, b*: CIE b* (yellowness) parameter
*, **, *** Significant at $p < 0.05$, 0.01 and 0.001, respectively.

Table 4.7 Correlation coefficients among phenolic compounds, antioxidant capacity, and color values measured on lentil seed coat Cont.

Variable	FLNES	PRC	LP	ABTS	DPPH	MC	L*	a*
PRC	0.80***							
LP	0.78***	0.97***						
ABTS	0.73***	0.97***	0.95***					
DPPH	0.71***	0.96***	0.92***	0.97***				
MC	0.73***	0.97***	0.95***	0.99***	0.98***			
L*	-0.69***	-0.55**	-0.53**	-0.43*	-0.38	-0.45*		
a*	0.35	0.21	0.27	0.12	0.05	0.16	-0.75***	
b*	-0.57**	-0.40	-0.36	-0.28	-0.28	-0.31	0.73***	-0.48*

FLNES: flavones, PRC: procyanidins, , LP: inhibition of phospholipid peroxidation, ABTS: ABTS free radical scavenging activity, DPPH: DPPH free radical scavenging activity, MC: Fe^{2+} ion chelation activity, L*: CIE L* (lightness) parameter, , a*: CIE a* (redness) parameter, b*: CIE b* (yellowness) parameter

*, **, *** Significant at $p < 0.05$, 0.01 and 0.001, respectively.

Moreover, correlation analysis showed strong positive correlations between flavon-3-ols and procyanidins ($r = 0.91$) which shows that higher concentrations of procyanidins could be expected at higher concentrations of flavon-3-ols. This relationship is obvious as the procyanidins are the polymeric flavan-3-ols. The same relationship has also been exhibited by the high correlation coefficient between TFC and CTC. From correlation analysis between phenolic compounds and antioxidant activities, it was found that significant ($p<0.001$) positive correlations existed between TPC and DPPH radical scavenging activity ($r = 0.96$), ABTS radical scavenging activity ($r = 0.95$), metal chelation activity ($r = 0.94$) and phospholipid peroxidation inhibition activity ($r = 0.93$). These results showed that the total phenolic content could be used with reasonable accuracy to predict antioxidant activity of water extracts of lentil seed coat. These findings are in agreement with those reported by Xu et al. (2007), Zhang et al. (2011) and Zhang et al. (2015). Among different phenolic classes, procyanidins showed the greatest correlation coefficients ($r>0.96$) with all antioxidant assays. Strong positive correlations were also found between the flavon-3-ols and DPPH radical scavenging activity, ABTS radical scavenging activity, metal chelation activity and phospholipid peroxidation inhibition activity with correlation coefficients at 0.95, 0.95, 0.87 and 0.94 respectively. Moreover, the antioxidant assays correlated with flavones, dihydroflavonols and hydroxybenzoic acids, however, with relatively lower correlation coefficients (r values between 0.74 and 0.92). Phenolic classes, hydroxybenzoic acids and flavonols showed moderate correlations with antioxidant assays. These correlation results suggested that different phenolic

contents might have different degree of contribution for overall antioxidant activity of water extracts. The higher correlation exhibited in TFC and CTC with antioxidant assays shows that the presence of high concentrations of flavonoids and condensed tannin results in superior antioxidant potential of lentil seed coat water extract which could be related to their chemical structures. As described in previous sections, the antioxidant potential of phenolic compounds appears to depend on the pattern and number of free -OH groups on the molecule (Brewer et al., 2011). For example, it has been shown that proanthocyanidins B-2 was better than proanthocyanidins B-4 or (–)-epicatechin to scavenge hydroxyl radical and superoxide anion (Zhao et al., 2006) while the antioxidant activity of flavan-3-ols was found to increase from monomer to trimer and then decrease from trimer to tetramer (Plumb et al., 1998; Aron and Kennedy, 2008). The more significant positive correlation coefficients observed between procyanidins and antioxidant assays show the tendency of having the stronger antioxidant capacity in extracts with high concentrations of procyanidin.

Studies have reported that legumes with dark seed coat color possessed a high antioxidant activity due to the presence of high amounts of phenolic compounds (Xu et al., 2007). Correlation analysis was therefore conducted to determine linear relationships with phenolic compounds and antioxidant performance with the color attributes. Results did not show strong associations between different parameters. Among different phenolic classes, phenolic acids, flavonols, flavones and procyanidins showed moderate negative correlations (-0.55 to -0.69) with L* value showing that darker seed coat tended to have higher level of phenolic compounds. However, no correlations between TPC and color values were found. In terms of antioxidant activity only L* value of seed coat were moderately and negatively correlated with ABTS radical scavenging activity ($r = -0.43$), ferrous ion chelation activity ($r = -0.45$) and phospholipid peroxidation inhibition activity ($r = -0.53$), but not with DPPH radical scavenging activity. With respect to a* and b* values, no strong associations were observed between phenolic compounds and antioxidant assays. Hence, a* and b* values of seed coat color would not be strong indicators of antioxidant activity or phenolic concentration in lentil seed coat.

4.5.6 Principal component and hierarchical clustering analysis

Principal component analysis (PCA) followed by hierarchical clustering analysis was conducted to classify the lentils as a function of phenolic concentration and antioxidant capacity of their seed coat water extracts. The results of PCA (Figure 4.7) showed that the first two principal components (Dim 1 and Dim 2) explained 93% of the variability in data, with the Dim 1 being the component with the highest explanatory power (85%). The bi-plot (Figure 4.7a) and variable contribution plots (Figures 4.6b and 4.6c) explained that flavon-3-ols, flavonols, ABTS radial scavenging capacity, ferrous ion chelation capacity, liposome peroxidation capacity and DPPH radial scavenging capacity had higher influence on the component one (Dim 1) and they had strong positive correlation with each other. This reveals that the largest variation among the cultivars exists on these variables. Component 2 (Dim 2) was mostly influenced by the variables; flavones, stilbenes, hydroxycinnamic acids and procyanidins.

Lentil cultivars were classified into three groups by hierarchical clustering analysis. Lentils with gray background color seed coat (CDC Maxim and CDC SB-3) and brown color (CDC Robin) seed coat were grouped and isolated from green seed coat lentils (CDC Greenland, CDC Greenstar) suggesting similarity in properties between gray and brown seed coat types. Two zero tannin genotypes had relatively similar properties with respect to the antioxidant capacity and phenolic composition which were far below the normal tannin genotypes, hence they have been separated from the normal tannin genotypes. Antioxidant capacity as measured by all assays and flavon-3-ols and procyanidin contents decreased from cluster one through cluster three. Accordingly, cluster one consisting of green lentil cultivars showed the highest antioxidant potential. Though, it was noted that the differences in the overall antioxidant capacities between the two clusters of normal tannin cultivars varied between 6% and 9%. Flavon-3-ols and procyanidins were the phenolic classes which showed the strongest connections with antioxidant capacity of water extracts. Mirali (2016) reported that two independent genes (Ggc and Tgc) are involved in the determination of seed coat background color in lentil. Based on the phenolic profiles of aqueous acetone extracts, Mirali (2016) reported that seed coats with the homozygous recessive tgc allele (green and gray seed coats) had higher content of flavan-3-ols, proanthocyanidins and some flavonols than in brown (Ggc Tgc) seed coat. And further she concluded that production of some phenolic compounds, specifically the proanthocyanidins, is controlled by the Tgc seed coat color gene. In the present study, the water extracts of brown seed coat (CDC Robin) showed fairly similar phenolic profile
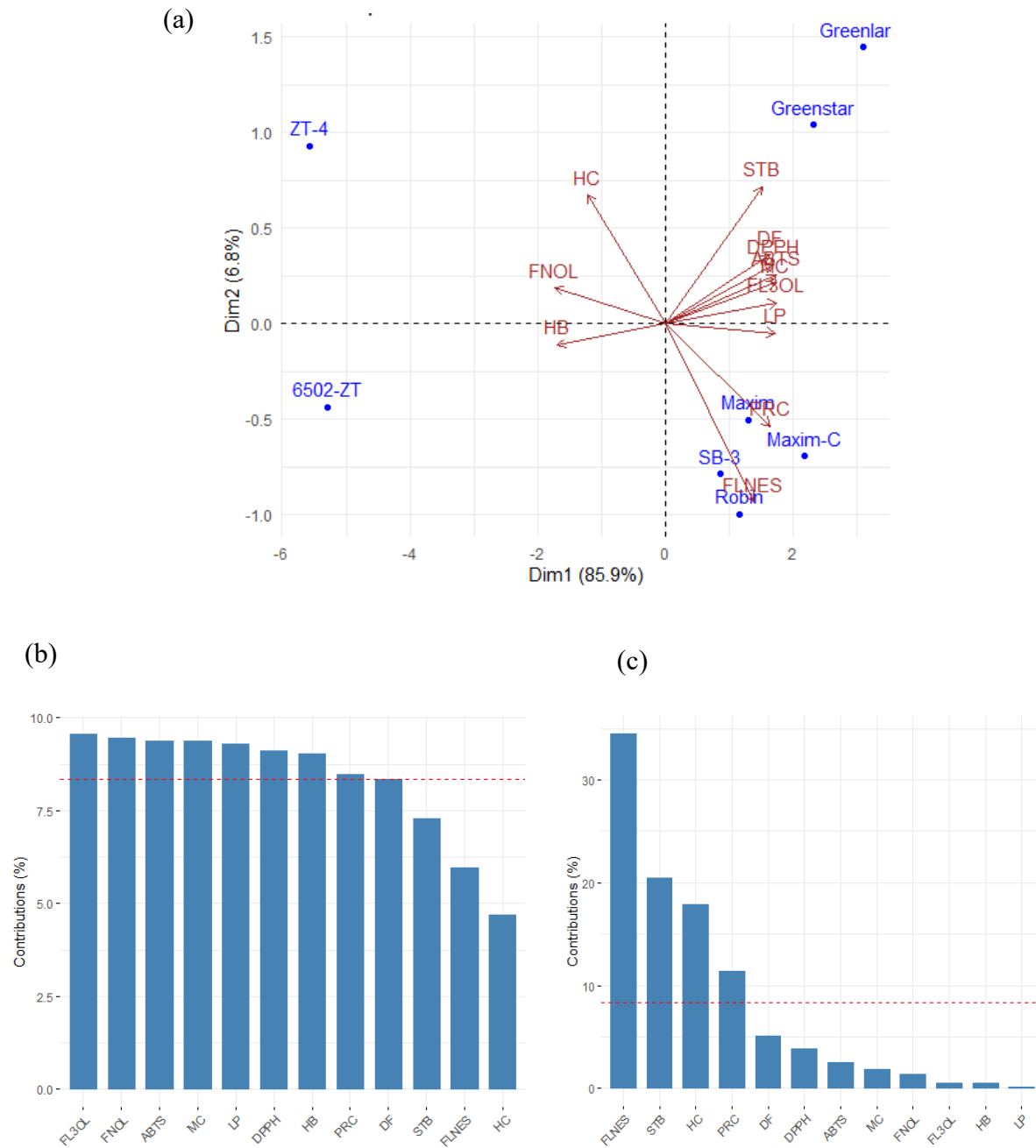
Figure 4.7 Principal component analysis for phenolic contents and antioxidant capacity
(a) biplot (b) contribution of variables to Dim 1 (c) contribution of variables to Dim 2
HC: Hydroxybenzoic acids, HB: Hydroxycinnamic acids, STB: Stilbenes, FNOL: Flavonols, FL3OL: Flavon-3-ols, DF: Dihydroflavonols, FLNES: Flavones, PRC: Procyanidins, LP: Inhibition of phospholipid peroxidation, ABTS: ABTS free radical scavenging activity, DPPH: DPPH free radical scavenging activity, MC: Fe^{+2} ion chelation activity.

and antioxidant capacity to the gray seed coat (CDC Maxim). This may indicate that there is no major difference in the water-soluble phenolics between the gray and brown seed coat. The water-soluble phenolics might play a major role in biological systems and food systems as they compose the phenolic fraction that is readily soluble in aqueous medium.

Green and red lentils are the two major market classes of lentil. The green market class which has green color seed coat are generally consumed as whole seed. The findings of this work emphasized the value of consuming whole green lentil seeds. The consumption of whole lentil rich in bioactive phenolic compounds may provide the health beneficial effect via several antioxidant mechanisms including the scavenging of free radicals and chelation of transition metals. Spanish brown (CDC SB-3) with gray seed coat with dotted pattern has a niche market, and current results add more value to possible nutritional benefits of this category as well. On the other hand, red market class (gray, brown seed coat) which is consumed as dehulled football or split lentils, generate seed coats as a byproduct. Currently, this byproduct is used as livestock feed and have limited economic value. This study demonstrated the potential of the utilization of normal tannin seed coat as a source of natural antioxidants for fortification of foods and nutraceutical applications which ultimately increase the economic value of seed coat.

4.6 Conclusions

This study demonstrated that lentil seed coat is an excellent source of phenolic compounds. As hypothesized, the phenolic composition and antioxidant activity of water extracts were affected by cultivar. Seed coat contains a mixture of several phenolic compounds belonging to different classes ranging from low molecular weight phenolic acids to high molecular weight phenolic compounds such as procyanidins. Of the 30 phenolic compounds identified in the water extracts, normal tannin seed coat consisted of mainly kaempferol tetraglycoside, catechin-3-glucoside and procyanidins whereas, kaempferol tetraglycoside was the dominant phenolic compound in zero tannin seed coats. With respect to the antioxidant activity, all cultivars except zero tannin genotypes showed significant activity. Correlation analysis showed a strong relationship ($r > 0.93$) between antioxidant capacity of seed coat and phenolic concentration. Clustering analysis classified the cultivars into three groups showing green lentils having higher phenolic concentrations and stronger antioxidant capacity than genotypes with gray and brown seed coat and lastly zero tannin seed coat cultivars had very low antioxidant capacity. It has been shown that normal tannin seed

coat water extract has the potential to be used as a source of natural antioxidants and to be used in the development of functional foods.

4.7 Connection to next study

Results of study I and II displayed that normal tannin lentil seed coat is a potential source of antioxidants and has significant antioxidant effects against the oxidation of lipids in MSC. This indicated the possibility of using lentil seed coat components as an alternative for synthetic antioxidants used in meat products, which is an important area to investigate due to the increasing consumer demand for clean label products. Commercially in meat products, different types of antioxidant compounds are used, which usually offer multifunctions. Although the first two studies demonstrated that lentil seed coat components are potential antioxidants, their efficacy to replace synthetic antioxidants in meat products is still unclear. Phosphates are one of the most common additives in most meat products and they mainly impart antioxidant and water holding properties. Thus, for the replacement of these compounds, the lentil should be able to impart both antioxidant and water holding properties. Lentil seed coat proved that it possesses antioxidant properties, and lentil flour would be a potential meat binder as it is a rich source of starch and protein. Therefore, the next study was designed to investigate the efficacy of the combination of lentil flour and seed coat to replace the phosphates in MSC bologna. Among the lentil cultivars studied in the present study, CDC Greenland showed relatively higher phenolic content and antioxidant capacity. Hence, seed coat from this cultivar was used for the next study.

5. STUDY III: EFFECTIVENESS OF LENTIL SEED COMPONENTS FOR REPLACING PHOSPHATES IN MECHANICALLY SEPARATED CHICKEN BOLOGNA

5.1 Abstract

The present study was conducted to investigate the efficacy of lentil seed components as a replacement for phosphates in non-cured sausage. Bologna-type sausages were prepared from mechanically separated chicken by incorporating combinations of infrared heated lentil flour (6%) and ground seed coat or seed coat water extracts equivalent to 300 or 500 ppm of total phenolics. These were compared with controls containing 0.3% sodium tripolyphosphate with 6% lentil flour (STPP+LF) or without lentil flour (STPP-no binder). Exclusion of STPP in the formulations resulted in a 6 to 7-fold increase in raw batter viscosity. No significant differences were found in cook loss and proximate composition except for moisture content among different formulations. Formulations containing lentil flour showed lower ($p<0.05$) purge loss compared to STPP-no binder control even though their pH was lower (6.23 to 6.27) than the STPP-no binder (6.47). The addition of lentil flour led to a firmer texture but adding ground seed coat resulted in a mushier texture. Replacement of STPP with lentil seed components did not affect the oxidative stability of the products. Samples containing lentil flour and seed coat, or seed coat water extracts had lower ($p<0.001$) lipid oxidation throughout the 56 days of storage at 4°C (TBARS lower than 0.72 mg MDA/kg) compared to STPP-no binder control (1.83 mg MDA/kg) and was similar to STPP+LF control (0.86 mg MDA/kg). A 12-member sensory evaluation panel showed that addition of ground seed coat reduced color desirability scores and increased foreign flavor notes, and after taste, however, water extracts had no effect on texture and flavor attributes. In terms of overall acceptability, all products were scored within the "liking" range of the scale except the sample with seed coat (LF+500SC). Overall, the results of this study suggested that the combination of lentil flour and seed coat water extracts are potentially effective systems to deliver the techno-functional properties of synthetic phosphates in chicken bologna without compromising texture and sensory properties of the product.

5.2 Introduction

Phosphates are salts of phosphoric acid available in different chemical forms such as orthophosphates, pyrophosphates and tripolyphosphates. Food grade phosphates are widely used as multifunctional ingredients in meat and poultry products as they contribute to processing properties and functional characteristics. Alkaline phosphates improve the water retention properties and textural properties of cooked meat products by increasing pH and ionic strength (Long et al., 2011; Sebranek, 2015; Xiong, 2012). In addition, they improve shelf-life of meat products by protecting the flavor and color. Phosphates have very strong antioxidant effects against oxidation of lipids in cooked meat products during storage primarily by binding metal ions that act as catalysts for oxidation (Kılıç et al., 2014). Conversely, there are some health concerns about the use of phosphates in foods. Some studies suggested that phosphates form insoluble salts with calcium, which leads to impaired calcium absorption and bone calcification (Calvo, 1993; Sherman and Metha, 2009; Kemi et al, 2006). Moreover, excessive dietary intake of phosphates might increase the potential risk of chronic kidney diseases (Uribarri, 2009). High dietary phosphate also has significant effects on cardiac fibrosis and arterial wall thickening, especially in patients with chronic kidney disease, resulting in enhanced cardiac risk (Amann et al., 2003; Block et al, 2004; Foley et al, 2009). Apart from these nutritional drawbacks, phosphates have a negative connotation with the natural and clean label trend since phosphates used in the meat products are chemically synthesized additives. Thus, to eliminate or reduce the use of synthetic phosphates in meat products, studies were focused on replacers of natural origin (Jarvis et al., 2012; Cho et al., 2017; Bae et al., 2017). However, researchers have found difficulty in discovering phosphate substitutes which are capable of replacing all inherent functional properties of phosphates as such as improving WHC, texture and yield of meat products as well as their stability against oxidative deterioration (Petracci et al., 2013; Bae et al., 2017).

Plant-based ingredients derived from different legumes and cereals have been found to be beneficial to improve the quality and yield of emulsion type meat products (Talukder and Sharma, 2010; Cortez-Vega et al., 2013; Kurt and Kilinccerker, 2012). Protein-rich ingredients such as soy products have been found to increase viscosity, stabilize fat and form a strong gel upon heating whereas high starch materials such as wheat flour, corn starch, and potato starch are beneficial in improving cook stability, water holding capacity and overall yields in emulsion type meat products (Verma et al., 1984; Asgar et al., 2010; Pietrasik et al., 2012;). Some of these have shown some

antioxidant function (Ulu, 2004; Singh et al., 2014) but for the most part, they may not be able to deliver this functionality when used to replace phosphates.

Meat materials such as mechanically separated chicken (MSC) are prone to oxidation, and a useful model to study the effectiveness of natural ingredients to replace phosphates in food systems. MSC is a valuable co-product of chicken meat processing, obtained by application of mechanical force (pressure and/ or shear) to chicken bones left after the bulk of meat has been manually removed. Therefore, MSC contains many more lipids which originates from bone marrow and bone tissues than hand deboned meat (Pussa et al., 2009). A significant part of the released lipids are phospholipids rich in polyunsaturated fatty acids (Spiteller et al., 2001; Pussa et al., 2009). Due to this compositional nature of the MSC and the aeration and excessive stress applied during mechanical separating process (Dawson and Gartner, 1983), MSC is considered to be highly susceptible to oxidation.

Lentil flour is rich in starch and protein and shows some antioxidant properties (Li, 2017). In addition, various phenolic compounds with potent antioxidant properties are concentrated in the lentil seed coat (Dueñas et al., et al., 2002) and could further enhance phenolic content if the seed coat was included in the flour when milling or added later. Moreover, application of extracts of seed coat would be an alternative delivery vehicle to increase the phenolic concentration. Legume products are associated with off-flavors developed through the action of oxidative enzymes (Asgar et al, 2010). Removal of off flavors has been a primary concern with the utilization of legume products; hence heat treatments are employed to eliminate oxidative enzymes. Infra-red (IR) heating is used as a pre-heat treatment in lentil processing. It was found that IR treated lentil flour has improved cooking and water holding properties and antioxidant properties (Der, 2010; Pathiratne, 2014; Shariati-levari et al., 2016; Li, 2017). Therefore, the utilization of IR treated lentil flour is suggested to be beneficial in meat applications and is now available commercially. The improved physico-chemical and functional characteristics together with the presence of phenolic compounds would make lentil a promising ingredient in emulsion type meat products to enhance textural properties and oxidative stability. Therefore, this study aimed to evaluate the applicability of lentil seed components as replacers for phosphates in MSC bologna. To achieve this, products formulated with infra-red (IR) treated lentil flour, seed coat and aqueous extracts of seed coat of lentil was compared with products formulated with synthetic phosphates.

Hypothesis:

The components of lentil seeds (flour, ground seed coat, and seed coat water extracts) will be capable of delivering water-holding properties and antioxidant properties similar to synthetic phosphates in bologna formulated with MSC without compromising their texture and sensory properties.

5.3. Materials

Fresh mechanically separated chicken meat (MSC) was purchased from a commercial processing slaughter plant (Prairie Pride Natural Foods Inc., Saskatoon, SK, Canada) and stored at -30°C until processing. Infra-red heated (IR) lentil flour was provided by InfraReady Products Ltd. (Saskatoon, SK, Canada). Certified lentil seeds (Cultivar CDC Greenland) was purchased from a commercial grower and used as the source of seed coat. The three replicate seed lots were provided from three different fields. Food grade STPP, sodium erythrobate, sodium chloride (salt) and spices were purchased from local stores. Sodium hydroxide, boric acid, sulfuric acid, diethyl ether, trichloroacetic acid, thiobarbituric acid, 1, 3, 3-tetramethoxypropane, used were of ACS grade.

5.4 Methods

5.4.1. Preparation of seed coat extracts

Lentil seeds were dehulled and seed coat was milled following essentially the same procedure as described in section 3.5. Lentil seed coat extracts were prepared from 3 replicate lots of seed coat separately. Ground lentil seed coat was mixed with water (1:10 ratio) in a plastic container and mixed on a magnetic stirrer for 15 h at room temperature. The extract was separated by centrifugation at 3000 g for 10 min at 4°C followed by vacuum filtration through Whatman No. 1 filter paper. The residue was re-extracted two more times under the same conditions. The extracts were then transferred into sterilized glass Erlenmeyer flasks and pasteurized at 72°C for 15 sec in a water bath. (Lindberg Blue M Water Bath Model WB1122A) at 75°C. The extracts were cooled to 20°C immediately after heat treatment by placing the flasks in an ice water bath. The extracts were analyzed for total phenolic content (Section 3.5) and kept at 1°C until processing (used within 2 days).

5.4.2. Formulations of bolognas

Seven different bologna formulations were prepared from mechanically separated chicken by incorporating IR lentil flour (LF), ground (250 µm particle size) lentil seed coat (SC) or water extract of lentil seed coat (WE). The seven bologna formulations include: (STPP-no binder): 0.3% STPP and no binder, (STPP+LF): 0.3% STPP and 6% IR treated lentil flour (equivalent to 128 ppm of total phenolics), (LF): 6% IR heated lentil flour, (LF+300SC): 6% IR heated lentil flour and 0.66% seed coat (equivalent to 300 ppm of total phenolics), (LF+500SC): 6% IR heated lentil flour and 1.10% seed coat (equivalent to 500 ppm of total phenolics), (LF+300WE): 6% IR heated lentil flour and 15.80% water extract of seed coat (equivalent to 300 ppm of total phenolics), (LF+500WE): 6% IR heated lentil flour and 26.34% water extract of seed coat (equivalent to 500 ppm of total phenolics). All other non-meat ingredients were kept constant in all formulations except water. Nitrite curing salt was not included in any of the formulations. The detailed formulas are shown in Table 5.1. The protein content in all formulations was adjusted to meet the Canadian regulations for minimum total protein and meat protein contents of 11% (w/w) and 9.5% (w/w), respectively in all formulations.

Table 5.1 Formulations of chicken bolognas formulated with IR treated lentil flour, seed coat and water extracts of seed coat

Ingredient (%)	Treatment						
	STPP-no binder	STPP+LF	LF	LF+300SC	LF+500SC	LF+300WE	LF+500WE
MSC	64.00	64.00	64.00	64.00	64.00	64.00	64.00
LF	-	6.00	6.00	6.00	6.00	6.00	6.00
SC	-	-		0.66	1.10	-	-
WE	-	-	-	-	-	15.80	26.34
STPP	0.30	0.30	-	-	-	-	-
Salt	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Spices	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Water	33.74	27.75	28.05	27.38	26.94	12.24	1.70

MSC: mechanically separated chicken, LF: IR treated lentil flour, SC: ground seed coat, WE: water extract of seed coat, STPP: sodium tripolyphosphate

5.4.3. Processing of bolognas

Bologna were prepared in the meat processing pilot plant located at the University of Saskatchewan using commercial processing conditions. The temperature of the pilot plant was maintained around 5°C during processing. Three replicate batches of bologna were prepared separately and within each replication, different treatments (7 kg batch per each treatment) were processed in random order. Meat (MSC) thawed at 1°C for 24 to 48 h was passed through a meat grinder (3.9 mm end plate, Biro Grinder, model AMFG-24, Marblehead, OH, U.S.A.). The detailed processing procedure is illustrated in Figure 5.1. The temperature of the meat batter was about 5, 7 and 10°C after chopping, milling and vacuum tumbling, respectively. The stuffed bologna was held overnight at 1°C followed by cooking in air-agitated water in a retort (DIXIE Canner Co. Athens, Georgia, USA). Bologna chubs were immersed in water preheated to 50°C and the temperature of the water was increased in three steps to 60, 70 and 80°C. Water temperature was held constant by controlling the steam supply and water agitation until the product temperature reaches the water temperature at each step. A thermocouple connected to a data logger (Barnant Scanning Thermocouple Thermometer, Burrington, III) was inserted at the geometric center of a bologna to monitor the internal temperature of the bologna. The cooking was stopped when the product end point temperature of 78°C was reached. Bolognas were immediately cooled to 4°C by transferring them into an ice-water bath. Bologna treatments were stored at 1°C until slicing.
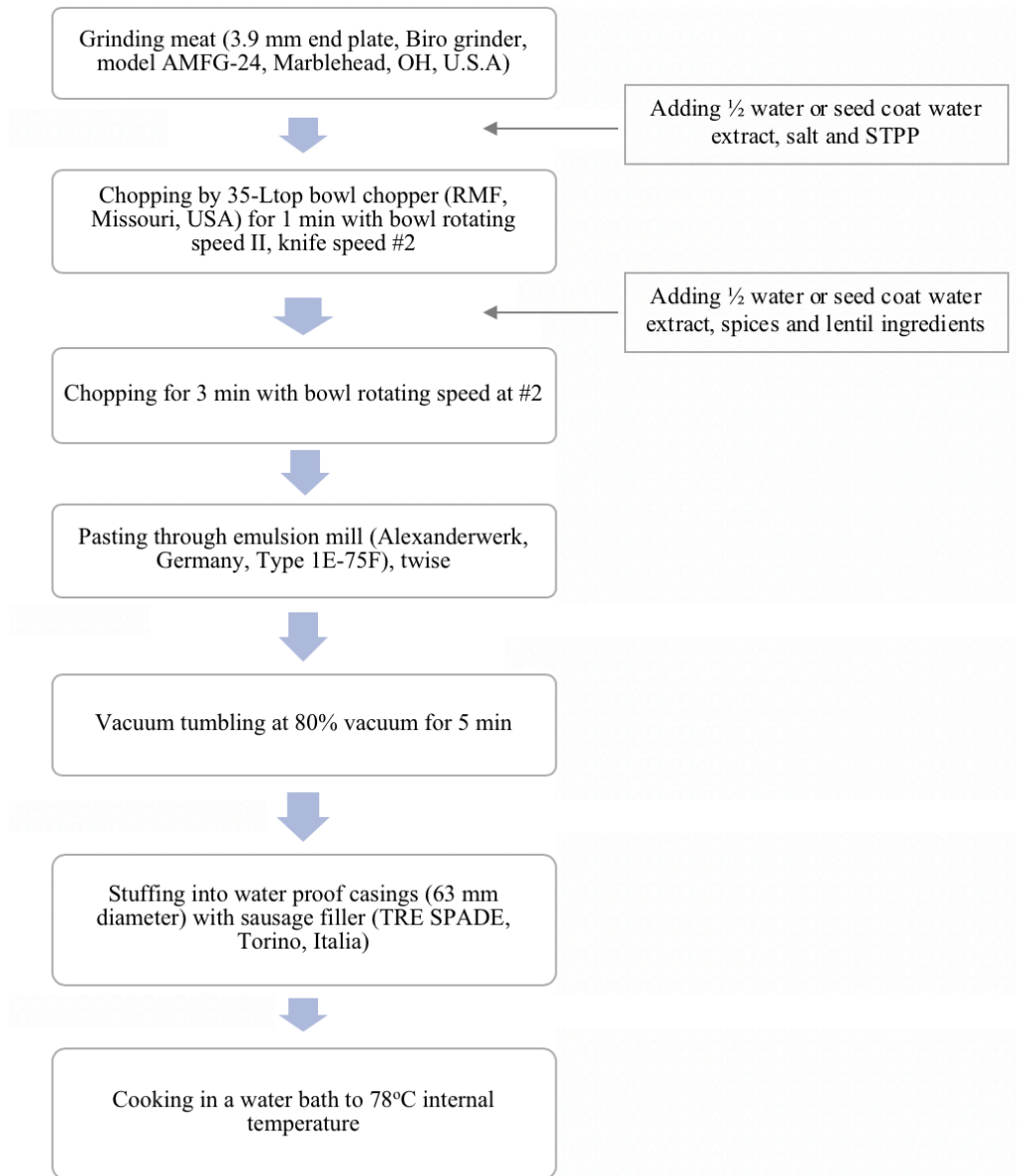
Figure 5.1 Processing flow chart of chicken bologna products with lentil components

5.4.4. Slicing and storage of bolognas for analysis

Three chubs of bologna from each treatment was sliced into different thicknesses as shown in the diagram below (Fig 5.2) for the analysis of chemical and textural properties. Samples of bologna was vacuum packed in high water vapor and oxygen barrier polyethylene pouches (O_2 permeability < 25 cm^3 /m^2/24h) and stored in the dark at 4°C. One chub of bologna was left unopened and held at 1°C for sensory evaluation.
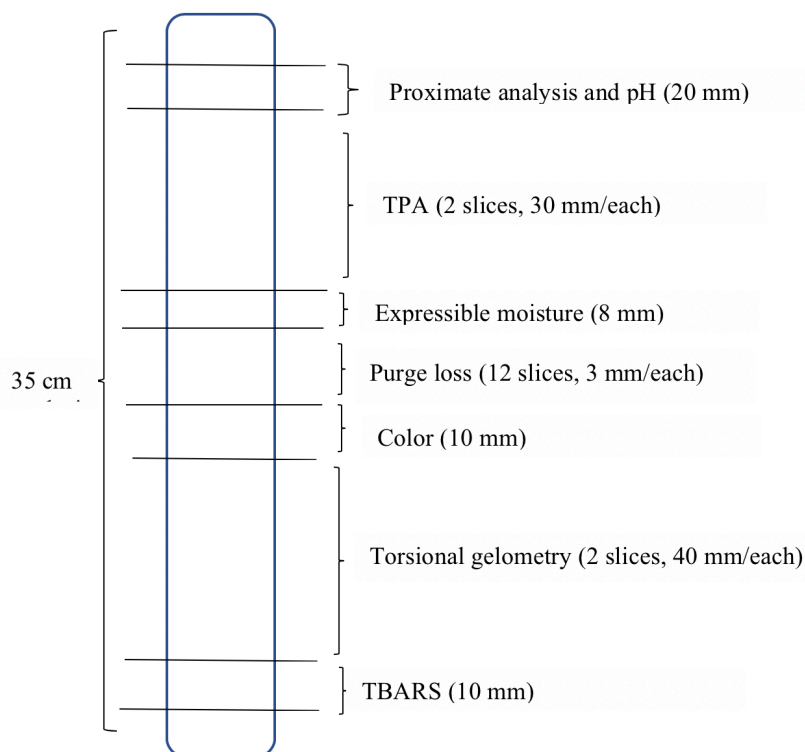
Figure 5.2 Portions of cooked bologna sliced for measuring various properties

Labels in figure:
- Proximate analysis and pH (20 mm)
- TPA (2 slices, 30 mm/each)
- Expressible moisture (8 mm)
- Purge loss (12 slices, 3 mm/each)
- Color (10 mm)
- Torsional gelometry (2 slices, 40 mm/each)
- TBARS (10 mm)
- 35 cm

5.4.5. Determination of batter viscosity

Viscosity of raw batter was measured in triplicate as described by Shand (2000). Briefly, the viscosity of raw batter was measured using a programmable rheometer (Brookfield Engineering Laboratories Inc. Middleboro, MA, USA, Model DV-III+). The No 7 spindle was set into the center of the batter-filled 250 mL cup and the viscosity was recorded after rotating the spindle at 10 rpm for 30 sec. The viscosity of the batter was reported in centipoises together with the batter temperature.

5.4.6. Determination of cook loss

The cook loss of bologna was estimated based on the differences between the weights of the raw products and cooked products. The weight of each bologna chub was recorded soon after stuffing. After bologna was cooked and chilled for 24 h, three chubs per treatment were opened, blotted gently with a paper towel and reweighed. The cook yield was calculated as follows:

$$\text{Cook loss (\%)} = \frac{\text{Initial weight of sausages (g)} - \text{Cooked weight of sausages(g)}}{\text{Initial weight of sausages (g)}} \times 100 \qquad (5.1)$$

5.4.7. Determination of purge loss

The purge accumulation was measured for cooked sliced product on duplicate samples of each treatment per replication. Six slices (3 mm thick) of bologna was packed as two stacks in a pre-weighed polyethylene bag and vacuum packaged (95% vacuum, Model 550A, Sipromac, Quebec, Canada,). After packaging, the bags were stored in the dark at 4°C for 14 days. The purge loss was determined by reweighing the blotted slices from two packages after each storage interval and was expressed as a percentage of the initial sample weight.

$$\text{Purge loss (\%)} = \frac{\text{Initial sample weight} - \text{Saple weight after storage}}{\text{Initial sample weight}} \; X \; 100 \qquad (5.2)$$

5.4.8. Determination of expressible moisture

The expressible moisture was measured following the method described by Shand (2000). Within each treatment formulation, three core samples (1.5 ± 0.3g) were placed in the thimble shaped filter papers (Whatman # 3 and # 50 in a 50 mL Falcon plastic tube). After centrifugation for 15 min at 750 g at 4°C (Beckman, J2-HC centrifuge with Beckman Coulter JA-17 rotor, MN, U.S.A.), the weight of the core samples was measured. Results were expressed as the percentage of ratio of moisture released from the sample to the initial core sample weight.

$$\text{Extressible moisture (\%)} = \frac{\text{Initial sample weight} - \text{Saple weight after sentrifugation}}{\text{Initial sample weight}} \; X \; 100 \qquad (5.3)$$

5.4.9. Determination of proximate composition and pH

For each product, moisture, protein, fat and ash content were measured according to the methods of AOAC 950.46, 981.10, 960.39a and 920.153, respectively (AOAC, 1990). Samples were prepared for analysis by blending samples of bologna (100 g) for 30 sec in a food processor (Cuisinart® Mini-Prep® Plus Processor). Duplicate samples of each treatment per replication were analyzed.

For the determination of pH, a sample (20 g) in a filter bag (Whirl-Pak® Filter Bags, Sterile, Nasco®) was homogenized in 80 ml of deionized water for 3 min using a stomacher lab blender (Stomacher® 400 Lab Blender Series, Seward, US). Then pH of the filtrate was measured using a pH meter.

5.4.10. Determination of CIE color

The color of sliced (1 cm) cooked bologna was measured using a Hunter Lab MiniScan XETM (Hunter Associates Laboratories Inc., Reston, VA) based on CIE L*, a*, and b* values with illuminant A and 10° standard observer. All the samples were done in duplicate.

5.4.11. Texture profile analysis

The texture profile analysis was carried out using TMS-Pro Texture Press (Food Technology Corp., Sterling, VA, U.S.A.) interfaced with a computer, using the software supplied by Texture Technologies Corp. (Texture Lab Pro, version 1.13-002). Four core samples (25 mm height and 35 diameter) were removed from each treatment and compressed twice to 50% of their original height at a constant speed of 100 mm/min. The TPA parameters were recorded and expressed in terms of hardness (N), chewiness (N*mm), springiness (mm), fracturability (N) and cohesiveness.

5.4.12 Torsional gelometry

Torsion analysis was carried out according to the method described by Kim et al. (1986) with modifications. Briefly, bologna was cut into 30 mm thick samples and core samples were prepared using a 20 mm cork borer (#12). Plastic disks (1 mm thick) designed to fit the torsion apparatus were glued onto each end of the prepared core samples with cyanoacrylate glue (Loctite® 404, Instant adhesive, Loctite Corporation, Newington, CT, U.S.A.). The cored samples were then milled into dumbbell shape with minimum of 10 mm at the mid-section using a bench-top grinder (KCI-24A2, Bodline Electric Company, Chicago, IL, U.S.A.). The dumbbell shaped sample were mounted on torsion fixture in the torsion device (Brookfield digital viscometer model DV-I, Gel Consultants Inc., Raleigh, NC, U.S.A.) and twisted at 2.5 rpm until structure failure occurs. Shear stress (torsion rigidity) and strain at failure (deformation) were recorded and texture of the samples were described in terms of brittle, mushy, tough and rubbery.

132

5.4.13 Assessment of lipid oxidation

Thiobarbituric acid reactive substances (TBARS) was assayed according to the method of Bedinghaus and Ockerman (1995). Briefly, a sample of 2.5 g was placed in a sampling filter bag (Whirl-Pak® Filter Bags, Sterile, Nasco®). Sample was homogenized with 25 mL of trichloro acetic acid (TCA) solution (20% TCA containing 1.6% phosphoric acid) and 25 mL of cold deionized water for 3 min using a stomacher blender (Stomacher® 400 Lab Blender Series, Seward, US). The mixture was filtered through Whatman No. 1 filter paper. The volume was brought up with TCA solution and deionized water (1:1). Five mL of filtrate was transferred into centrifuging tubes and 5 mL of 0.02 mol/L of thiobarbituric acid was added. The tubes were placed in a boiling water bath for 35 min and then were cooled for 10 min in ice water. The absorbance of the filtrate was measured at 532 nm against a blank. A standard curve prepared from a series of solutions of 1, 1, 3, 3-tetramethoxypropane with different concentrations, was used for the calculation of TBARS values (mg malondialdehyde equivalents/kg meat).

5.4.14 Sensory evaluation

The cooked bologna was evaluated after 2 weeks of storage at +1°C. Sensory evaluation was conducted in the sensory evaluation facility located at the University of Saskatchewan which is equipped with individual booths. The panels consisted of 12 assessors who had prior experience in the assessment of bologna products. Panel training was performed in two sessions (1 h per session) over a period of two days prior to the analysis. The panel was trained in the description and intensities of color, flavor and texture attributes, evaluation procedure and use of the scale. Prior to serving, bologna was diced into cubes (10 mm x 10 mm x 10 mm) and these were placed in 15 mL white color plastic cups marked with 3-digit sample numbers and then covered by a lid. The prepared samples were kept in a refrigerator at 4°C until serving. Each assessor was served 5 pieces from each sample of bologna. Samples from all treatments were evaluated in one session in order to reduce the session-to-session variation and three replicates of each treatment was analyzed on separate days. The serving order was randomized according to sample, replicate and assessor. Water and unsalted crackers were served for cleansing the palate between samples and samples were evaluated under white fluorescent light (~ 400 lux). A total of 15 attributes were evaluated using eight-point category scales.

This study was accepted on ethical grounds by the University of Saskatchewan Behavioral Research Ethics Board (BEH#18-14).

5.4.15 Data analysis

All experiments were performed in three replicates. Statistical analysis was conducted using SAS version 9.4 (SAS Inst. Inc., Cary, N.C., USA). Analysis of variance (ANOVA) followed by multiple comparisons using Tukey's test was performed to compare the differences between treatments. Significant difference was defined at $p < 0.05$. Associations among physicochemical and sensory attributes were assessed by Pearson's correlation analysis. Principal component analysis and hierarchical cluster analysis were performed using FactoMineR in R statistical system.

5.5. Results and Discussion

5.5.1. Raw batter viscosity

The viscosity of raw batter of all formulations was measured under consistent temperature conditions ($4.3 - 4.8\,^{\circ}C$) and mean values are given in Table 5.2. The batter viscosity was primarily affected by the ingredients added. Addition of STPP resulted in significant lower values ($p < 0.001$) for viscosity than that of other formulations which were prepared without adding STPP. The relatively higher water content in STPP- no binder formulation could be one reason for the lower viscosity observed in that sample. In the absence of any plant-based extender, Claus et al., (1990), Sanjeewa (2008) and Xu (2017) also noted a reduction in batter viscosity in bologna formulations with more water. Although the STPP-LF control had a nearly comparable water content in its formulation relative to the other formulations extended with LF without added STPP, it showed reduced batter viscosity ($p < 0.001$) indicating that STPP could play a significant role in batter viscosity. When phosphates are added to meat products, they dissociate the acto-myosin complex formed by sequestration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations during rigor mortis and, as a result, the meat becomes more tender (Aberle et al., 2005; Petracci et al., 2013). This might explain the reduced viscosity of the batters with added STPP. Except for the STPP+LF sample, the inclusion of LF in bologna formulations led to a 6 to 7-fold increase in the viscosity of the raw batter. The increased viscosity of meat batter to a certain extent offers easy handling of the batter

Table 5.2 Effect of STPP and lentil seed components on raw batter viscosity of chicken bologna (n = 3)

Treatment	Viscosity (cP) x 10^4	Temperature (°C)[NS]
STPP-no binder	2.64^a	4.3
STPP+LF	5.15^a	4.8
LF	14.82^b	4.8
LF+300SC	15.88^b	4.6
LF+500SC	16.31^b	4.8
LF+300WE	17.70^b	4.6
LF+500WE	15.51^b	4.3
SEM[1]	1.112	0.02

[1]Standard error of mean
[a,b]Means with the different letters in the same column are significantly different (P<0.05)
[NS] Means within the same column are not significantly different (p>0.05)
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

in some processing procedures such as stuffing (Shand, 2000). The inclusion of seed coat or water extract had no significant effect on batter viscosity.

5.5.2. Water holding capacity of cooked bologna

One of the major functions of phosphates in meat products is increasing the water holding capacity. Cook loss, purge loss and expressible moisture content were measured as indicators of water holding capacity of different formulations and the results are shown in Table 5.3. In general, none of the experimental treatments showed significant difference in cook loss. The cook loss of the samples with STPP alone tended to be lower compared to the samples prepared without STPP, however, the difference was not large enough to make a significant difference (P=0.04). Insignificant differences observed among the treatments for cook loss might be due to the use of water-proof casings.

Table 5.3 Effect of STPP and lentil seed components on water holding properties of chicken bologna (n = 3)

Treatment	Cooking loss (%)[NS]	Purge loss (%)	Expressible moisture (%)
STPP-no binder	0.79	9.45[a]	20.4[a]
STPP+LF	1.09	4.87[c]	16.6[c]
LF	1.79	5.86[b]	16.7[bc]
LF+300SC	1.43	5.58[bc]	18.0[abc]
LF+500SC	1.32	5.44[bc]	20.0[ab]
LF+300WE	1.70	5.70[bc]	17.1[bc]
LF+500WE	1.63	5.71[bc]	17.6[abc]
SEM[1]	0.208	0.186	2.77

[1]Standard error of mean
[a-c]Means with the different letters in the same column are significantly different (P<0.05)
[NS]: means within the same column are not significantly different (p>0.05)
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

Purge loss, the percentage weight loss of the sample after 14 days of storage to the sample's initial weight, was found be significantly different among formulations. Purge loss of the STPP-no binder control was significantly higher (p<0.001) than that of all other treatments. The significantly higher purge loss in STPP-no binder control (9.45%) compared to the STPP+LF treatment (4.87%) showed that STPP alone was not able to retain water in cooked chicken bologna during the storage as effectively as the combination of STPP and LF. However, purge loss observed for the sample added with only LF (5.86%) was significantly higher (p<0.05) than that of STPP+LF treatment. The basis for improvement in water holding capacity in phosphate treated meat products includes increased ionic strength, pH and cleavage of actomyosin cross bridges allowing more water to retain in the meat matrix (Aberle et al., 2012). Incorporation of lentil flour together with seed coat components (seed coat or water extract) resulted in similar purge loss to the STPP+LF and the addition level (300 ppm or 500 ppm TPC equivalents) had no significant effect.

The fluid released from the application of centrifugal force as expressible moisture is considered the free or loosely bound water (Resconi et al., 2015). Expressible moisture values for

cooked bologna ranged from 16.56% to 20.35%. The highest expressible moisture content was observed for STPP-no binder control, LF+300SC, LF+500SC and LF+500WE treatments; however, most LF treatments did not differ from STPP+LF. In general, the addition of lentil seed components in chicken bologna had similar effects on water holding capacity as the addition of synthetic phosphates. The increased water holding capacity observed in LF treatments could be associated with the water-binding ability of proteins and starch of LF. Protein and starch are biological macromolecules that can imbibe water and form gel matrices when heated. They could form a complex 3D gel network in the presence of meat protein involving various forces such as van der Waals, electrostatic and hydrogen bonding, which traps fine particles of emulsified meat or the meat matrix simply traps the starch and non-meat proteins as fillers (Sanjeewa et al., 2010). The flour components have high affinity for water and help retain water in meat products during cooking and storage (Aberle et al., 2012). In agreement, several researchers reported that the water holding capacity of comminuted meat products increased with the addition of legume flours (Dzudie et al., 2002; Modi et al., 2003; Serdaroglu et al., 2005; Sanjeewa, 2008).

5.5.3. Proximate composition and pH of cooked bologna

The mean values for proximate composition of cooked bologna are given in Table 5.4. The substitution of water with equal amount of flour resulted in a significant reduction of moisture content in flour added bologna. Thus, STPP-no binder control had significantly higher moisture content compared to all flour added samples and significant differences were not found among the formulation with flour. Furthermore, no significant differences were found for fat and ash content among all samples. The content of fat and ash ranged from 6.98% to 7.55% and 2.40% to 2.75%, respectively. The protein content of the bologna ranged between 11.02% and 11.66% and all treatments complied with the Canadian regulations for protein content (9.5% meat protein and 11% total protein). The flour-added bologna tended ($p<0.10$) to have high protein content compared to STPP-no binder control. This slightly higher protein content could be due to the protein contributed from the lentil flour. The IR treated lentil flour contained approximately 25% protein and 43% starch. It was found that protein content increased with extending the comminuted meat products with cowpea four (Prinyawiwaktkul et al., 1997), soy, black gram and green gram flour (Modi et al., 2003) and black eye bean, chickpea and lentil flour (Serdaroglu et al., 2005). However, the incorporation of bean flour in beef sausages decreased the protein content due to the replacement

of part of meat with flours and this increased the carbohydrate content of the product (Dzudie et al., 2002). Overall, no differences (p>0.05) have been found for any analyzed component due to the removal of phosphates from the bologna formulations. Thus, the replacement of synthetic phosphates (STPP) by lentil seed components, in the concentrations and conditions described, has no effect on the composition of the chicken bologna.

The effects of treatment and storage on pH values of bologna are shown in Table 5.4. As expected, the addition of STPP resulted in an increase in pH (p<0.001) with pH values of 6.47 and 6.44 for STPP-no binder and STPP+LF control samples, respectively at 0 weeks. The pH is an important parameter in meat products as it influences factors such as color and ability to retain water. When using alkaline phosphates, an increase in pH and the associated increase in net negative charges on meat protein results in more charged groups available for water binding (Aberle et al., 2012). The pH of bologna formulated without STPP ranged between 6.23 to 6.27, with a slight variation among the formulations. During the storage, pH value of STPP-no binder control increased significantly (p<0.001) but the overall change was small (0.04 units).

Table 5.4 Proximate composition and pH of cooked chicken bologna (n = 3)

Treatment	Moisture (%)	Fat[NS] (%)	Protein[NS] (%)	Ash[NS] (%)	pH[2] 0 weeks	15 weeks
STPP-no binder	78.89[b]	6.98	11.02	2.40	6.47[b]	6.51[a]
STPP+LF	74.35[a]	7.13	11.35	2.75	6.42[c]	6.44[bc]
LF	74.32[a]	7.12	11.43	2.56	6.26[def]	6.26[def]
LF+300SC	73.89[a]	7.06	11.66	2.56	6.23[f]	6.25[def]
LF+500SC	73.60[a]	7.29	11.43	2.59	6.24[ef]	6.25[def]
LF+300WE	74.21[a]	7.48	11.60	2.54	6.27[de]	6.28[d]
LF+500WE	73.99[a]	7.55	11.37	2.47	6.25[def]	6.27[de]
SEM[1]	0.397	0.101	0.160	0.087	0.006	

[1]Standard error of mean
[a,f]Means with the different letters in the same column are significantly different (P<0.05)
[2] Means with the different letters among pH values are significantly different (P<0.05)
[NS]: means within the same column are not significantly different (p>0.05)
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

Such an increase in pH might be attributed to the accumulation of microbial metabolites and deamination of protein (Goddard et al., 1996). However, significant changes in pH were not observed for bologna formulations with lentil seed components after 15 weeks of refrigerated storage. This might be due to the antimicrobial properties of the lentil seed components. The bologna formulated with lentil seed components had the phenolics from flour and seed coat. The effectiveness of phenolic compounds as antimicrobial agents in foods has been documented (Rauha et al., 2000; Puupponen-Pimiä et al., 2001; Heinonen, 2007; Estevinho et al., 2008; Lee and Lee 2010). Further study of the effects of the phenolics on microflora may be warranted.

5.5.4 CIE color of cooked bologna

Color is an important factor for consumer acceptance of meat and its products. The differences in the color of cooked bologna were determined by the instrumentally measured color parameters L*, a* and b* (Table 5.5). All formulations of bologna were prepared without adding sodium nitrite or other curing agents and myoglobin and hemoglobin contents were similar among the formulations as same amount of meat was used in all formulations. Therefore, the color differences between the bologna was mainly influenced by the combined effects of thermally denatured myoglobin and hemoglobin blended with the lentil seed components added and the amount of water in the formulations. As seen in Table 5.5, all color values were affected by the addition of seed coat components. The data indicated that samples treated with seed components had lower L* values indicating that those samples were darker than the STPP-no binder control. The STPP-no binder sample displayed the highest lightness values of 68.44 whereas the lightness value was similar across all treatments with seed components. This darker appearance was likely due to the color imparted by lentil seed components. It may have also been influenced by a reduction in the overall light scattering properties associated with relatively lower amount of water in the products. Similar results have been reported by Sanjeewa (2008) for low-fat chicken bologna extended with chickpea (variety Desi) flour.

In terms of a* value (redness), a similar trend was observed showing that bologna formulated without binders appeared redder than flour added bologna. STPP-no binder bologna showed the highest a* value (13.93) which was significantly higher (p<0.001) than the other treatments except STPP+LF sample. Binders may mask the color of myoglobin resulting a less red

Table 5.5 Effect of STPP and lentil seed components on CIE color of cooked chicken bologna (n = 3)

Treatment	L	a*	b*
STPP-no binder	68.44[a]	13.93[a]	18.05[a]
STPP+LF	65.01[b]	8.83[ab]	15.83[ab]
LF	63.18[b]	7.96[b]	14.94[b]
LF+300SC	63.31[b]	8.83[b]	17.18[ab]
LF+500SC	63.14[b]	8.46[b]	17.08[ab]
LF+300WE	64.64[b]	8.40[b]	16.53[ab]
LF+500WE	64.96[b]	9.08[b]	16.58[ab]
SEM[1]	0.694	1.171	0.769

[1]Standard error of mean
[a,b]Means with the different letters in the same column are significantly different (P<0.05)
[NS]: means within the same column are not significantly different (p>0.05)
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

color product. Similarly, Prinyawiwatkul et al. (1997) observed that addition of chickpea flour in chicken nuggets results in reduction of a* value compared to no binder controls.

Different formulations had only minor effects on b* value. STPP-no binder showed the highest b* value (18.05) while the LF treatment had the lowest b* value (14.94) among treatments. Overall, when comparing the CIE color values between the treatments with and without STPP, the substitution of phosphates appeared to have no significant effect on the color of chicken bologna. However, there was a color change with lentil flour addition which may need to be masked with spices if found to be objectionable by consumers. In the present study bologna products were non-cured. The application of LF in cured meat systems may not have a significant effect on color.

5.5.5. Texture profile analysis (TPA) of cooked bologna

The texture is one of the most significant sensory attributes in emulsified meat products such as bologna. It is directly related to the fat and water holding capacity of the meat matrix, which is influenced by ionic strength and protein functionality (Horita et al., 2014). Thus, the removal of phosphates could have an effect on texture properties as a result of decreased ionic strength or by altering the charge density thereby reducing the ability to hold water and contribute to gel strength. Parameters from TPA of bologna are presented in Table 5.6. Addition of lentil flour resulted in higher TPA hardness values than those of the STPP-no binder control samples. The softer texture in the no-binder control might be due to the higher moisture content in that formulation. The hardness of the LF and STPP+LF formulation did not differ showing that removal of STPP from the bologna extended with lentil flour did not make a significant effect on their hardness. Similarly, for the attributes; cohesiveness, springiness, and chewiness the sample containing lentil flour lower values (p<0.001) than the STPP-no binder control but did not differ from the STPP+LF formulation.

Besides, removing STPP from flour added bologna did not have a significant impact on their TPA cohesiveness. The addition of water extracts of seed coat did not affect the texture of bologna. However, the bologna incorporated with seed coat showed the lowest values for TPA cohesiveness among the flour added bologna samples and increasing the level of seed coat from 0.66% (LF+300SC) to 1.1% (LF+500SC) resulted in a significant decrease in cohesiveness. The springiness of the bologna decreased significantly at the seed coat level of 1.1% whereas it was comparable with the STPP-no binder control and other flour added bologna. The incorporation of seed coat in the formulations significantly reduced (p<0.005) the chewiness of bologna and was similar to the chewiness of STPP-no binder control. These results revealed that the seed coat imparts a softer and musher texture to the product which could be attributed to the incorporation of fiber associated with the seed coat. Based on the proximate composition of the lentil seed coat, it contains around 60% of total carbohydrates; which could mainly be comprised of fiber. Xu (2017) observed similar effect on the texture of pork bologna added with pea fiber fractions at 2.5% level. Gradual decrease in hardness of pork sausages with the increasing level of oatmeal was reported by Yang et al. (2007). Conversely, the addition of inulin in mortadella, a Spanish cooked meat product, resulted in increased hardness as compared to control products (Garcia et al., 2006).

Table 5.6 Effect of STPP and lentil seed components on TPA texture of cooked chicken bologna (n = 3)

Treatment	Hardness (N)	Cohesiveness	Springiness (%)	Chewiness (Nmm)
STPP-no binder	55.43[b]	0.55[a]	82.84[a]	322.49[d]
STPP+LF	88.20[a]	0.51[b]	81.92[a]	503.60[a]
LF	91.63[a]	0.51[b]	82.50[a]	513.12[a]
LF+300SC	81.40[a]	0.49[bc]	80.65[ab]	422.68[bc]
LF+500SC	80.40[a]	0.45[d]	77.41[b]	380.59[cd]
LF+300WE	86.43[a]	0.52[b]	81.64[a]	480.07[ab]
LF+500WE	88.63[a]	0.52[b]	81.73[a]	493.21[a]
SEM[1]	3.002	0.008	0.890	18.421

[1]Standard error of mean
[a,b]Means with the different letters in the same column are significantly different (P<0.05)
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

Similar findings were also observed by Viuda-Martos et al. (2010) in mortadella with orange dietary fiber.

5.5.6. Torsional gelometry of cooked bologna

The shear stress and strain of bologna with different lentil components and with or without STPP observed in the torsional gelometry analysis are shown in Table 5.7. Torsional gelometry (stress and strain at failure) testing is a fundamental method that can be used to evaluate the textural properties of finely comminuted meat products. Based on the results of torsion analysis, the meat product can be described by the four sensory descriptors; brittle, mushy, tough and rubbery. In general, removal of phosphates from the formulations of bologna did not have a significant effect on the shear stress and strain at failure. However, the different lentil seed components in the formulations showed significant effects (p<0.001) on the shear stress and strain of bologna. Except the bologna with seed coat (LF+300SC and LF+500SC) lentil flour-containing bologna showed higher (p<0.001) shear stress values than that of the STPP-no binder control.

Table 5.7 Effect of STPP and lentil seed components on torsional texture of cooked chicken bologna (n = 3)

Treatment	Stress (kPa)	Strain	Rigidity (kPa)
STPP-no binder	20.37^c	1.96^{ab}	10.56^d
STPP+LF	26.31^b	1.88^{bc}	14.03^b
LF	25.81^b	1.97^a	13.15^{bc}
LF+300SC	18.68^{cd}	1.53^d	12.39^c
LF+500SC	17.47^d	1.40^e	12.51^c
LF+300WE	28.88^a	1.86^c	15.59^a
LF+500WE	26.42^b	1.92^{abc}	13.76^{bc}
SEM[1]	2.530	0.022	1.430

Values are the means of ten measurements of three replicates
[1]Standard error of mean
[a-d]Means with the different letters in the same column are significantly different (P<0.05)
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

Among the flour added bologna, the highest shear stress was observed for the bologna LF+300WE while no significant differences were noted between STPP+LF, LF and LF+500WE. The lack of a significant difference in the shear stress between STPP+LF and LF treatments revealed that phosphates had no significant effect on the shear stress of bologna. Seed coat addition resulted in a reduction (p<0.001) in shear stress in comparison to all other treatments.

The shear strain, which represents the elasticity behavior of gelled meat products, was found to be significantly higher in STPP-no binder control compared to other samples except LF (6% flour) and LF+500WE which showed similar shear stress. Similar to the shear stress the addition of seed coat to bologna resulted in lower (p<0.001) shear strain than all other samples. The bologna with lentil flour and STPP (STPP+LF) showed lower elasticity (p<0.05) than the bologna with only lentil flour (LF). Significant differences in the shear stress and strain between samples resulted in significant differences (p<0.001) among the rigidity values for bologna. The lowest rigidity was reported for the STPP-no binder control whereas LF+500WE had the highest rigidity. The rigidity of the bologna with seed coat (LF+300SC and LF+500SC) was lower than that of the STPP+LF bologna and was similar to other flour containing bologna.

Figure 5.3 shows the texture map of the bologna. As can be seen, the rubbery texture of the STPP-no binder control was relatively higher compared to the other samples. This observation is in agreement with the results of Sanjeewa (2008). He studied the effect of the inclusion of wheat and chick pea flour in pork bologna and observed that the bologna with no binders were rubberier
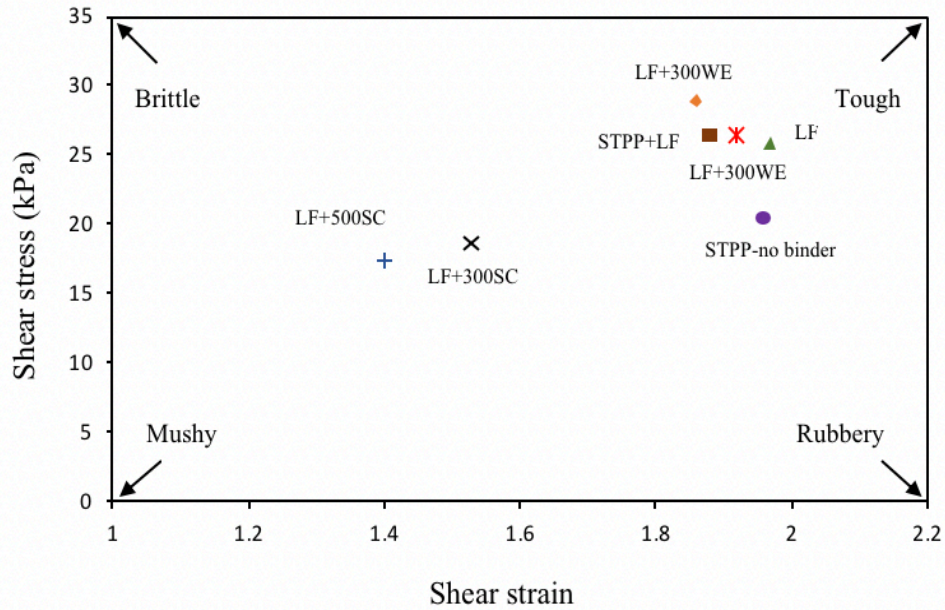
Figure 5.3 Torsion texture map of bologna containing different lentil seed components
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

than the bologna with added flour. In the present study, lentil flour made the bologna tougher than the STPP-no binder control and was signified by higher shear stress and strain values. This suggests the formation of strong gel structure. The STPP together with lentil flour (STPP+LF) reduced the tougher texture compared to adding lentil flour alone. However, the elasticity or the rubbery nature of LF (lentil flour only) was higher than that which contains the combination of STPP and lentil flour. The texture of bologna moved towards mushy with the addition of seed coat and this could possibly due to the decrease in shear strain. This softer texture in seed coat added bologna might be linked to the fiber fraction of the seed coat. In agreement, Xu (2017) observed that the inclusion of pea fiber makes pork bologna softer and less cohesive than the control which was produced

without any binders. Similarly, Ktari et al. (2014) observed a reduction in these properties when dietary fiber products (beta-glucan, cellulose and potato fiber) were added in beef sausage.

Cooking of meat emulsions causes structural changes in the muscle protein, which favor intermolecular protein interactions. This transformation from raw batter to a gel are associated with change in viscoelastic properties, and an increase in rigidity can be viewed as the development of gel matrix structure (Foegeding, 1998). It was reported that the incorporation of polysaccharides such as starch, carrageenan, and xanthan gums in muscle protein gels may decrease the stress and strain at failure, and the heated mixture becomes more paste-like than gelled (Foegeding and Ramsey, 1987). This behavior is ascribed to the disruption of the muscle protein gelation due to the competition for available protein, which depends on the type and concentrations of the added nonmeat ingredients (Foegeding, 1998). Components of lentil flour did not reduce stress, may compliment meat protein better than some other binders.

5.5.7. Correlations between water holding properties, instrumental texture properties and proximate composition

Pearson correlation analysis was performed to analyse whether linear relationships exist between water holding and instrumental texture properties and proximate composition of chicken bologna. Table 5.8 shows the correlation coefficients for those properties which showed any significant correlations. The cook loss showed moderate negative correlation with the moisture content ($r = -0.60$), as the cook loss leads to lower product moisture content and vice versa. Cook loss also positively correlated with instrument hardness ($r = 0.66$) indicating that the products that can retain more moisture would have harder /firmer texture. Purge loss had moderate negative correlation ($r = -0.59$) with ash content. This may be due to the enhanced water holding properties due to the addition of phosphates (STPP) as samples with STPP had higher ash content (Table 5.4). Negative associations were also noted between purge loss and hardness ($r = -0.81$) and chewiness ($r = -0.67$). For proximate composition protein content showed moderate correlation with purge loss ($r = -0.44$), showing their positive contribution to retain water within the product matrix. There were some significant associations among texture attributes. Positive correlations existed between springiness and cohesiveness ($r = 0.86$), chewiness and hardness ($r = 0.91$) as these texture properties are generally determined by the same factors such as strength of protein matrix and moisture content.

Table 5.8 Correlation coefficients (r) among water holding and texture properties and proximate composition (n=21)

	1	2	3	4	5	6	7	8	9	10	11
1. Cook loss	1										
2. Purge loss	-0.40										
3. Expressible moisture	-0.21	0.34									
4. Ash	0.11	-0.59**	0.02								
5. Protein	0.42	-0.44*	-0.24	0.01							
6. Moisture	-0.60**	0.82***	0.21	-0.38	-0.42						
7. Hardness	0.66**	-0.81***	-0.46*	0.30	0.54*	-0.80***					
8. Cohesiveness	-0.18	0.47*	-0.29	-0.23	-0.44*	0.57**	-0.29				
9. Springiness	-0.06	0.22	-0.49*	-0.25	-0.22	0.32	-0.00	0.86***			
10. Chewiness	0.58**	-0.67***	-0.56**	0.21	0.36	-0.57**	0.91***	0.12	0.37		
11. Shear stress	0.28	-0.24	-0.62	-0.01	-0.06	-0.26	0.48*	0.53*	0.60**	0.71***	
12. Shear strain	0.06	0.21	-0.12	-0.01	-0.31	0.35	0.00	0.82***	0.69***	0.36	0.51*

*, **, *** = Significant at P<0.05, 0.01 and 0.001, respectively.

5.5.8. Lipid oxidation in cooked bologna

TBARS analysis was employed to monitor lipid oxidation in cooked chicken bologna during storage at 4°C (Figure 5.4). The treatments and storage time had a significant effect (p<0.001) on TBARS values. The initial TBARS values of cooked bologna differ significantly (p<0.001) among the treatments even though the starting meat material and processing conditions were the same for all treatments. Therefore, the differences observed in the initial TBARS values among treatments could be attributed to the oxidation taking place during cooking. Since heat is a factor of promoting lipid oxidation, the cooking process is believed to increase the oxidized fat content of the food (Pikul et al., 1984b). Many researchers found that cooking increases the concentrations of malonaldehyde in chicken meat by as much as 5 to 20 times than that of raw meat samples (Siu and Draper, 1978; Igene et al., 1979b; Newburg and Concon, 1980). In the present study, the highest TBARS values were reported for the STPP-No binder control sample (0.81 mg MDA/kg sample), while the lowest values were occurred in the sample LF+300WE and LF+300SC (0.33 mg MDA/kg sample). The TBARS values were lower in all samples treated with lentil seed components, except the samples which was treated with only LF, as compared to the STPP-no binder control. These results suggested that lentil seed coat components have a strong (p<0.001) antioxidant activity against lipid oxidation during cooking. Similar findings have been obtained in Study 1 (Section 3.5.4.6), where SC and WE showed strong protective effects against lipid oxidation during cooking.

Significant treatment, storage time and treatment x storage time interaction effects (p<0.001) were observed for TBARS values of cooked bologna stored at 4°C for 56 days. The TBARS values of all samples increased with refrigerated storage time. The TBARS values of the STPP-no binder control increased steadily throughout the storage period and were significantly higher than other samples except for the sample treated with only lentil flour (LF). The sample LF showed similar behavior to STPP-no binder control, although the TBARS values at each time interval and the overall TBARS values were slightly lower than that in the STPP-no binder control.
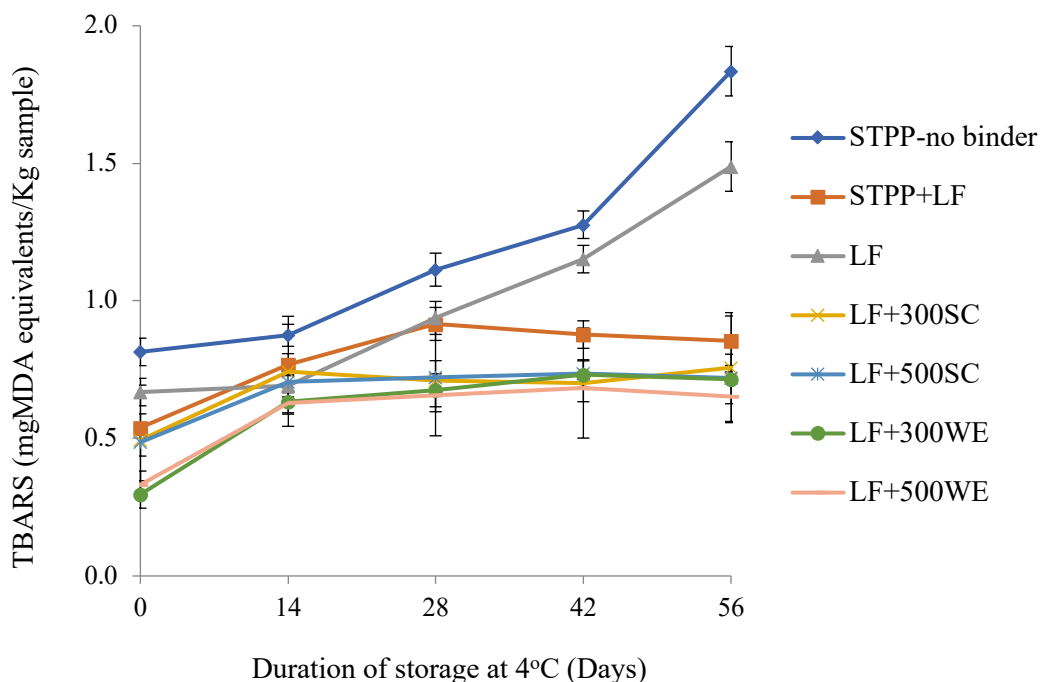
Figure 5.4 TBARS values of chicken bologna containing lentil components stored at 4°C
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics, Values are the means and standard error of duplicate assays from three replicates.

The TBARS values of the STPP+LF sample increased at the beginning of the storage and then began to decrease from day 28. This decrease in TBARS values could be due to the reaction of malonaldehyde compounds with protein or due to their decomposition (Melton, 1983). Comparing the TBARS values of the STPP-no binder control, STPP+LF and LF samples, the addition of the combination of 0.3% STPP and 6% lentil flour appeared to be more effective in controlling lipid oxidation than when they were added separately in chicken bologna. Moreover, the data indicated that the samples which contained SC, or WE had lower (p<0.001) lipid oxidation throughout the storage compared to the other samples. The TBARS values of these samples increased during the first 14 days and then remained almost stable without further increase to the end of 56 days of refrigerated storage. In addition, TBARS values of these samples remained well below the threshold value (1 mg MDA/ kg) for sensory perception of warmed-over flavor

throughout the entire storage. As determined by a trained panel, the threshold of TBARS to detect oxidized flavors in cooked meat was between 0.50–1.0 mg MDA/kg meat (Tarladgis et al., 1960). The minimum level of TBARS that untrained panelists found for off-flavor was 0.6-2 mg MDA / kg (Greene and Cumuze, 1982). The lower TBARS values observed in the bologna treated with lentil seed and water extracts may be ascribed to their antioxidant properties. Study I (Sections 3.5.3 and 3.5.4) and II (Section 4.5.4) of the present wok demonstrated the strong antioxidant potential of lentil sed coat. In addition, the antioxidant capacity of lentil seed coat was also reported by Li (2017). He observed that beef burgers with added lentil seed coat (0.6%) developed lower level of TBARS than control burgers during the refrigerated storage for 7 days and for 12 weeks under frozen (-20°C) storage.

Overall, results of this experiment revealed that the addition of lentil seed coat components was equally effective as the addition of STPP in controlling lipid oxidation in chicken bologna extended with lentil flour thus, the replacement of synthetic phosphates by lentil seed components may not have negative effects on the oxidative stability. The antioxidant properties of phosphates are thought to be associated primarily with their capacity to sequester metal catalysts, making them unavailable to engage in oxidation reactions (Aberle et al., 2012).

The significant antioxidant activity of the seed coat components might be due to the presence of various phenolic compounds. In Study I (Sections 3.5.3 and 3.5.5) and II (Section 4.5.4), lentil seed coat were found to have substantial antioxidant activity when measured as 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity, Fe^{2+} ion chelating activity and phospholipid peroxidation inhibition activity. The antioxidant activity exhibited by the water extracts of green seed coat (CDC Greenland) at 300 ppm of TPC in DPPH, ABTS, Fe^{2+} ion chelation and phospholipid peroxidation inhibition assays were 63%, 79%, 71% and 44%, respectively, whereas the corresponding values at 500 ppm were 76%, 91%, 81%, and 61%, respectively. These results indicated that SC and WE have antiradical properties and the ability to chelate metal catalysts of lipid oxidation. In agreement, Xu and Chang (2008) found that lentil had the highest antiradical capacity in comparison with green pea, yellow pea and chickpea. Further, lentil had higher Oxygen Radical Absorbance Capacity (ORAC) than most of the common fruits including apple, plums, blackberries, cherries, figs, peaches, pears and oranges (Haytowitz and Bhagwat, 2010). The previous experiments (Study II, Section 4.5.3) demonstrated that kaempferol tetraglucoside

(flavonols) catechin glucosides (flavon-3-ols) and proanthocyanidins were the dominant water-soluble phenolics in green lentil seed coat from the same cultivar used in this study (CDC Greenland) and they exhibited strong positive correlation with antioxidant activity. In comparison to the lentil seed coat, lentil flour (cotyledon) had lower total phenolic content (2.14 mg GAE/g), thus the antioxidant activity of lentil flour was relatively lower compared to the seed coat components. In the present experiment, the phenolics added through the seed coat in LF+500SC treatment was equivalent to the phenolic content in 4 times of flour. Other than the phenolic compounds, Li (2017) reported that lentil seed coat and cotyledon contain water-soluble proteins which have antioxidant properties. Moreover, the soluble protein in seed coat were shown to have higher antiradical activity than those obtained from cotyledon, which was suggested to be due to the higher amount of protein-bound phenolic compounds.

With respect to the TBARS values, no significant difference was observed between SC and WE treatments at both concentrations (300 ppm and 500 ppm) of TPC. This might be due to that both systems contained the same concentrations of TPC. These results are in good agreement with the finding of study I (Section 3.5.4.7) which showed similar antioxidant properties of SC and WE when added in cooked MSC model system. Further, changing the concentration of total phenolics (300 ppm or 500 ppm) in either SC or WE treatments did not show a significant impact on TBARS values indicating that 300 ppm of TPC was high enough to replace the antioxidant properties exerted by 0.3% of the STPP in this meat system with lentil flour. The TPC contributed by both LF and SC/WE into LF+300SC or LF+300SC treatments was 428 ppm. Generally, seed coat represents around 10% of the entire seed weight (Dueñas et al., 2002; Dueñas et al., 2006; Li, 2017); therefore, the whole lentil flour needed to contribute an equivalent amount of TPC in this meat system would be 10%. Adding this much flour in meat products may sometimes have practical limitations including meeting the product requirement for meat protein content and sensory acceptability. Thus, an extract may provide the advantage of adding additional phenolics to a meat system with minimal effect on its composition and, consequently, on sensory properties.

5.5.9 Sensory evaluation of cooked bologna

The sensory properties of the cooked bologna were evaluated by a 12-member semi-trained panel and data are presented in Table 5.9. Adding lentil flour and components such as seed coat affected color desirability (Figure 5.5). The bologna added with lentil seed components could be distinguished from others in terms of color. The highest score which indicates "moderately desirable" was given to the STPP-no binder control while adding LF had slightly lower desirability which may related to the color change observed in Table 5.5. The application of lentil seed coat in bologna formulations affected the color liking scores and the mean score was between 3 and 4, which indicates that color desirability was between 'slightly undesirable' and 'moderately undesirable'.
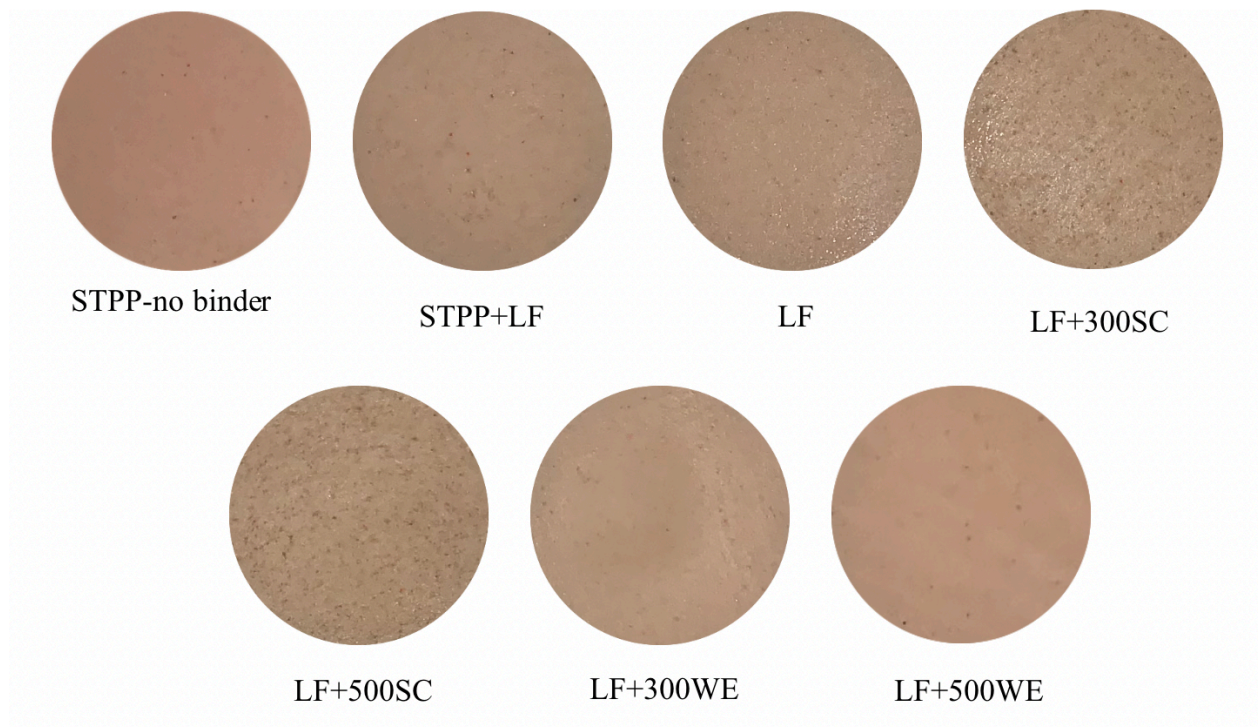
Figure 5.5 Cross sectional view of cooked bologna

STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics.

Table 5.7 Sensory properties of chicken bologna incorporated with lentil seed components (n = 12 panelists x 3 replicates)

Treatment	Color Desirability	Surface moisture	Springiness	Hardness	Cohesiveness	Denseness	Chewiness
STPP-no binder	5.97[a]	6.56[a]	5.86[a]	4.33[b]	4.83[ab]	4.86[bc]	5.33[a]
STPP+LF	4.75[b]	5.61[bc]	5.28[b]	5.28[a]	5.08[a]	5.36[ab]	5.33[a]
LF	4.61[b]	5.61[bc]	5.17[b]	5.25[a]	5.03[a]	5.50[a]	5.19[a]
LF+300SC	3.83[c]	5.25[cd]	4.47[c]	4.22[b]	4.39[bc]	4.39[c]	4.03[b]
LF+500SC	3.31[d]	4.94[d]	4.03[c]	3.53[c]	4.14[c]	3.75[d]	3.22[c]
LF+300WE	4.89[b]	5.81[b]	5.44[ab]	5.50[a]	5.11[a]	5.44[ab]	5.47[a]
LF+500WE	4.78[b]	5.89[b]	5.11[b]	5.39[a]	4.92[a]	5.33[ab]	5.31[a]
SEM[1]	0.104	0.161	0.112	0.144	0.149	0.122	0.168

[1] Standard error of mean.

[abc]Means with the different letters in the same column are significantly different (P<0.05)

Color desirability: 8 = extremely desirable, 1 = Extremely undesirable

Surface moisture: Extremely moist: 8 = extremely moist, 1 = Extremely dry

Springiness: 8 = extremely elastic, 1 = Extremely rigid

Hardness: 8 = extremely firm, 1 = Extremely soft

Cohesiveness: 8 = Extremely cohesive, 1 = Extremely crumbly

Denseness: 8 = Extremely compact, 1 = Extremely loose

Chewiness: 8 = Extremely chewy, 1 = Extremely mushy

STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

Table 5.9 Sensory properties of chicken bologna incorporated with lentil seed components (n = 12 panelists x 3 replicates) Cont.

Treatment	Juiciness	Graininess	Saltiness	Overall flavor intensity	Foreign flavor intensity	Aftertaste	Overall acceptability
STPP-no binder	5.92[a]	2.81[c]	4.42[ab]	4.58[b]	3.97[b]	4.06[d]	4.67[b]
STPP+LF	5.06[b]	4.56[b]	4.69[ab]	4.56[b]	4.14[b]	4.56[bc]	5.00[ab]
LF	4.81[bc]	4.25[b]	4.42[ab]	4.78[ab]	4.14[b]	4.39[c]	5.19[ab]
LF+300SC	4.44[cd]	5.50[a]	5.08[a]	5.22[a]	4.69[a]	4.86[ab]	4.69[ab]
LF+500SC	4.14[d]	6.03[a]	4.72[ab]	5.06[ab]	4.92[a]	5.03[a]	3.78[c]
LF+300WE	5.14[b]	4.53[b]	4.36[b]	4.97[ab]	4.28[b]	4.56[bc]	5.19[ab]
LF+500WE	4.97[bc]	4.53[b]	4.83[ab]	5.00[b]	4.00[b]	4.58[bc]	5.33[a]
SEM[1]	0.144	0.138	0.139	0.139	0.093	0.104	0.136

[1] Standard error of mean.
[abc]Means with the different letters in the same column are significantly different (P<0.05)
Juiciness: 8 = Extremely juicy, 1 = Extremely dry
Graininess: 8 = Extremely grainy, 1 = Extremely smooth
Saltiness: 8 = Extremely intense, 1 = extremely bland
Overall flavor intensity: 8 = Extremely intense, 1 = Extremely bland
Foreign flavor intensity: 8 = Extremely intense, 1 = Extremely bland
Aftertaste: 8 = Extremely intense, 1 = Extremely bland
Overall acceptability: 8 = Extremely acceptable, 1 = Extremely unacceptable
STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

However, the addition of seed coat extracts had relatively lower effect on color compared to the addition of seed coat and were similar to the other treatments with LF. The treatments STPP+LF, LF, LF+300WE and LF+500WE were similar in relation to the color desirability and they were scored as 'like slightly'. These results indicate that, the incorporation of WE may be a preferred delivery vehicle to add more phenolic compounds to the products.

For springiness, mean scores of bolognas ranged between 4 (slightly rigid) and 6 (moderately elastic). Incorporation of lentil flour were perceived to reduce the springiness. LF added samples except the samples containing seed coat (LF+300SC and LF+500SC) were ranked as slightly elastic while STPP-no binder sample was moderately elastic. The bologna with seed

coat were given the lowest scores which were within the "rigid" range of the scale while those with WE were not different from most other treatments with LF. These findings are in agreement with the results of texture profile analysis (TPA) and the torsional gelometry analysis which showed higher ($p<0.05$) springiness and rubberier texture for STPP-no binder control. The springiness scores of WE samples were slightly higher in comparison to SC treatments, and they did not differ from LF and STPP-LF samples showing that the application of WE had a limited impact on springiness.

Almost similar trends were observed in the sensory scores for hardness, cohesiveness, denseness, and chewiness. STPP-no binder control was given a lower hardness score (by one unit) than that of LF added samples except for samples with SC (LF+300SC and LF+500SC) showing the contribution of starch from LF to the bologna texture. The samples with seed coat had a slightly softer texture compared to other flour added samples. Cohesiveness data showed a similar trend to hardness with lower scores given to the STPP-no binder control and bologna samples with SC. All treatments showed similar chewiness except the treatment with SC, which had lower scores than that of other samples. In agreement with the results of the torsional gelometry analysis (Figure 5.3), panelists scored bolognas with SC as slightly mushy for cohesiveness while other treatments containing LF were similar in chewiness to the STPP-no binder control.

Overall, the sensorial texture scores were in accordance with the results obtained from texture profile analysis and torsional gelometry analysis. No significant differences were noted between LF and STPP-LF treatments indicating that exclusion of STPP from the formulations with LF had no significant impact on the perception of texture. Moreover, the springiness, hardness, cohesiveness, denseness, and chewiness scores of WE treatments did not differ from LF and STPP-LF samples showing that the application of more phenolics as WE have limited impact on sensory perception of bologna texture. The texture scores of treatments with SC had significantly different scores from the other treatments, which could be due to the fiber added from SC.

Application of lentil seed components especially SC increased the grainy texture in comparison to STPP-no binder control. The lowest and highest scores for graininess were given to the STPP-no binder control (moderately smooth) and samples with SC (moderately grainy), respectively. No significant differences were found between other treatments indicating that WE had no significant effect on the grainy perception of chicken bologna. A possible solution to reduce the grainy appearance would be further reducing the particle size of the lentil ingredients, especially

the particle size of SC, which was ~250 µm in the present study. Similar to the results of the present study, Xu (2017) reported that bologna products with 2.5% lentil bran received higher sensory scores for grainy texture (more graininess) compared to the no binder control. Furthermore, Sanjeewa (2008) also observed higher scores for graininess in pork bologna with chickpea flour compared to the no binder control. He suggested that these higher scores could be due to the finely ground seed coat contained in the flour.

The inclusion of legume products in meat systems can cause off-flavors. Compared to the STPP no-binder control, SC added samples had stronger foreign flavor. In addition, aftertaste was higher in the samples with seed coat compared to the control. It was noteworthy that the foreign flavor intensity of the samples with WE did not differ significantly from the control groups (STPP-no binder and STPP+LF), whereas the aftertaste was comparable to the STPP-LF and more intense than the STPP-no binder control. Similar to these results, the mean scores for foreign flavor in bologna with 2.5% lentil bran were found to be significantly higher than the control with no binders (Xu, 2017). It was found that adding WE had limited negative effects on bologna flavor and after taste than adding SC and were similar to other treatments with LF. The aftertaste of the STPP-no binder control and LF+500SC were scored as slightly bland and slightly intense, respectively whereas, the mean scores of the other samples were between the two categories.

For overall acceptability, all samples received scores within the "liking" range of the scale except the bologna LF+500SC, which was rated as "slightly dislike". However, the acceptability of LF+300SC bologna was similar to the STPP-no binder control, although it had relatively lower color desirability and higher graininess, aftertaste and foreign flavor. It is important to note that, the overall acceptability of bologna formulated with WE (LF+300WE and LF+500WE) were similar to the acceptability of samples formulated with STPP or LF alone. However, the overall acceptability in this study was based on analysis performed by 12 semi trained panelists, therefore, product acceptability should be confirmed with a wider panel of consumers.

In summary, sensory results revealed that the replacement of STPP with LF and WE have limited impact on sensory attributes. However, application of SC showed some negative effects; hence, reducing the SC particle size would be a way to minimize the effects related to appearance of the products. As mentioned in mentioned in section 5.5.8, flour from only non-dehulled lentil necessary to provide total phenolic content equivalent to LF+300SC system was nearly 10% which may result in color, texture and flavor properties. Therefore, the application of lentil flour, which

generally would have a finer particle size in combination with WE, would be the best way to add high levels of phenolic compounds to meat systems.

5.5.10 Principal component analysis (PCA) and hierarchical cluster analysis

Principal component analysis (PCA) followed by hierarchical cluster analysis was conducted on water holding and texture properties, oxidative stability and sensory data to investigate the associations among these variables and bologna treatments. (Figures 5.5 and 5.6). The first two principal components (dimensions) explained 87% of total variance. Among the 28 variables analyzed, the first dimension was strongly associated with sensory attributes; springiness, color desirability, graininess, after taste, juiciness, surface moisture, foreign flavor intensity, chewiness, springiness, cohesiveness; instrumental cohesiveness and shear strain, CIE L* value (Figure 5.6). Dimension two, which explained 34% of variation was defined by instrumental (TPA) hardness, shear stress and rigidity, CIE a* and b*, purge loss and cook loss, and sensory attributes hardness, denseness, and overall acceptability.

The results of hierarchical cluster analysis are in accordance with PCA results, and bologna treatments were divided into three distinct groups (Figure 5.7). The STPP-no binder control which was characterized by relatively springy texture was loaded on the negative direction of dimension two. Further, STPP-no binder sample showed weaker water holding properties (purge loss and expressible moisture) and oxidative stability compared to the other LF incorporated treatments even though it contained STPP. The two seed coat treatments (LF+300SC and LF+500SC) were clustered into one group and loaded on the negative direction of dimension one of PCA biplot. These treatments mainly differed from other treatments having a softer texture, and relatively intense graininess, after taste and foreign flavor. Treatments STPP+LF, LF, LF+300WE and LF+500WE clustered as one group showing that they have relatively similar characteristics. This further confirms that the exclusion of STPP from bologna formulations containing LF have limited impact on water holding properties, texture and sensory attributes and oxidative stability of the product. Further, these results reveled that, application of WE also have limited effect on the properties of bologna extended with LF indicating that it would be the better way to deliver more phenolics into the meat system.

156
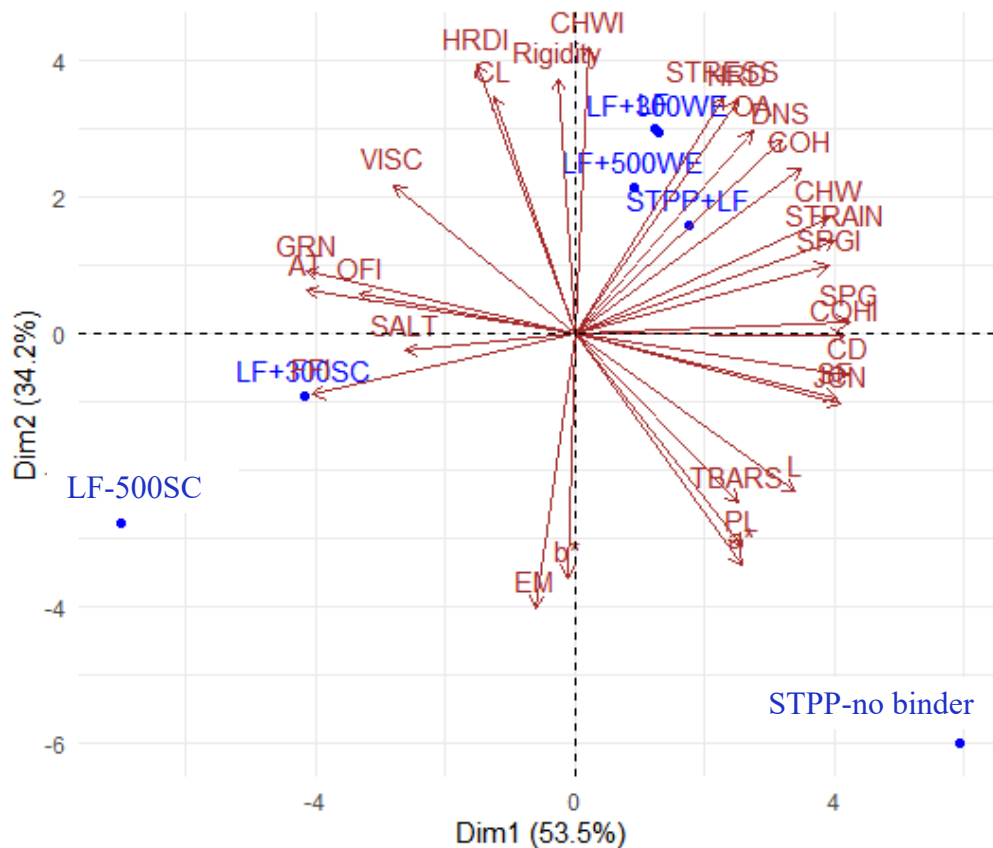
Figure 5.6 Principal Component Analysis (PCA) biplot showing the multivariate variation among chicken bologna in terms of water holding properties, instrumental texture properties, oxidative stability and sensory attributes.

VISC: viscosity, CL: cook loss, PL: purge loss, EM: expressible moisture, HRDI: instrumental hardness, COHI: instrumental cohesiveness, SPGI: instrumental springiness, CHWI: instrumental chewiness, STRESS: torsional gelometry shear stress, STRAIN: torsional gelometry shear strain, L*: CIE L* value, a*: CIE a* value, b*: CIE b* value, TBARS: thiobarbituric acid reactive substances, CD: color desirability, SF: surface moisture, SPG: springiness, HRD: hardness, COH: cohesiveness, DNS: denseness, CHW: chewiness, JCN: juiciness, GRN: graininess, SALT: saltiness, OFI: overall flavor intensity, FFI: foreign flavor intensity, AT: aftertaste, OA: overall acceptability, STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics
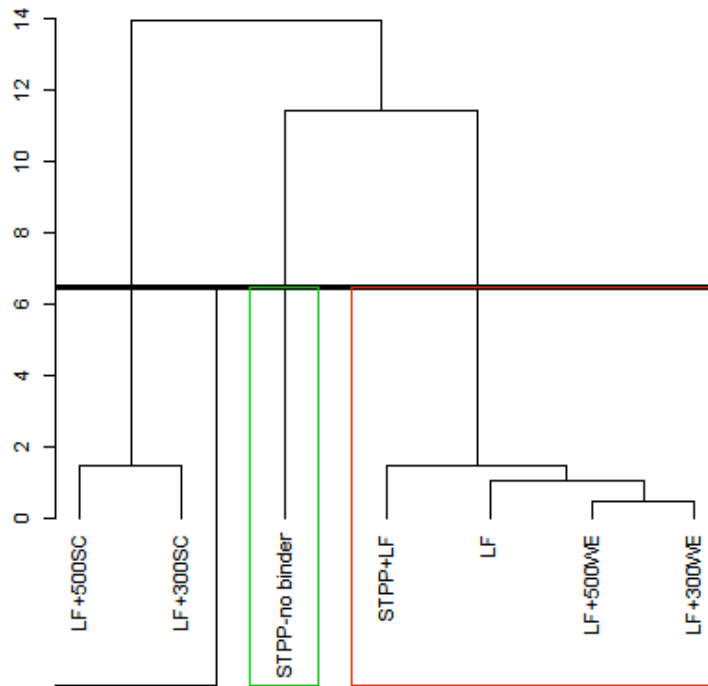
Figure 5.7 Hierarchical cluster analysis dendrogram of chicken bologna evaluated in terms of water holding properties, instrumental texture properties, oxidative stability and sensory attributes.

STPP-no binder: 0.3% STPP only; STPP+LF: 0.3% STPP +6% lentil flour; LF: 6% lentil flour; LF+300SC: 6% lentil flour + seed coat equivalent to 300 ppm of total phenolics; LF+500SC: 6% lentil flour + seed coat equivalent to 500 ppm of total phenolics; LF+300WE: 6% lentil flour + seed coat water extract equivalent to 300 ppm of total phenolics; LF+500WE: 6% lentil flour + seed coat water extract equivalent to 500 ppm of total phenolics

5.6 Conclusions

Overall, results showed that components of lentil seeds are capable of delivering water holding and antioxidant properties in bologna similar to STPP. Additions of IR treated lentil flour increased the raw batter viscosity. Cook loss, expressible moisture content, and purge loss did not increase through replacement; thus, lentil flour was able to bind water effectively. Texture properties of the bologna with lentil flour and water extract of seed coat were comparable to those with lentil flour and 0.3% of STPP and better than that with only 0.3% STPP and no binder. The addition of seed coat in chicken bologna had negative effects on sensory properties, particularly color and flavor. However, the addition of water extract containing 300 ppm or 500 ppm TPC did not adversely affect the sensory properties. The addition of infrared heated lentil flour together

with seed coat or water extract of seed coat was able to control the lipid oxidation in chicken bologna as effectively as STPP. Therefore, the combination of IR lentil flour and water extract of seed coat would be a suitable candidate to contribute the functional properties of phosphates and would be beneficial in controlling lipid oxidation without compromising the texture and sensory properties particularly in products where nitrite is not included.

5.7 Connection to next study

In this study, lentil flour was found to contribute to water holding properties and antioxidant capacity in chicken bologna, showing that lentil flour would be a potential binder with some antioxidant activity in meat systems. Therefore, the primary objective of the next study was to investigate the performance of IR heated lentil flour alone as a binder in meat products. IR heated lentil flour was used as a binder in a commercial formulation of a wiener type sausage without further modifications of the processing procedures and recipe except the type of binder. For comparison, isolated soy protein and corn starch were used as they are widely used binder ingredients in the industry. The performance of lentil flour was evaluated based on physico-chemical, textural, and sensory properties of finished processed products. As a secondary objective, antioxidant efficacy of lentil flour was assessed in comparison to sodium nitrite. Further, consumer acceptability of the products was evaluated in a cross-cultural consumer study to assess the acceptability of the products between cultures that are strongly and less familiar with lentils.

6. STUDY IV: SENSORY CHARACTERISTICS, OXIDATIVE STABILITY AND CROSS-CULTURAL CONSUMER ACCEPTABILITY OF CHICKEN SAUSAGES FORMULATED WITH LENTIL FLOUR

6.1 Abstract

The efficacy of infra-red heated (IR) lentil flour as a binder in wiener type chicken sausages were evaluated based on physicochemical properties, oxidative stability, and sensory characteristics of the final products. Sausages were prepared from mechanically separated chicken meat (MSC) by incorporating lentil flour at 4%, 6%, and 8% levels and one formulation (4% lentil flour) without sodium nitrite. Control samples included a formulation without any binder and a commercial formulation containing isolated soy protein (1.75%) and modified corn starch (4%). Total starch content was adjusted to 4% in all formulations except in the no-binder control. In general, water holding properties and instrumental texture properties of the sausages containing IR lentil flour were similar to the commercial formulation and was not affected much by the level of lentil flour added. The thiobarbituric acid reactive substances values of all samples were below 2 mg MDA/kg sample throughout the storage at 4°C (42 days) and -18°C (56 days) and did not differ significantly among formulations. Moreover, the oxidative stability of the no-nitrite sausages was similar to the sausages containing lentil flour, showing that the exclusion of nitrite from the sausage formulations containing IR lentil flour had no significant effect on their lipid oxidation. Sensory descriptive attributes of the sausages were evaluated by two descriptive attribute panels. Overall, no drastic differences were observed in the sensory parameters between lentil flour added formulations and the commercial formulation. The liking of the sausages was investigated in consumer tests with Canadian and Sri Lankan consumers (total of 180 panelists). No apparent differences in acceptability were found between the consumer groups, but exclusion of nitrite reduced color acceptability. The overall acceptability of lentil flour added sausages were similar to the commercial formulation. Consequently, this study demonstrated that IR lentil flour had potential for utilization as a binder in chicken sausages without any marginal decline in physicochemical and sensorial characteristics.

6.2 Introduction

Lentil is an edible legume with unique nutritional and functional characteristics. It is a good source of carbohydrates (60%), protein (26%), and fiber (30%) with readily available energy (Tiwari and Singh, 2012). In addition, lentil contains many health promoting components such as vitamins, minerals, and phytochemicals, which include phenolic compounds (Faris et al., 2013). Phenolic compounds of plant origin have been well studied as antioxidants that can delay or prevent oxidative damage and thus prevent the occurrence of diseases associated with oxidative stress in the human body (Willett, 1994; Kushi et al., 1999; Yochum et al., 1999; Kris-Atherton et al., 2002). It has been shown that increased lentil consumption has potential health benefits such as reduced risk of cardiovascular disease, hypertension, cancer, diabetes, and gastrointestinal diseases (Tharanathan and Mahadevamma, 2003, Jacobs and Gallaher, 2004, Lutsey et al., 2007) which might be related with its beneficial phytochemicals. Lentil has been incorporated into different world cuisines and is predominantly consumed in Mediterranean and Asian regions of the world. However, its consumption in western countries is limited due to traditional eating customs, and lack of consumer understanding, processing techniques, and diversified food products (Zhang et al., 2015). Development of convenience products may open the avenues for not only increased consumption but also new opportunities for the pulse industry to increase the demand for value-added lentil ingredients.

Non-meat ingredients from different plant sources are used as binders and extenders to reduce cost and serve as functional ingredients in comminuted meat products. In particular, non-meat ingredients rich in protein and starch such as soy proteins (Gujral et al., 2002), buck wheat protein (Bejesano and Corke, 1998), common bean four (Dzudie al., 2002), bengal gram, green gram and black gram flours (Modi et al., 2003), wheat flour (Ulu, 2004), corn flour (Serdaroglu and Degirmencioglu, 2004) and chick pea flour (Serdaroglu et al., 2005; Sanjeewa et al., 2010, Shariati-levari et al., 2016) added in comminuted meat products have resulted in increased water and fat binding capacity, decreased cooking loss and modification of emulsion properties, texture and taste. Being rich in starch and protein, lentil flour may serve as a potential source of extender or binder in meat products and reduce cost by replacing some of the meat in the formula. In addition, lentil flour also has the advantage of imparting antioxidant properties coming from phenolic compounds. They could play a significant role in stabilizing the oxidative stability of meat products as effectively as synthetic antioxidant compounds commonly added in meat products such

as nitrites. Thus, the addition of lentil flour is expected to exert beneficial effects on the overall taste, texture, flavor and appearance of the products and the stability of these properties throughout their shelf life.

Thermal processing can be used to enhance the physico-chemical and functional properties of pulses (Cenkowski and Sosulski, 1997). For example, infra-red heating has been employed in the pulse processing industry as a pre-heat treatment. Studies have shown that infra-red heating can inactivate oxidative enzymes, improve cooking properties and enhance the shelf stability of lentil flour (Der, 2010; Pathiratne, 2014; Li, 2017). Therefore, the utilization of IR treated lentil flour is suggested to be beneficial in meat applications rather than using untreated lentil flour. For a successful integration of lentil flour into meat products, technology needs to be developed to optimize product formulations and processing parameters ensuring optimum quality and storage stability, which are vital for determining their suitability for large-scale production.

All industries have a strong trend to expand their horizons and compete on a global basis. Thus, producers and trade organizations are faced not only with understanding the relationship between consumer preferences and product characteristics but also how this relationship depends on the cultural context. Although research in this area is not new, there are only a few research studies done on cross-cultural differences particularly with reference to foods. Some studies have shown that chemosensory perception is largely similar across cultures (Druz and Baldwin, 1982; Prescott, 1998). In contrast, Prescott and Bell (1995) reported that sensory perception and preferences tend to differ between cultures. For example, there were substantial differences in the level of sweetness preferred by Koreans and Nigerians compared to Canadians (Druz and Baldwin, 1982). Bertino et al. (1983) reported that differences exist between Taiwanese and US students with respect to preferred sweetness in cookies. As for most products, the acceptance for meat was also found to be unique to different countries or cultures (Ladele et al., 1996). Prescott et al. (2002) found that food choice in different cultures is largely influenced by several factors including sensory appeal, convenience, health concerns, natural content, price and ethical concerns. Further, Prescott (1998) emphasized the need for understanding acceptability of relatively novel foods for export markets because of the complexity of food preferences influenced by both sensory and non-sensory influences among different cultures. The limited research done exploring the cross-cultural consumer preferences for processed meat products necessitates its need when developing meat products for export markets.

In this context, the present study was intended to investigate the functional behavior of IR treated lentil flour in a meat system. The study was designed to evaluate the effect of different levels of lentil flour on physicochemical properties and sensory profile of wiener type sausages prepared from mechanically separated chicken and to assess their lipid stability, storage quality and consumer acceptability among consumers in Canada and Sri Lanka. The purpose of selecting these consumer groups were to compare the perceptions between the consumers with high (Sri Lankan) and low (Canadian) lentil consumption experience. On the other hand, the introduction of lentil flour as a meat binder would benefit Sri Lankan consumers as it would help reduce the production cost of meat products. In comparison to Canada and other developed countries, the per capita consumption of meat is low in Sri Lanka mainly due to poor affordability (Jayathissa et al, 2014). The application of lentil flour in MSC meat systems will help lower product prices while increasing the overall protein content.

Hypothesis:

I. Inclusion of IR treated green lentil flour in sausage formulations will improve the water holding, textural, antioxidant, and sensory properties of wiener type MSC sausages.

II. Canadian and Sri Lankan consumers will have different perceptions toward IR treated green lentil flour incorporated wiener type MSC sausages.

6.3 Materials

MSC (71.84% moisture, 15.80% protein 11.67% fat, and 0.80 ash) was obtained on the same day of processing from the Prairie Pride Natural Foods Inc. (Saskatoon, Canada) and stored at -30°C until processing. Meats were sourced on three different production dates. MSC (67.76% moisture, 17.56% protein 12.98% fat, and 0.94 ash) used in Sri Lanka were similarly sourced by Keells Products PLC. Infra-red (IR) treated lentil flour was provided by the Infraready Products Ltd. (Saskatoon, Canada). It had the following composition: 8.26% moisture, 24.63% protein, 63.35% total carbohydrates, 18.0% dietary fiber, 1.06% fat and ash 2.71%. Food grade sodium tripolyphosphate (STPP), sodium erythrobate, sodium chloride (salt), sodium nitrite (cure salt) and spices were purchased from the local market. Modified corn starch and isolated soy protein (ISP) were purchased from Markham Meat Industry Supplies Inc. (Aurora, Ontario, Canada).

6.4 Methods

6.4.1 Formulations of sausages

The formulations of sausages were developed based on commercial recipes used by Keells Food Products PLC. (Ja-Ela, Sri Lanka), who is a major meat processor in Sri Lanka. This study was designed with six treatments. The total starch content in each formulation of sausages was adjusted to 4%, except the control which was formulated without binders. Isolated soy protein and modified corn starch were used as a comparison because they are commonly used as binders or extenders in commercial meat products in Sri Lanka and around the world. The test formulations were developed by incorporating infra-red heated lentil flour to replace the protein and starch content delivered by isolated soy protein and modified corn starch partially or completely. Sodium nitrite was added in all formulations except one in order to evaluate the antioxidant efficacy of lentil flour compared to nitrite.

The no-binder control contained 1.8% sodium chloride, 125 ppm of sodium nitrite, 0.16% spices, 0.3% STPP and 0.04% sodium erythrobate. Treatment two (ISP+CS) was a commercial formulation and contained 1.75% ISP and 4% modified corn starch. The other treatments were formulated by adding IR treated lentil flour at 4% (4%LF), 6% (6%LF) and 8% (8%LF) levels replacing ISP and adjusting modified corn starch so that total starch content remained at 4% of the formulation. The treatment 4%LF-NO2 had similar formulation to the treatment 4%LF except sodium nitrite. The MSC content was kept at 60% in all formulations except the control (66%). The other ingredients which were used in the control were added in similar concentrations in all other treatments (Table 6.1). Formulation was based on material weight.

6.4.2 Processing of sausages

The processing of sausages was carried out at two locations; Keells Food Products PLC (Ja-Ela, Sri Lanka) which is a commercial meat processing facility and the meat processing pilot plant at University of Saskatchewan (Saskatoon, Canada) under commercial processing conditions. Each product was prepared in triplicate.

The frozen MSC was thawed at 1°C for 48 h. Thawed MSC was ground through 4.7 mm grinder plate (Biro Grinder, model AMFG-24, Marblehead, OH, U.S.A.) and separated for each treatment (8 kg and 20 kg batches in Canada and Sri Lanka, respectively) which were processed in random order. Meat was mixed with STPP for 25 seconds in a bowl chopper with four bladed knife

Table 6.1 Formulations of chicken sausages

Ingredient (%)	No-binder	ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2
			Treatment			
MSC	66.00	60.00	60.00	60.00	60.00	60.00
Lentil Flour (IR treated)	-	-	4.00	6.00	8.00	4.00
Isolated soy protein (ISP)	-	1.75	-	-	-	-
Modified corn starch	-	4.00	2.18	1.27	0.36	2.18
Salt	1.80	1.80	1.80	1.80	1.80	1.80
Spices	0.16	0.16	0.16	0.16	0.16	0.16
Sodium Nitrite	0.01	0.01	0.01	0.01	0.01	-
Sodium erythrobate	0.04	0.04	0.04	0.04	0.04	0.04
Sodium tripolyphosphates (STPP)	0.30	0.30	0.30	0.30	0.30	0.30
Water	31.69	31.94	31.51	30.42	29.33	31.53

(35 L RMF Steel, Kansas City, MO, U.S.A.) at speed 1 (4,000 rpm knife speed). Then the meat mixture and salt, cure salt and half of the ice water were mixed for 1 min in the bowl chopper at same speed. The remaining ingredients were added, and chopping was continued at speed 2 (8000 rpm knife speed) for 4 min. The average meat batter temperature after chopping was 8°C. The chopped batter was then tumbled (Model: Glass VSM 150 vacuum mixing machine, Rostfrei, Germany) under vacuum conditions (80%) for 5 min to remove the air trapped in the meat batter. The average temperature of the meat batter after vacuum tumbling was 10°C. At this point, samples were taken to measure the batter viscosity. The meat batter was kept overnight at 1°C for proper distribution of the ingredients. On the following day, each meat batter was stuffed using a Handtmann VF80 stuffer (Biberach / Riss, Germany) into artificial cellulose casings (21 mm diameter), hand linked at 10 cm and smoked and cooked in a smokehouse to an internal temperature of >75°C. When the endpoint temperature was reached, the sausages were chilled under shower of cold water to an internal temperature of 40°C followed by holding in a cold room at 4°C for 12-14 h. The sausages were hand peeled and vacuum packed in high water vapor and oxygen barrier polyethylene bags (O_2 permeability < 25 cm^3 /m^2/24h) as one layer of sausages per bag. The processing of sausages in the Keells Food Products PLC, Sri Lanka followed the same procedure,

however, the stuffing, linking, peeling and packaging were done using a commercial automated stuffer, linker, skinning and packaging line. Sausages made in Sri Lanka were sampled for proximate analysis, pH, and sensory evaluation only and all other analysis were done on those produced in Canada.

6.4.3 Storage of samples

Samples of sausages were stored in dark at 4°C in a walk-in cooler and at -18°C in a chest freezer for the storage study. Sampling and analysis for oxidation stability was carried out at day 0, 14, 28 and 42 days of storage and texture properties was analyzed at 0 and 28 days in the samples stored at 4°C. The samples stored under frozen conditions were analyzed at 0, 28, 56 and 84 days for oxidation stability and at 0 and 56 days for texture properties. The frozen samples were thawed overnight at 1°C before analysis. All analysis was performed following triplicate sample production.

6.4.4 Determination of batter viscosity

Viscosity of raw batter was measured in triplicate as described by Shand (2000) with a Brookfield Synchro-Lectric viscometer (Model RVT; Brookfield Engineering, Stoughton, MA., U.S.A.) within one hour after the preparation of batter. The number seven spindle was positioned in a 250 mL plastic cup filled with batter. The spindle was allowed to rotate (10 rpm) for 30 sec and the apparent viscosity was measured. The viscosity at two locations in each cup was measured.

6.4.5 Determination of cook loss

The smokehouse cook loss of sausages was determined as the weight difference between raw and cooked sausages. The weight of all the sausages was recorded before smoke house cooking and weight of cooked sausages was recorded after 12-24 h chilling. The cook loss was calculated as follows:

$$\text{Cook loss (\%)} = \frac{\text{Initial weight of sample (g)} - \text{Cooked weight of the of sample (g)}}{\text{Initial weight of sample (g)}} \text{ X } 100 \qquad (6.1)$$

6.4.6 Determination of purge loss

Purge loss (drip loss) was determined on three vacuum packaged bags containing six sausages from each treatment. After packaging in preweighed plastic pouches (as described in section 6.4.2), the products were stored for 14 and 28 days at 4°C and 28 and 56 days at -18°C. Purge loss was determined by reweighing blotted product from packages after each storage time and was expressed as a percentage of the initial weight.

$$\text{Purge loss (\%)} = \frac{\text{Initial sample weight} - \text{Sample weight after storage}}{\text{Initial sample weight}} \, X \, 100 \qquad (6.2)$$

6.4.7 Determination of expressible moisture

Expressible moisture (EM) was determined on cooked product as outlined by Shand (2000). Briefly, two pieces of Whatman #3 filter paper (9 cm in diameter) and one piece of Whatman #50 (7 cm diameter), were folded into a thimble shape to fit inside a 50 mL Falcon centrifuge tube. A core sample of sausages (1.5 ± 0.3 g) was placed on the filler papers and centrifuged for 15 min at 750 g (Beckman, J2-HC centrifuge with Beckman Coulter JA-17 rotor, MN, U.S.A.). The sample was taken out with a tweezer and reweighed. All treatments were analyzed in triplicate and results were expressed as the percentage of moisture released from the sample to the initial sample weight.

$$\text{EM(\%)} = \frac{\text{Initial sample weight (g)} - \text{Final sample weight (g)}}{\text{Initial sample weight (g)}} \, X \, 100 \qquad (6.3)$$

6.4.8 Determination of proximate composition and pH

Samples were prepared for analysis by blending approximately 100 g of the products for 1 min in a food processor (Cuisinart® Mini-Prep® Plus Processor). The frozen samples were thawed overnight at 1°C before sample preparation. Moisture, protein, fat, and ash contents were determined according to the AOAC Methods (1990) 950.46, 981.10, 960.39a and 920.153, respectively.

The pH of ground sausages was evaluated using a pH meter (Fisher Scientific Accumet, 60 ON, Canada, Model AR15). About 20 g of sample was blended with 80 mL of deionized water in a filter bag (Whirl-Pak® Filter Bags, Nasco®, WI, USA) using a Stomacher Lab-Blender (Seward

Ltd, Bury St. Edmunds, UK, Model BA6021) for 3 min. The pH electrode was immersed into the filtered solution and pH value was recorded.

6.4.9 Texture profile analysis

The instrumental texture properties of sausages were performed using a TMS-Pro Texture Press (Food Technology Corp., Sterling, VA, U.S.A.) fitted with a 1000 N load cell. The chilled samples were kept at room temperature for 30 min before the analysis. Six core samples from each formulation were cut (13 mm in diameter and 6.5 mm height) and axially compressed twice to 50% of their original height at a constant crosshead speed of 100 mm/min.

The following texture parameters were determined.

Hardness: peak force on first compression (N)

Chewiness: hardness x cohesiveness x springiness (Nmm)

Springiness: distance the sample recovered after the first compression (mm)

Fracturability: force required to fracture gels (N)

Cohesiveness: ratio of the active work done under the second force-displacement curve done under the first compression curve (dimensionless)

6.4.10 Determination of shear force

Shear force was measured on six samples from each treatment. Samples were cut in to 10 mm x 10 mm wide strips ensuring that edges of the product were excluded. Prepared samples were stored in plastic bags and equilibrated with room temperature. The shear force was determined by shearing the samples using a Warner-Bratzler shearing device attached to TMS-Pro Texture Press Displacement (mm) 52 (Sterling, VA, U.S.A.). The full-scale load was set at 1000 N with the crosshead speed set at 100 mm/min and peak shear force was recorded.

6.4.11 Measurement of CIE color

Instrumental color was measured using a Minolta Chroma Meter with a 10° observer angle and illuminant A standardized against white and black tiles. The color measurements were taken on the outer and cut surfaces and expressed according to the color coordinates: lightness (L*), redness (a*, +red to -green) and yellowness (b*, +yellow to -blue).

6.4.12 Determination of phenolic contents and antioxidant activity in water extracts of lentil flour

6.4.12.1 Preparation of water extracts

IR-treated lentil flour extracts were prepared according to the method described by Aguilera et al. (2010). Sample of flour (2 grams) was weighed and mixed with 20 mL of deionized water in 50 mL screw-capped plastic tubes and extracted using a shaking water bath at 100 rpm for 15 h at 23 °C. The mixture was then centrifuged at 3000 g at 4°C for 10 min. The supernatant was separated and brought to 20 mL volume with deionized water and filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C. Extracts were prepared from triplicate samples of flour and all assays were done in duplicate.

6.4.12.2 Determination of phenolic contents

The Total phenolic content (TPC) and total flavonoid content (TFC) were determined according to the procedure described in section 3.4.4.1 and 3.4.4.2, respectively. The condensed tannin content (CTC) of extracts was determined according to the procedure described in section 4.4.4.3.

6.4.12.3 Determination of antioxidant activity

The DPPH and ABTS free radical scavenging activity, ferrous ions chelation activity and Inhibition of phospholipid peroxidation were determined according to the procedures described in section 3.4.5.1, 3.4.5.2, 3.4.5.3 and 3.4.5.4, respectively.

6.4.13 Determination of lipid oxidation

Samples of sausages stored under refrigerated and frozen conditions were analyzed for TBARS at specified time intervals (Section 6.4.3) according to the method described by Bedinghaus and Ockerman (1995). Briefly, samples were prepared for the analysis by pulverizing the samples of product (approximately 100 g) using a kitchen style food processor (Cuisinart® Mini-Prep® Plus Processor) for 1 min with an intermittent mixing at 30 sec with a spatula to obtain a homogenous mixture. The TBARS in the samples were extracted by adding 25 mL of 20% (w/v) trichloroacetic acid (TCA) solution containing 1.6% (w/v) phosphoric acid to 2.5 ± 0.02 g of the ground sample in filter bags (Whirl-Pak® Filter Bags, Nasco®, WI, USA) and homogenizing for 2

min using the stomacher. Homogenization was continued for another 1 min after the addition of 25 mL of deionized water. The extract was filtered through Whatman No. 1 filter paper and subsequently 5 mL of the filtrate was reacted with 5 mL of 0.02 M thiobarbituric acid (TBA) reagent in a water bath at 95°C for 35 min to form pink colored TBA-malonadialdehyde complex. The mixture was then cooled in ice water for 10 min. The absorbance of this complex was measured at 532 nm using a spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) against a blank mixture which was prepared according to the same procedure without the sample. 1,1,3,3-tetramethoxypropane (TMP) was used as the standard.

6.4.14 Sensory evaluation

The sensory evaluation of sausages was conducted both in Canada and Sri Lanka using Canadian and Sri Lankan consumer panelists to evaluate the cross-cultural preferences and acceptability of the developed products. Sensory evaluation consisted of descriptive sensory analysis using semi-trained panels and consumer affective tests. The consumer panelists were recruited thorough telephone interviews, emails and posters. This study was accepted on ethical grounds by the University of Saskatchewan Behavioral Research Ethics Board (BEH # 07-188) and the Ethics Review Committee of the Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka (20180H102).

6.4.14.1 Descriptive analysis
(a) Panel selection and training

Panelists were selected for proper aptitude on the basis of their availability and time, good health and no allergy to any food and willingness to participate by interviewing them individually. The sensory panel used in Canada were selected from the employees who were working in the Saskatchewan Food Industry Development Centre Inc. (Food Centre), Saskatoon Canada. Panel consisted of seven female panelists and five male panelists aged between 25 to 50 and with 1- 20 years of experience in the sensory assessment of food products.

For the sensory panel used in Sri Lanka, the 12 panelists recruited (six male and six female) were in the same age range of the group of the panelists selected in Canada. The panelists were selected from the employees of the Keells Food Products PLC., Ja-Ela, Sri Lanka and they were also had 1-20 years of experience in sensory evaluation of meat products.

Panel training was performed in three sessions each lasting 1-2 h, over a period of three days and included group discussions and individual ratings. The panelists were seated at a conference-type table to facilitate communication. During the training sessions the panelists received detailed explanation of the methodology of the assessments and description of the sausages, familiarized with the description of the evaluation procedure and lexicons, and agreed on precise definitions of the sensory attributes and sample size for the evaluation and finally the use of the score cards to assess sausages. The test products were included in the training sessions; however, the panelists were unaware that those are the products that they would evaluate in the test.

(b) Sample preparation and testing

Samples of sausages were prepared by placing them in boiling water for about 5 min until the internal temperature reached >80°C. The temperature was monitored using a cooking thermometer inserted lengthwise in one of the sausages in each heating. Sausages from each formulation was boiled in separate vessels and prepared in random order. After draining the water, the sausages were placed in stainless steel dishes and covered with a sheet of aluminum foil. The cooked samples were then kept in an incubator set at 70°C to keep them warm for about 30 min. Just before serving the sausages were cut into 2.5 cm long pieces. Samples (three pieces from each sample) were placed in 50 mL plastic cups coded with three-digit random numbers, covered with plastic lids and served warm.

Evaluation was done on each production replication on three separate days to minimize the flavor carryover and fatigue effects. In order to reduce the session-to-session variation, samples from all treatments were evaluated in one session (45 min sessions). Panelists evaluated samples from only five formulations. The control formulation was not included in the study as our objective in sensory evaluation was to evaluate the effectiveness of lentil flour as a binder in meat products in comparison to the commercial ingredients; isolated soy protein and modified corn starch and further to reduce the sensory load on the panelists. Between the samples, the panelists were asked to cleanse the palate by eating a piece of unsalted cracker and drinking a mouthful of water. A total of 21 attributes categorized in the order of perception; first sight and touch, first bite with front teeth, chewing with molar teeth and after swallow were evaluated using 8-point rating scales.

In the Food Centre, Canada, the sensory evaluations were performed in a sensory lab with partitioned booths, free from noise and odors, and under white fluorescent light. Whereas at the Keells Food Products PLC, Sri Lanka, panelists worked in a room at separate tables as this facility did not have a sensory lab with partitioned sensory booths. However, the room environment was maintained free from noise, odor and samples were evaluated under white fluorescent light.

6.4.14.2 Consumer affective test

The same sausage treatments assessed in the descriptive analysis were evaluated for their acceptability in the consumer test. The consumer tests were performed both in Canada and Sri Lanka using three consumer groups.

(a) Recruitment of panelists

The three consumer groups, each consisted of 60 panelists were employed. These groups were Group SL: Sri Lankan consumers who were born and living in Sri Lanka, Group SL-CA: Sri Lankan consumers who were born in Sri Lanka but living in Canada and Group CA: Canadian consumers who were born and living in Canada.

The consumers for the group SL was recruited from the faculty, staff and students of the Wayamba University of Sri Lanka. Consumer group SL was comprised of 25 males and 35 females aged between 18-60 years. The group SL-CA and CA were comprised of the consumers from the staff and students of the University of Saskatchewan and general public whom were selected to closely match the demographics of group SL. The groups SL-CA and CA were comprised of 24 male: 36 female and 23 male: 37 female ratios, respectively and were within the same age range. During the recruitment of the panelists for all 3 groups the following primary selection criteria were also considered. The consumer panelist should be at least 18 years of age, not allergic to any food or food ingredient, available to participate in the test on a particular testing date and time, regular consumers of meat (poultry) products and willingness to participate.

(b) Sample preparation and testing

Samples of sausages were prepared and served according to the same procedure used in the descriptive analysis. Samples from the five formulations were evaluated in one session which last about 30 min. Prior to the product evaluation the participants were asked to complete a consumer survey questionnaire which included questions related to demographic and socioeconomic status such as age, gender, education level and household income. A few questions were included to assess their knowledge of the health benefits of the incorporation of lentils in meat products and the awareness of the use of additives in meat products and their food purchasing behavior.

The groups SL-CA and CA conducted the sensory evaluations at the University of Saskatchewan in a room specially designed for sensory studies with separated booths, positive air flow, free from odors and under fluorescent light (400 lux). The consumer analysis in Sri Lanka was carried out at the University of Wayamba, Sri Lanka. The participants were seated in an air-conditioned room, with separate tables, free from distracting odors and noise and under fluorescent lighting.

The participants were instructed on and provided with a written procedure to be followed and sensory descriptors. The samples were served warm, in random order and coded with 3-digit number. Participants were asked to taste and evaluate the sensory attributes of products on a 6-point scale.

6.4.15 Data analysis

All experiments except the consumer study were performed in triplicate. Statistical analysis of was conducted using SAS version 9.4 (SAS Inst. Inc., Cary, N.C., USA). Analysis of variance (ANOVA) followed by multiple comparisons using Tukey's test were performed for significant differences in means for chemical, textural and sensory properties among samples. The results are expressed as the mean and SEM. The differences were considered significant at $p < 0.05$ level. Repeated measures ANOVA was performed to analyze the treatment and storage time effects on TBARS values and Pearson correlation analysis was performed to analyze the correlations between variables. To examine the possibility of explaining the variation within the samples with fewer parameters, principal components analysis and hierarchical cluster analysis was done for descriptive and consumer data using FactoMineR in R statistical system 2.15.1.

6.5 Results and Discussion

6.5.1 Raw batter viscosity

Mean values for the viscosity of raw sausage batters are given in Table 6.2. Batter viscosity is a measure of the resistance of meat emulsion to flow and it determines the flow behavior of the product before it cooks. Viscosity influences the texture of cooked product as well as ease of handling during processing. The viscosity of meat batters ranged between 2.02 x 10^4 and 5.36 x 10^4 cP. Batter viscosity increased gradually with the increasing concentration of lentil flour, which could be due to the slightly reduced water content in the formulation and the increased water binding ability of the batter. However, viscosities in formulations with ISP+CS and 4% lentil flour were not different from no binder control. Commercial sausage formulation (ISP+CS) showed a viscosity similar to the batters with 4% and 6% lentil flour indicating that ISP plus corn starch and lentil flour up to 6% have similar effect on batter viscosity. The sausage formulation with the highest lentil flour content (8% LF) showed the highest viscosity (p<0.05) which was significantly different from the no-binder control and commercial formulation (ISP+CS).

Table 6.2 Effect of binders on viscosity of raw meat batter (n=3)

Treatment	Viscosity (cP) x 10^4	Temperature (^{o}C)^{NS}
No-binder	2.02^c	4.3
ISP+CS	3.09^{bc}	4.8
4%LF	3.77^{abc}	4.8
6%LF	4.17^{ab}	3.6
8%LF	5.36^a	4.8
4%LF-NO2	3.09^{bc}	4.6
SEM[1]	0.399	

Values are presented as means
$^{a-c}$Means within the same column with different superscripts are significantly different (p<0.05)
^{NS} No significant difference among treatments within the column
[1]Standard error of mean
No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

In the present study, all formulations except no binder control had the same starch content. Although, the total starch content was similar among the formulations with binders, the content of starch coming from lentil were 0, 1.82, 2.73, 3.64 and 1.82, for ISP+CS, 4%LF, 6%LF, 8%LF and 4%LF-NO2, respectively. Therefore, the differences observed in batter viscosity could be attributed to the functional properties of the protein fraction or the type of starch. In agreement, Sanjeewa et al. (2010), observed that the batter viscosity was significantly affected by the type and level of flour added. They reported that the addition of chickpea (Kabuli and Desi cultivars) and pea flour at 5% resulted in significantly higher values for viscosity than that of the no binder control and the 2.5% level of flours. In addition, IR heated starch has a higher water holding capacity (Der, 2010), which may increase batter viscosity.

6.5.2 Water holding properties

Data for the water holding properties of cooked sausages are presented in Table 6.3. Cook loss is an important parameter for the meat industry in predicting the product behavior influenced by non-meat ingredients during cooking (Pietrasik and Chan, 2002). Denaturation of meat proteins during cooking decreases water holding capacity (WHC), in contrast the incorporation of extenders can help improve the WHC in comminuted meat products (Petracci et al., 2013). The inclusion of binders tended to decrease the cook loss in sausages even though the differences among treatments were not high enough to be significant (P>0.05).

Cook loss of the sausages ranged between 16.63% and 18.33%. In contrast to these results, a significant decrease in cook loss was reported for low fat beef burgers extended with IR treated lentil and chickpea flour compared to the control (Shariati-Ievari, 2016). Similarly, Dzudie et al. (2002) observed lower cook loss in beef sausages formulated with common bean flour. However, Sanjeewa et al. (2010) observed similar cook loss in bologna with 2.5% chickpea, pea and wheat flour and control (no binder) showing that the addition of those flours at 2.5% level had no significant effect on cook loss. The cook loss values observed in the present study (16.63% and 18.33%) were higher than those reported by Choi et al. (2010) for pork frankfurters added with vegetable oil and rice bran (6.59%). The cooking yield of sausages depends on the cooking temperature, cooking time and the ingredients (Hughes et al., 1997; Huang et al., 2005; Kim and Chin, 2007; Banon et al., 2008). The water bath cooking practiced in sausage manufacturing

Table 6.3 Effect of binders on water retention properties of cooked chicken sausages during refrigerated and frozen storage (n = 3)

Treatment	Cook loss[NS] (%)	Purge loss (%)			Expressible moisture (%)		
		14 days at 4°C	28 days at 4°C	56 days at -18°C	0 days	28 days at 4°C	56 days at -18°C
No-binder	18.33	2.20[a]	6.82[a]	17.10[a]	16.68[a]	23.91[a]	17.00[a]
ISP+CS	17.27	0.71[ab]	1.20[b]	3.10[b]	8.19[b]	14.81[b]	9.06[bc]
4%LF	17.25	0.81[ab]	1.00[b]	7.38[b]	8.58[b]	12.52[bc]	9.40[bc]
6%LF	16.96	0.69 [ab]	1.37[b]	5.18[b]	8.70[b]	9.29[bc]	8.29[bc]
8%LF	16.87	0.41[b]	1.04[b]	5.07[b]	8.87[b]	8.40[c]	7.25[c]
4%LF-NO2	16.63	0.88[b]	1.09 [b]	6.65[b]	9.67[b]	12.19[bc]	9.93[b]
SEM[1]	1.597	0.340	0.276	2.1047	0.664	1.234	0.466

[a-c]Means within the same column with different superscripts are significantly different (p<0.05)
[NS] No significant difference among treatments within the column
[1]Standard error of mean
No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

of these studies as well as the differences in ingredients probably have resulted lower cook loss compared to our study. The smoke house cooking can result in higher cook loss compared to water bath cooking due to the air flow, and the addition of binders either ISP, corn starch or lentil flour were not able to cause a significant change in cook loss.

Purge loss determined after 14 days of storage ranged between 0.41% to 2.20% (Table 6.3). Purge loss decreased slightly as the amount of lentil flour increased; however, differences were not large enough to be significant. Formulations with lentil flour at 4% level did not decrease the purge loss compared to the control (No-binder). However, the sausages extended with 6% and 8% lentil flour had significantly low purge loss (p<0.05) compared with the control treatment at 14 days of storage. Moreover, results revealed that the purge loss of the commercial formulation (ISP+CS) was similar to the formulations containing lentil flour. During the 28 days of refrigerated storage considerable increase in purge loss were noted in the no-binder control samples. The addition of binders decreased the purge loss significantly in sausages probably due to the ability to keep the moisture in the matrix. This observation was previously reported for various meat products. The

addition of chickpea, pea and wheat flour in low fat pork bologna had reduced the purge significantly compared to the bologna without binders (Sanjeewa, 2008). Similarly, increased water retention was reported for buffalo meat burgers extended with soya, Bengal gram, and green gram flours (Modi et al., 2003) and chicken nuggets extended with cowpea and peanut flour (Prinyawiwatkul et al., 1997). Reducing high purge losses are economically significant not only because of loss of weight, but because the lost water contains high quality meat proteins (Aberle et al., 2012). High purge loss may lead to dryness and toughness of the products. After 56 days of frozen storage, a considerable increase in purge loss was observed for all samples. However, still the purge loss was significantly higher in the no-binder control sample (17.10%). Purge loss of samples extended with binders ranged between 3.10% and 7.38% and significant differences were not noted among the samples irrespective of the level of lentil flour added and the lentil flour added samples showed water holding capacity similar to the commercial formulation (ISP+CS). Increased purge loss observed in all samples might be linked to the structure damage attributable to freezing.

Expressible moisture values measured in terms of the percent amount of moisture removed from the samples due to centrifugal force are illustrated in Table 6.3. The addition of binders reduced the expressible moisture significantly compared to the no-binder control. There was no significant difference in initial expressible moisture between the sausages extended with binders which had expressible moisture values ranging from 8.19% to 9.67%. These values were significantly ($p<0.05$) lower than that of no- binder control (16.68%). The expressible moisture values of present study were lower than those reported for other plant binders (Sanjeewa et al., 2010; Wei, 2019) even at fairly similar product protein contents (13% -15%) showing that a strong matrix had been formed with good water holding capacity. Sanjeewa et al. (2010) reported expressible moisture values ranging from 10.28 % to 12.52% for low fat pork bologna extended with Desi chick pea (5% level) and pea flour (5% level); whereas 11.27%, 10.80% and 9.77% were observed for pork bologna with pea starch, faba starch and faba protein, respectively, each added at 3% level (Wei, 2019). These differences might be partly due to the differences in the formulations such as meat species and added water. The storage at refrigerated and frozen conditions resulted in a significant increase in expressible moisture in control samples. The expressible moisture contents were 23.9% and 17.00% in the no-binder control samples stored at 4°C (28 days) and -18°C (56 days), respectively. Expressible moisture contents remained significantly lower in samples with binders compared to the control even after both refrigerated

and frozen storage. This shows that, water added appeared to be satisfactorily retained within the meat matrix by the added binders. Meat protein could form complex 3D gel network involving electrostatic forces, hydrogen bonding, van der Walls forces and entanglement which bind water and entraps flours added as binders (Aberle et al., 2012, Sanjeewa et al., 2010). Starch and protein from legume and cereal flours are biological molecules and also could imbibe water and form gels upon heating (Tolstoguzov and Braudo 1983; Li and Yeh, 2003, Sanjeewa et al., 2010). Therefore, flour components added may contribute to hold water and fat within the meat matrix during cooking and storage.

The commercial formulation which contained ISP and modified corn starch showed similar expressible moisture to the sausages extended with lentil flour indicating that both types of binders function similarly to retain moisture and fat in sausages. However, the sausages extended with 8% lentil flour (8%LF) showed significantly lower expressible moisture content compared to the commercial formulation (ISP+CS) after the storage at 4°C for 28 days, indicating a particular advantage for this level of lentil flour.

Part of the explanation for this observation could be the differences in the composition of starch between the two formulations. The functionality of starch may differ owing to the differences in botanical origin (Li and Yeh, 2003). Although the total starch content of sausages was consistent between these treatments, ISP+CS contained starch from corn whereas in 8%LF major portion of starch was from lentil. This may indicate that the lentil starch had better ability to retain its water holding properties during the storage under refrigerated conditions. The lentil and corn starch differ on amylose to amylose pectin ratio. The amylose content of lentil and corn starch were 32% and 25%, respectively and the higher amylose to amylopectin ratio was associated with the lower degree of crystallinity and enthalpy of gelatinization. (Joshi et al., 2013). Moreover, Joshi et al. (2013) reported that lentil starch gels exhibited higher viscoelastic solid properties suggesting the formation of stronger gels compared to corn starch.

Studies have shown that incorporating extenders with high protein content leads to a more stable meat protein matrix that reduces the loss of water and fat (Serdaroglu et al., 2005, Sanjeewa, 2008). In the present study, sausages containing 8% lentil flour had a higher protein content (p<0.05) than commercial formulation (Table 6.4). Thus, the lower expressible moisture content observed in 8%LF could also be influenced by the protein content.

6.5.3 Proximate composition and pH cooked sausages

The proximate composition and pH of cooked sausages prepared in Canada and Sri Lanka are shown in Table 6.4. The proximate composition of sausages prepared at both locations exhibited the same trend for all formulation. The ANOVA reveled significant differences only for moisture and protein content across the formulations. Products had a moisture, protein fat and ash content ranging from 66.67 - 74.14%, 12.84 - 16.04, and 2.52 - 2.96%, respectively. The moisture content of flour added samples were significantly lower than that of the control treatment due to the replacement of meat with the binders. With respect to the protein content, ISP+CS, 6%LF and 8%LF formulations prepared in Sri Lanka had higher protein content (about 1% difference) than the corresponding values of sausages prepared in Canada. As the lentil flour level increased, the protein content also increased slightly, and the formulation with 8% lentil flour showed the highest protein content. Moreover, the protein content of this formulation was significantly higher than that of the commercial formulation (ISP+CS). Increasing the protein content is beneficial in the nutritional point of view, as well as the ability to increase the overall protein content of a product with less meat can reduce the cost of meat products. Further, from a health perspective, it is beneficial to substitute meat protein with plant protein as it can reduce the level of saturated fatty acids and cholesterol content of the product.

The fat and ash contents (%) in the no-binder control were 74.14 and 2.88, respectively. The addition of binders did not change the fat and ash content in the sausages ($p > 0.05$). However, the overall fat and ash content was higher in the sausages formulated in Sri Lanka than in Canada. This discrepancy may be due to slight differences in composition of ingredients, processing conditions and analytical equipment used between two locations.

The effects of the addition of binders and storage on the pH of sausages are shown in Table 6.5. The initial pH ranged from 6.43 to 6.49 and was not significantly ($p>0.05$) different among formulations and between the sausages produced at two locations. Moreover, pH did not change during storage at both refrigerated and frozen conditions. Overall, sausages prepared in both countries were not identical but close in composition.

Table 6.4 Proximate composition of cooked chicken sausages prepared in Canada and Sri Lanka (n = 3)

Treatment	Moisture	Protein	Fat [NS]	Ash [NS]	pH [NS] 0 days	pH [NS] 28 days at 4°C	pH [NS] 56 days at -18°C
Canada							
No-binder	74.14[a]	13.52[cd]	8.06	2.88	6.47	6.45	6.46
ISP+CS	70.02[b]	13.47[cd]	7.45	2.91	6.45	6.46	6.40
4%LF	67.62[cde]	12.90[d]	7.64	2.93	6.49	6.43	6.39
6%LF	68.23[cd]	13.70[c]	7.70	2.95	6.48	6.46	6.45
8%LF	67.17[e]	14.59[b]	7.46	2.96	6.43	6.43	6.41
4%LF-NO2	68.18[cd]	12.84[d]	7.61	2.86	6.49	6.38	6.43
Sri Lanka							
ISP+CS	68.36[c]	14.48[b]	8.08	2.52	6.37	ND	ND
4%LF	67.36[cde]	13.29[cd]	8.24	2.54	6.33	ND	ND
6%LF	67.73[cde]	14.80[b]	8.32	2.81	6.35	ND	ND
8%LF	66.67[e]	16.04[a]	8.07	2.46	6.44	ND	ND
4%LF-NO2	67.74[cde]	13.22[cd]	8.25	2.52	6.39	ND	ND
SEM[1]	0.232	0.160	0.442	0.153	0.022	0.051	0.016

Values are presented as means

Means within the same column with different superscripts are significantly different (p<0.05)

[NS] No significant difference among treatments within the column

[1]Standard error of mean

ND: not determined

No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

6.5.4 Texture profile analysis (TPA) of cooked sausages

The textural properties of comminuted meat products are influenced by the degree of extraction of myofibrillar proteins, content of stromal protein, degree of comminution and type and level of non-meat ingredients (Serdaroglu et al., 2005). In terms of the initial hardness (Table 6.5), the sausages with 8% lentil flour showed a higher hardness (p<0.05) compared to the control

Table 6.5 Effect of binders on TPA texture properties of chicken sausages (n = 3)

Texture attribute	Storage period	Treatment						
		No-binder	ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2	SEM[1]
Hardness (N)	0 days	8.37[b]	11.73 [ab]	12.40 [ab]	12.03 [ab]	12.83 [a]	10.93 [ab]	1.051
	28 days at 4°C	9.67[b]	12.60[a]	13.37[a]	13.17[a]	14.20[a]	11.70[ab]	1.115
	56 days at -18°C[NS]	14.03	19.33	20.87	17.60	19.27	16.73	2.063
Cohesiveness	0 days	0.64[a]	0.60[b]	0.59[b]	0.59[b]	0.58[b]	0.60[b]	0.010
	28 days at 4°C	0.63[a]	0.62 [ab]	0.60[ab]	0.60[ab]	0.59[b]	0.61[ab]	0.011
	56 days at -18°C[NS]	0.59[a]	0.58[a]	0.55[ab]	0.51[b]	0.53[ab]	0.56[ab]	0.016
Springiness (%)	0 days	77.36	78.53	78.24	77.87	77.63	77.42	1.116
	28 days at 4°C	78.05	80.62	80.29	79.23	78.99	79.12	1.579
	56 days at -18°C[NS]	80.32	79.78	80.12	76.74	78.74	79.35	1.851
Chewiness (Nmm)	0 days [NS]	22.84	32.53	32.5	32.33	33.87	29.62	3.572
	28 days at 4°C	26.15[b]	37.36 [ab]	38.20 [a]	37.43 [a]	39.65 [a]	33.21[ab]	5.525
	56 days at -18°C[NS]	41.17	56.88	60.40	46.96	51.04	46.94	7.465
WBSF[NS]	0 days	4.6	5.03	5.37	5.27	5.03	5.1	0.495
	28 days at 4°C	5.13	6.67	6.60	6.57	7.17	5.67	0.822
	56 days at -18°C[NS]	9.80	8.77	9.80	9.40	8.67	7.77	0.651

[a,b]Means within the same row with different superscripts are significantly different (p<0.05)
[NS] No significant difference among treatments within the row
[1]Standard error of mean
No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

formulation suggesting a strong structure formation despite the use of 6% less meat. However, the hardness did not increase significantly as the level of lentil flour in the formulations increased from 4% to 8 % likely as the total starch content was similar between treatments. Similar observation was also reported for low fat pork bologna extended with chickpea and wheat flour (Sanjeewa et al., 2010). They reported that these flours added at 2.5 and 5% levels had no significant effect on the hardness. In contrast, in the same study they found that the level of pea flour added had

significant effect on hardness of bologna. Those results indicated that the effect of binders on hardness of bologna could vary depending on the type of flour. However, in the present study significant differences in hardness were not found between the commercial formulation (ISP+CS) and other formulations with lentil flour. TPA hardness of sausages were significantly affected by both storage conditions. Hardness of stored samples were significantly higher than initial sample hardness which might be attributed to the purge loss. It also may reflect some starch retrogradation. During cold storage, amylose is more susceptible to retrogradation than amylopectin (Egharevba, 2019). Since, lentil starch contains more amylose than that of corn starch, more starch retrogradation could be expected in sausages containing more lentil flour.

In terms of cohesiveness (Table 6.5), addition of binders either the ISP and corn starch or lentil flour resulted in lower cohesiveness than those found in the no binder control. However, after the 28 days of refrigerated storage, only the formulation with 8% lentil flour had significantly lower cohesiveness than the control and cohesiveness values ranged between 0.59 and 0.63. In contrast, Sanjeewa (2008) reported that that the addition of chickpea flour increased the cohesiveness of pork bologna. However, addition of rice bran, faba starch and protein, carrageenan in meat products did not result in significant change in their cohesiveness compared to the control without binders. For the attributes of TPA chewiness, springiness and WBSF, no differences were reported among formulations (p>0.05) though, storage resulted in significant increase in chewiness and WBSF. Particularly, 56 days of storage at -18°C had the greatest effect on chewiness, springiness and WBSF, which could be associated to the moisture loss due to purge, starch retrogradation or movement of moisture with the protein matrix (Mortensen et al., 2006).

6.5.5 CIE color of cooked sausages

The data for the external and internal color of sausages are presented in Table 6.6. Meat color is one of the most important factors affecting consumers' decision when they purchase meat products. The color of cooked meat products is due to the combined effects of thermally denatured meat pigments blended with the other non-meat ingredients (Sanjeewa et al., 2010). Sodium nitrite is one of the common ingredients used in cured meat products which helps preserve the pink color of the product. However, nitrite is known to be potentially carcinogenic at certain processing or cooking conditions and at high concentrations (Bauer, 2014). Therefore, in the present study, one formulation (4%LF-NO2) was prepared without adding nitrite, in order to compare the consumer

Table 6.6 Effect of binders on the CIE color of cooked sausages (n = 3)

Treatment	External color			Internal color		
	L^{NS}	a*	b*	L^{NS}	a*	b*[NS]
No-binder	46.03	25.48[a]	29.25[a]	59.48	17.36[a]	14.82
ISP+CS	46.39	23.63[ab]	28.03[ab]	59.36	16.89[ab]	15.41
4%LF	44.55	20.97[bc]	26.32[ab]	56.92	14.45[bc]	14.80
6%LF	44.80	20.46[c]	25.74[b]	56.88	13.84[c]	15.16
8%LF	43.78	20.58[c]	27.39[ab]	58.33	13.07[c]	14.61
4%LF-NO2	44.89	17.02[d]	26.12[ab]	57.22	8.19[d]	16.05
SEM[1]	4.684	1.577	2.313	1.134	0.743	0.956

[a,b]Means within a same column with different superscripts are significantly different ($p<0.05$)
[NS]No significant difference among treatments within the column
[1]Standard error of mean
No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

preferences between cured and non-cured sausages and the differences in oxidative stability. The addition of lentil flour or the exclusion of nitrite had minor effect on the lightness (L*) of both external and internal color. The external L* value of sausages ranged between 43.78 and 46.39 and was darker than the internal color. Darker external color could be due to the brown color caused by smoking. The exclusion of the nitrite had no significant effect on either external or internal lightness.

Sausages formulated with lentil flour had lower a* (redness) values (20.46 to 20.97) than that of the no-binder control (25.48). The a* value of 4%LF was similar to the commercial formulation (ISP+CS) while other lentil flour added (6%LF and 8%LF) sausages had lower a* values. However, there was no significant difference between commercial formulation (ISP+CS) compared to no binder control. The lower a* value resulting due to the addition of lentil flour could be attributed to the dilution of myoglobin as there was less meat in these formulations and also the color masking effect from the binder color. The colour of ISP flour (L* = 85.07, a*= 4.94, and b * = 21.16) was close to that of lentil flour (L*= 85.81, a * = 3.29, and b* = 15.36), except for b*; however, the colour of corn starch was significantly lighter (L * = 96.71, a* = 0.49, and b * = 2.59)

than both ISP and lentil flour. Hence, the minimal impact on a* value observed in the commercial sausage (ISP+CS) was due to the higher proportion of corn starch in the formulation.

With regard to b*, higher values were found for external color. However, formulations had no significant effect on b* values. The omission of nitrite in sausage formulation had significant effect only on a* value. The external and internal a* values nitrite-free sausage (4%LF-NO2) were 17.02 and 8.19, respectively, and these values were significantly lower than any other nitrite-added formulations with values ranging from 20.46 to 25.48 and 13.07 to 17.36 for external and internal color, respectively. Previous studies have reported that legume and cereal binders show significant effect on color parameters of processed meat. Dzudie et al. (2002) noted that addition of 7.5–10% chickpea flour to beef sausages resulted in more yellowish products. Pietrasik & Janz, 2010 found that low fat pork bologna with 4% pea starch or wheat flour had higher a* value while wheat and barley flours had minimal effect on color of ultra-low fat pork bologna (Shand, 2000). In another study, Claus et al. (1990) noticed no significant change in the colour characteristics with beef bologna containing 2% ISP. These observations show that the effects of the addition of binders have varying effects on the color parameter of meat products which may depend on the type, concentration of the binders as well as the type of meat products. The possible explanations for the different results could be due to color imparted by the non-meat binders, dilution of the meat pigments, composition of the meat products and change in the light scattering properties associated with water and fat (Claus et al., 1991; Prinyawiwatkul et al., 1997; Serdaroglu et al., 2005; Pietrasik and Janz, 2010; Sanjeewa et al., 2010).

6.5.6. Correlations between water holding properties, instrumental texture properties and proximate composition

Table 6.7 presents the correlation coefficients among raw batter viscosity, water retention properties and proximate composition of sausages. Raw batter viscosity showed significant inverse relationship with purge loss (r = -0.68) and expressible moisture (r = -0.56). This may indicate that high viscous batters form strong gel structures with high water holding capacity. Moreover, negative correlation between purge loss and expressible moisture (r = -0.87) was observed indicating that expressible moisture would be an indicator of purge loss in meat products. In agreement, similar relationship was reported for batter viscosity and water holding properties in pork bologna (Sanjeewa, 2008). Protein content showed positive correlation with batter viscosity

Table 6.7 Correlation coefficients (r) among batter viscosity, water holding and texture properties and proximate composition (n=18)

	1	2	3	4	5	6	7	8	9	10	11	12
1. Viscosity	1											
2. Purge loss	-0.68**											
3. Expressible moisture	-0.56*	0.87***										
4. Cook loss	-0.17	0.09	0.17									
5. Hardness	0.58**	-0.73**	-0.67**	0.38								
6. Cohesiveness	-0.42	0.77***	0.80***	0.05	-0.55*							
7. Springiness	0.34	-0.16	-0.07	0.27	0.47*	0.35						
8. Chewiness	0.58**	-0.62**	-0.56*	0.44	0.93***	-0.33	0.68**					
9. WBSF	0.51*	-0.30	-0.21	0.09	0.41	0.08	0.71**	0.60**				
10. Ash	0.42	-0.21	0.01	0.16	0.26	0.21	0.60**	0.47*	0.54*			
11. Fat	-0.34	0.35	0.44	0.78**	0.01	0.17	0.07	0.12	0.13	0.15		
12. Protein	0.66**	-0.85***	-0.22	0.04	0.29	-0.45	-0.09	0.19	-0.11	0.14	-0.13	
13. Moisture content	-0.68**	0.84***	0.83***	0.21	-0.61**	0.74***	-0.02	-0.49*	-0.26	-0.17	0.41	-0.10

*, **, *** = Significant at P<0.05, 0.01 and 0.001, respectively.

(r = 0.66) and inverse relationship with purge loss (r = 0.85) showing the water holding effects of protein. Among the TPA texture properties, chewiness was correlated with hardness (r = 0.93) and springiness (r = 0.68).

6.5.7 Lipid oxidation

The influence of formulation and storage condition on lipid oxidation in chicken sausages is presented in Figure 6.1. The initial TBARS values ranged between 0.50 and 0.62 mg MDA/kg sample and no significant differences were found among formulations. During storage at 4°C, TBARS values of all formulations increased gradually, however, throughout the 42 days of storage TBARS values did not differ among the formulations (Figure 6.1a). It is noteworthy, that the TBARS values of the products formulated without nitrite (4%LF-NO2) was similar to the other formulations with nitrite.

Storage temperature had significant effect on TBARS as shown by considerably lower TBARS in frozen samples compared to refrigerated samples (Figure 6.1b). Similar to the samples stored at 4°C the TBARS values of the frozen samples increased gradually during the storage period. After 56 days of storage, TBARS values in samples ranged from 0.73 and 0.87 mg MDA/kg sample, which indicated only small oxidative changes in all mechanically separated chicken sausages.

Moreover, no significant differences were observed among the formulations and all samples had TBARS values below 2 mg MDA/kg product. The TBARS value is used as a measure of lipid oxidation in meat products and the rancid flavor is detected in meat products at TBARS values above 2 mg MDA/kg product (Greene and Cumuze, 1982). It was noted that the TBARS values of the no- nitrite formulation (4%LF-NO2) was comparable to the other formulations under both refrigerated and frozen storage conditions. This indicated that the exclusion of nitrite in the sausages extended with lentil flour had no significant negative effect on their oxidative stability. The antioxidant activity of nitrite is associated with its activity as a free radical scavenger and autoxidation chain reaction terminator (Aberle et al., 2012) and similar antioxidant activity exhibited by lentil flour to nitrite could be attributed to its phenolic compounds. Comparable antioxidant activity of lentil flour incorporated bologna has also been found in Study III (Section 5.5.8) in the present work. The chicken bologna added with 6% lentil flour showed significantly lower TBARS values during the 56-days storage at 4°C (0.86 mg MDA/kg) compared to the control
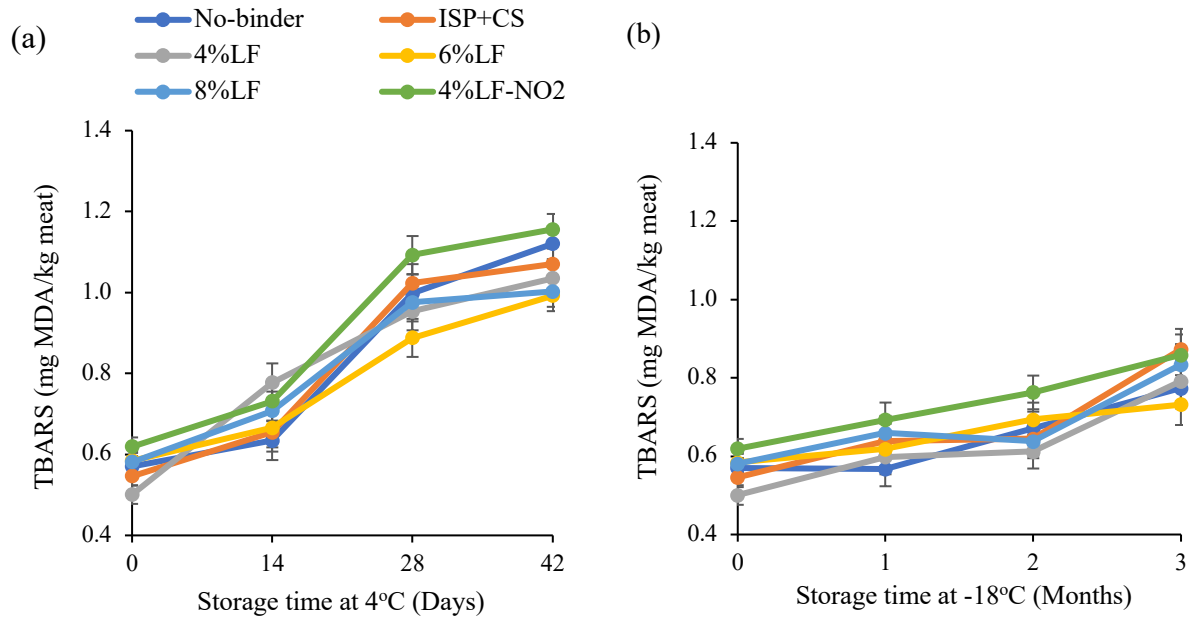
Figure 6.1 Effect of binders and storage on TBARS values of chicken sausages (a) storage at 4°C and (b) storage at -18°C

No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

prepared without binders (1.83 mg MDA/kg). Antioxidant potential of lentil flour on oxidative stability of meat products was also supported by other authors (Der, 2013; Pathiratne, 2014; Li 2017). According to Li (2017) who studied the effect of lentil flour added at 6% level in beef burgers, the samples with lentil flour had TBARS values lower than 2 mg MDA/kg sample while TBARS values were 2.35-3.05 mg MDA/kg sample for control burgers on week 12 of frozen storage.

Similar control of lipid oxidation by adding lentil flour in beef burgers was reported by Pathiratne (2014). These studies also suggested that the antioxidant activity of lentil flour was mainly coming from phenolic compounds (Oomah et al., 2011; Der, 2013; Pathiratne, 2014; Li, 2017). The water-soluble total phenolic compounds, total flavonoids and condensed tannin in lentil flour in the present study were found to be 2.14 (mg GAE/g), 0.72 (mg CE/g) and 1.12 (mg CE/g), respectively (Table 6.8). These results are consistent with the results reported by Pathiratne (2014) and Li (2017). They found that the water-soluble phenolic compounds in whole lentil flour was

Table 6.8 Phenolic contents and antioxidant activities of water extracts of IR treated lentil flour

TPC[1] (mg GAE/g)	TFC[2] (mg CE/g)	CTC[3] (mg CE/g)	DPPH (%)[4]	ABTS (%)[5]	MC (%)[6]	LP (%)[7]
2.14 ± 0.12	0.72± 0.06	1.12± 0.09	47.71 ± 1.45	50.94 ± 1.94	54.23± 1.50	30.45± 1.62

Values are presented as mean ± standard deviation

[1]TPC: total phenolic content expressed as mg gallic acid equivalents/ g sample, [2]TFC: Total flavonoid content expressed as mg (+)-catechin equivalents/ g sample, [3]CTC: Total condensed tannin content expressed as mg (+)-catechin equivalents/ g sample

[4]DPPH free radical scavenging activity, [5]ABTS free radical scavenging activity, [6]Fe^{2+} ion chelation activity, [7]Inhibition of phospholipid peroxidation

1.98 - 4.21 mg GAE/g sample. Pathiratne (2014) investigated the effect of seed tempering moisture and IR heating temperature on the phenolic compound and antioxidant activity of lentil flour. She concluded that there is a decrease in total phenolic compounds with increasing seed tempering moisture and increasing IR heating temperature, while hydroxyl radical scavenging activity, superoxide radical scavenging activity, and ferric ion reducing power was not affected. The TPC of lentil flour from non-IR heat treated and IR heat treated to 115°C (23% tempering moisture) lentil seeds were 3.37 mg GAE/g and 2.18 mg GAE/g, respectively.

Phenolic groups are excellent nucleophiles thus, they can act as free radical scavengers and metal ion chelators which cause oxidation (Han & Baik, 2008). In the present study, the water extracts of IR-treated lentil flour exhibited free radical scavenging activity of 47.71% and 50.94% as measured by DPPH and ABTS radical scavenging activity, respectively. The Fe^{2+} ion chelation activity was found to be 54.23%. These results indicate that the IR-treated lentil flour has antioxidant potential associated with free radical scavenging activity and metal ion chelation ability. Therefore, the application of lentil flour as a binder in meat products bring more advantages because of the multiple functions they provide.

6.5.8 Sensory properties
6.5.8.1 Descriptive analysis

One of the major concerns about the utilization of plant ingredients in meat products is their effect on sensory properties. In the present study, the sensory analysis was carried out using semi-trained descriptive panels. The average panelist ratings of descriptive analysis are shown in Table 6.9. As can be seen in Table 6.9, significant differences between sausages with regard to the external and internal color were found by both panels (Figure 6.2). The sausages prepared without

adding sodium nitrite (4%LF-NO2) had significantly lower desirability score compared to other sausages. Both groups scored the color of 4%LF-NO2 treatment as "slightly undesirable". The concentration of lentil flour (4%, 6% and 8%) in the formulation did not affect the color acceptability as evaluated by CA-T panel and the color of the lentil flour added sausages were similar to the color of the commercial formulation (ISP+CS). However, the SL-T panel rated the formulations with 6% and 8% lentil flour as less desirable with respect to the internal color while no significant difference was noted between the commercial formulation and the formulation with 4% lentil flour. The commercial formulation and lentil flour added formulations except 4%LF-NO2 were scored between "moderately desirable" and "very desirable" in internal color.

No significant differences among the sausages with regard to the texture attributes (harness, cohesiveness, springiness and denseness) were noted by the CA-T panel. These observations were consistent with the results of instrumental texture profile analysis which showed no significant difference among different treatments. However, SL-T panel were able to detect slight differences (one-unit difference) in hardness, cohesiveness and denseness of the sausages. The hardness, which was evaluated as the force required to bite through the sample, were found to be higher in 8%LF compared to the treatments with lower concentration of lentil flour (4%LF and 4%LF-NO2) and ISP+CS. The chewiness of the products was evaluated as the number of chews necessary to prepare sample for swallowing and cohesiveness was defined as the degree to which sample deforms before it ruptures. Similar to the hardness, 8%LF received higher scores for cohesiveness and chewiness compared to ISP+CS and formulations with 4% lentil flour (4%LF and 4%LF-NO2). The differences in the textural properties across the formulations could be attributed to the differences in the composition of the proteins and starch, although all formulations had consistent total protein and starch content. In support of the observations of the present study, Sanjeewa, (2008) reported that low fat pork bologna with 5% Kabuli and Desi flour obtained scores representing firmer texture than control with no binders and bologna with 5.0% wheat or 5.0% pea flour. Shand (2000) observed that low fat pork bologna with wheat flour or potato starch was significantly firmer than control group. Gramatina et al. (2012) reported that addition of 11% lentil flour increased hardness scores of pork sausages compared to the no binder control.

Table 6.9 Effect of binders on sensory properties of chicken sausages evaluated by Canadian (CA-T) and Sri Lankan (SL-T) descriptive panels (n=12 per panel x 3 replicates)

Sensory attribute	Panel	Treatment					
		ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2	SEM[1]
External color[y]	CA-T	6.67[a]	6.28[a]	6.28[a]	5.86[a]	4.64[b]	0.254
	SL-T	6.44[a]	6.00[b]	5.75[b]	5.86[b]	4.17[c]	0.084
Internal color	CA-T	6.56[a]	6.28[a]	6.00[a]	5.72[a]	3.83[b]	0.218
	SL-T	6.39[a]	6.47[a]	5.83[b]	5.78[b]	4.08[c]	0.083
Surface moisture[y,z]	CA-T[NS]	4.58	4.58	5.14	4.58	5.31	0.214
	SL-T[NS]	5.33	5.25	5.31	5.08	5.03	0.179
Surface smoothness[y,z]	CA-T	4.56[b]	5.39[ab]	5.67[ab]	5.39[ab]	6.28[a]	0.356
	SL-T[NS]	5.72	5.69	5.83	5.67	5.75	0.121
Springiness[y]	CA-T[NS]	5.69	5.39	5.86	5.67	5.94	0.265
	SL-T[NS]	5.47	5.33	5.25	5.47	5.31	0.07
Hardness[z]	CA-T[NS]	5.75	5.42	5.17	6.00	4.94	0.269
	SL-T	5.06[b]	4.83[b]	5.47[b]	6.42[a]	4.97[b]	0.176
Cohesiveness[y]	CA-T[NS]	5.64	5.31	5.81	5.75	5.83	0.181
	SL-T	4.97[b]	5.03[b]	5.14[ab]	5.58[a]	4.89[b]	0.101

Values are presented as mean

[a,b]Means within a same column with different superscripts are significantly different (*p*<0.05)

[NS]No significant difference among treatments within the column

[1]Standard error of mean

[y and z] Panel effect and Treatment x Panel interaction effect is significant, respectively

No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

CA-T: Semi-trained panel in Canada, SL-T: Semi-trained panel in Sri Lanka

External color desirability: 8 = extremely desirable, 1 = Extremely undesirable

Internal color desirability: 8 = extremely desirable, 1 = Extremely undesirable

Surface moisture: Extremely moist: 8 = extremely moist, 1 = Extremely dry

Surface smoothness: 8 = extremely smooth, 1 = Extremely rough

Springiness: 8 = extremely elastic, 1 = Extremely rigid

Hardness: 8 = extremely firm, 1 = Extremely soft

Cohesiveness: 8 = Extremely cohesive, 1 = Extremely crumbly

Table 6.9b Effect of binders on sensory properties of chicken sausages evaluated by Canadian (CA-T) and Sri Lankan (SL-T) descriptive panels (n=12 per panel x 3 replicates)

Sensory attribute	Panel	Treatment					
		ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2	SEM[1]
Uniformity	CA-T[NS]	5.75	5.61	5.94	5.69	6.19	0.333
	SL-T[NS]	5.67	5.83	5.78	5.64	5.36	0.110
Juiciness at first bite[y]	CA-T[NS]	5.39	6.03	5.14	4.92	5.25	0.277
	SL-T[NS]	5.86	5.92	5.83	5.75	5.72	0.117
Denseness	CA-T[NS]	5.22	5.14	5.25	5.67	5.64	0.305
	SL-T	5.00[c]	5.06[bc]	5.47[ab]	5.64[a]	5.00[c]	0.094
Chewiness[y]	CA-T[NS]	5.97	5.61	5.97	5.78	5.44	0.119
	SL-T	5.06[b]	5.11[b]	5.64[a]	5.72[a]	5.22[ab]	0.105
Juiciness[y]	CA-T[NS]	5.42	6.14	5.25	5.08	5.31	0.313
	SL-T[NS]	5.58	6.17	5.5	5.53	5.86	0.19
Graininess[y]	CA-T[NS]	4.72	4.69	4.67	4.75	4.14	0.272
	SL-T[NS]	4.28	3.64	4.50	4.44	3.64	0.345
Skin[y]	CA-T[NS]	6.11	5.83	5.83	6.03	5.08	0.198
	SL-T[NS]	4.94	5.11	5.31	5.19	4.89	0.112

Values are presented as mean

[a-c]Means within a same column with different superscripts are significantly different ($p<0.05$)

[NS]No significant difference among treatments within the column

[y and z] Panel effect and Treatment x Panel interaction effect is significant, respectively

[1]Standard error of mean

No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

CA-T: Semi-trained panel in Canada, SL-T: Semi-trained panel in Sri Lanka

Uniformity: 8 = Extremely even, 1 = Extremely uneven

Moisture release: 8 = Extremely moist, Semi 1 = Extremely dry

Denseness: 8 = Extremely compact, 1 = Extremely loose

Chewiness: 8 = Extremely chewy, 1 = Extremely mushy

Moisture release: 8 = Extremely juicy, 1 = Extremely dry

Graininess: 8 = Extremely grainy, 1 = Extremely smooth

Skin formation: 8 = Extremely distinct, 1 = Extremely indistinct

Table 6.9c Effects binders on sensory properties of chicken sausages evaluated by Canadian (CA-T) and Sri Lankan (SL-T) descriptive panels (n=12 per panel x 3 replicates)

Sensory attribute	Panel	Treatment					
		ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2	SEM[1]
Saltiness[y]	CA-T[NS]	5.53	5.69	5.53	5.47	5.64	0.202
	SL-T[NS]	5.03	5.03	5.28	5.28	5.19	0.100
Overall flavor intensity[y]	CA-T[NS]	5.61	5.64	5.39	5.67	5.47	0.257
	SL-T[NS]	5.28	5.08	5.36	5.58	4.97	0.193
Foreign flavor intensity[y]	CA-T[NS]	5.25	5.69	5.72	5.56	5.42	0.299
	SL-T	3.36[d]	3.56[d]	4.33[c]	5.19[a]	4.78[b]	0.076
Ease of swallow[y,z]	CA-T[NS]	5.72	6.19	6.22	5.72	6.83	0.16
	SL-T[NS]	5.61	5.81	5.69	5.92	5.64	0.082
Mouth coating[y]	CA-T[NS]	3.39	3.11	3.11	3.47	3.06	0.159
	SL-T	4.47[b]	4.25[ab]	4.39[ab]	4.31[ab]	4.14[b]	0.066
Aftertaste[y,z]	CA-T[NS]	4.69	4.67	4.53	4.44	4.61	0.158
	SL-T	3.61[b]	4.28[a]	4.58[a]	4.44[a]	4.31[a]	0.14
Overall acceptability[z]	CA-T[NS]	5.89	6.06	6.19	5.50	5.47	0.196
	SL-T	6.42[a]	6.33[a]	6.14[a]	5.64[a]	4.42[b]	0.169

Values are presented as mean

[a-c]Means within a same column with different superscripts are significantly different (*p*<0.05)

[NS]No significant difference among treatments within the column

[1]Standard error of mean

[y and z] Panel effect and Treatment x Panel interaction effect is significant, respectively

No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

CA-T: -trained panel in Canada, SL-T: Semi-trained panel in Sri Lanka

Saltiness: 8 = Extremely intense, 1 = Extremely bland

Overall flavor intensity: 8 = Extremely intense, 1 = Extremely bland

Foreign flavor intensity: 8 = Extremely bland, 1 = Extremely intense

Ease of swallow: 8 = Extremely easy, 1 = Extremely hard

Mouth coating: 8 = Extremely high, 1 = Extremely low

Aftertaste: 8 = Extremely intense, 1 = Extremely bland

Overall acceptability: 8 = Extremely acceptable, 1 = Extremely unacceptable
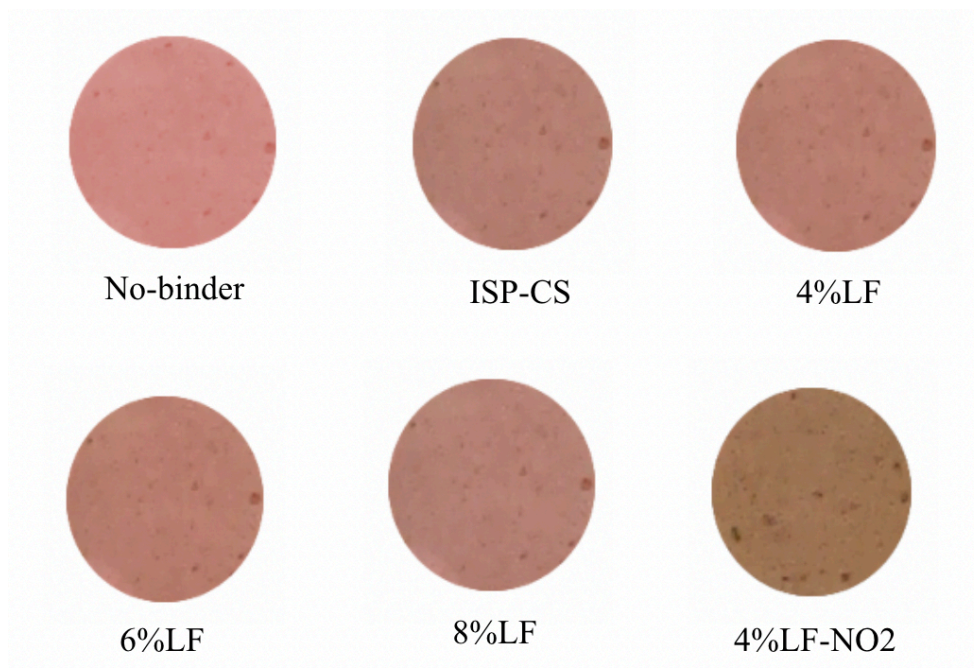
Figure 6.2 Internal color of chicken sausages

No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

All sausages were similar in surface moisture (degree to which the surface is moist or oily) which is related to the water and oil holding properties of the sausage matrix. "Slightly moist" surface moisture score showed that all formulations have fairly strong moisture retention properties.

The CA-T panel found that the lentil flour added sausages had smoother surface than the commercial sausage, however, SL-T panel did not distinguish significant differences between ISP+CS and lentil flour added sausages (4%LF, 6%LF, 8%LF and 4%LF-NO2) with regard to surface smoothness.

No significant differences between the sausages with lentil flour and ISP were noted by both panels with respect to the juiciness perceived at the first bite. All sausages received scores closer to 6 (moderately juicy) on the 8-point scale. Part of the explanation for this likely involves the relatively similar moisture content across different formulations which ranged between 67.17% and 70.02%. Non-meat extenders and binder are usually added in meat products to improve their water holding properties. The water holding properties of legume flours may depends on the concentration of protein, starch and fiber (Farouq et al., 2002). In the present study, all formulations of sausages contained reasonably similar concentrations of protein and starch and it seemed they

have same water holding properties. Furthermore, these results showed that the water retention properties of ISP and corn starch were similar to those of lentil flour and corn starch at the concentrations added.

Grainy texture score indicates the degree to which a sample contains small particles. Xu (2017) reported that inclusion of pea fiber fractions in bologna increased the graininess scores while bolognas with finer particle fiber products were given lower scores. However, in the present study the addition of lentil flour had no significant effect on the grainy texture compared to the ISP+CS and all sausages received scores in the "not grainy" range.

The term "skin" refers to the degree to which the skin is distinguishable from the rest of the sample during chewing. The properties of the meat emulsion as well as the cooking conditions might have an impact on skin formation in products. In the present study, SL-T panel ranked the skin attribute as "slightly distinct" whereas, CA-T panel's score ranged between "slightly distinct" and "moderately distinct". None of the panels detect significant differences between ISP+CS and lentil flour added sausages with regard to the skin.

Legume flours are often associated with foreign flavors which could be described as grass, beany or bitter astringent flavor. The foreign flavor was evaluated to test whether panelists could recognize any atypical or foreign flavors in sausages as this is one of the important factors that limit the application of legume ingredients in processed meat products. It is interesting to point out that the foreign flavor scores of the sausages extended with lentil flour was placed in the "bland" range. CA-T panel did not detect differences between the commercial formulation and lentil added sausages. However, SL-T panel noticed higher foreign flavor intensity (one-unit difference) in the sausages with 6% and 8% lentil flour compared to the sausages from commercial formulation (ISP+CS), though, they were within the "bland" range. The foreign flavor of legume flours is mostly derived from the enzymatic oxidation of linoleic and linolenic acid by the lipoxygenases (Roland et al., 2017). Therefore, heat processing has been employed to eliminate the foreign flavor development in legume products. The lentil flour used in the present study were infra-red heated to inactivate those lipoxygenase enzymes. The weak foreign flavors noted in the sausages extended with lentil flour could be due to this reason.

For after taste, the SL-T panel provided a lower score for ISP+CS, suggesting that panelists perceived slightly lower after taste in ISP+CS compared sausages with lentil flour. Conversely, panelists in the CA-T panel did not distinguish differences between the sausages and all sausages

were ranked between "slightly intense" and "slightly bland". The application of lentil flour in sausages had no significant effect on the oily mouthfeel. All sausages perceived to be with low oily mouthfeel.

SL panels' overall acceptance score for sausages formulated with lentil flour except 4%LF-NO2 was similar to the commercial formulation with ISP. The no nitrite sample received the lowest score and it was rated as "slightly unacceptable" while all other samples received scores between "moderately acceptable" and "very acceptable". CA panel's acceptance scores were relatively similar on all sausages with ratings between "slightly acceptable and very acceptable."

Overall, descriptive assessment showed that the sensory profiles of sausages obtained for each of the panels were practically similar likely due to the similar treatment composition of the products. The increasing level of lentil flour from 4% to 8% slightly increased the perception of hardness, cohesiveness, density, chewiness, foreign flavor, and after taste. Nitrite exclusion had only an effect on color of the sausages. However, the panel effect was significant for all attributes except internal color, hardness, uniformity and overall acceptability of the attributes showing that the weighting of the ratings given for those attributes were different between the groups. The Panel x Treatment interaction effect was significant for seven parameters which includes surface moisture, surface smoothness, hardness, foreign flavor intensity, ease of swallow, after taste and overall acceptability; hence, different formulations were not rated in the same way for these attributes.

6.5.8.2 Principal component analysis (PCA) and hierarchical cluster analysis of sausages based on sensory descriptive results

PCA was conducted on the descriptive analysis data from the CA-T and SL-T panels to evaluate the associations between sausage products and their sensory characteristics. Figure 6.3 shows scores and loadings of chicken sausages and sensory attributes evaluated by descriptive panels. The first two Dimensions (principal components) explained 78% of the total variation. The first dimension, which explained 44.9% of the variation, was associated with external and internal color, mouth coating, graininess, denseness, cohesiveness and overall flavor intensity in positive direction and ease of swallow and surface smoothness on negative direction. Dimension 2 explaining 33% variation was defined by denseness, cohesiveness and foreign flavor on positive direction and by juiciness attributes on the negative direction.

PCA scores showed that the sausage products could be divided into three clusters based on their sensory characteristics. Cluster 1 loaded on the negative side of Dimension 1 consisted of ISP+CS and 4%LF showing that sausages with 4% lentil flour have similar properties to the commercial products. Cluster 2 included the sausages with 6% and 8% lentil flour, and they were located on the positive direction of Dimension 1 and 2. These products primarily had slightly higher hardness, cohesiveness, chewiness, denseness, and foreign flavor. The sausage prepared without nitrite was separated from the other sausages and located on the negative side of Dimension 1. This sausage differed primarily from other sausages by having lower color acceptability.

Figure 6.4 shows the results of the hierarchical cluster analysis, which was performed on the descriptive analysis data to identify the sausages that were perceived as similar by the two panels. Both panels separated the 4%LF-NO2 from other formulations. Although the differences found among the formulations with lentil flour were limited, SL-T panels classified ISP+CS and 4%LF as one group and 6%LF and 8%LF as another group. However, CA-T panel clustered all formulations with lentil flour and commercial formulation as one group. Overall, these results showed that SL-T panel was more sensitive to treatment differences, perhaps due to their greater familiarity with sausage products, which are made daily in their processing environment.
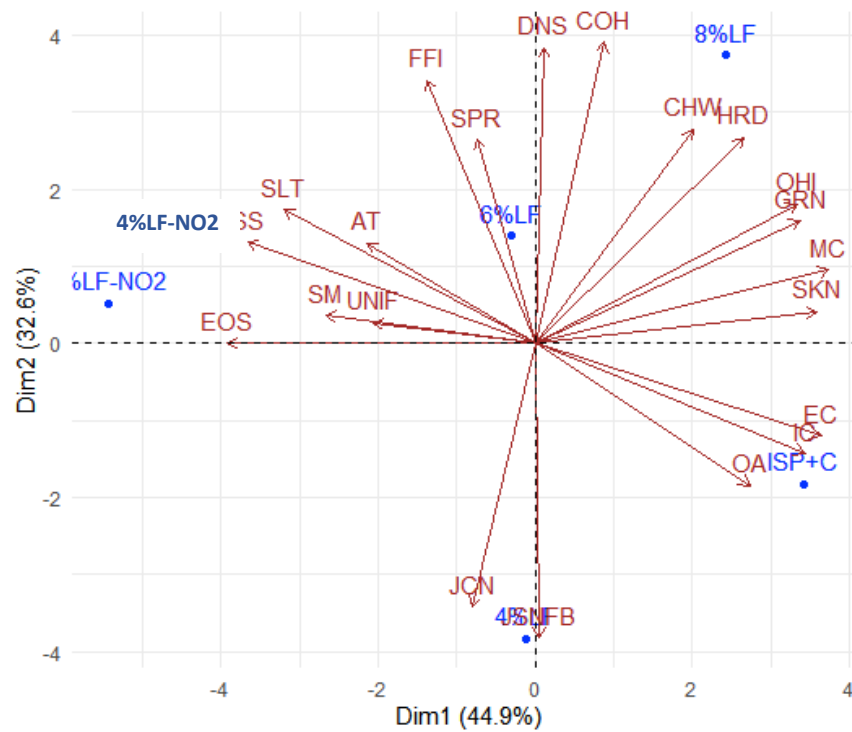
Figure 6.3 Biplot of principal component analysis showing the multivariate variation among chicken sausages in terms of descriptive sensory data

EC: external color, IC: internal color, SM: surface moisture, SS: surface smoothness, SPR: Springiness, HRD: hardness, COH: cohesiveness, UNIF: uniformity, JCNFB: juiciness at first bite, DNS: denseness, CHW: chewiness, JCN: juiciness, GRN: graininess, SKN: skin formation, SLT: saltiness, FI: overall flavor intensity, FFI: foreign flavor intensity, EOS: ease of swallow, MC: mouth coating, AT: Aftertaste, No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite
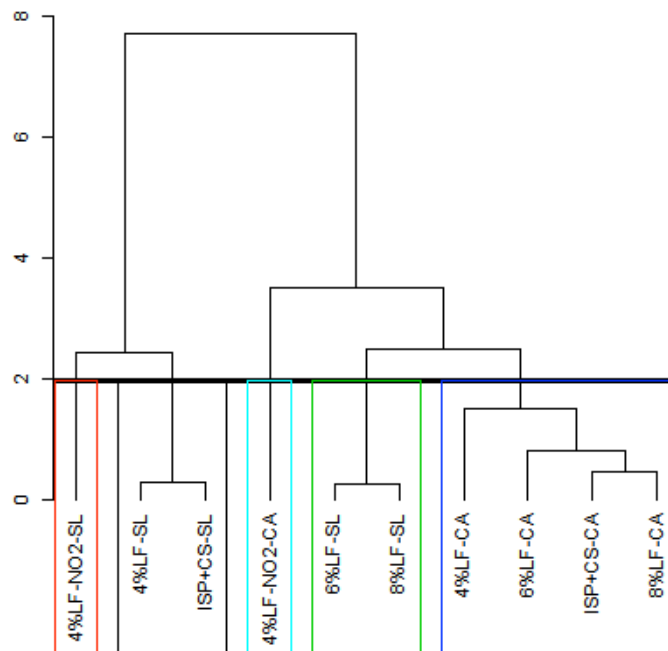
Figure 6.4 Hierarchical cluster analysis dendrogram of chicken sausages evaluated by Canadian and Sri Lankan panels

ISP+CA-CA, 4%LF-CA, 6%LF-CA, 8%LF-CA and 4%LF-NO2-CA are 1.75% ISP + 4% corn starch, 4% lentil flour + 2.18% corn starch, 6% starch + 1.27% corn starch, 8% lentil flour + 0.36% corn starch, and 4% lentil flour + 2.18% corn starch – no nitrite formulations, respectively, evaluated by Canadian semi-trained panel; ISP+CA-SL, 4%LF-SL, 6%LF-SL, 8%LF-SL and 4%LF-NO2-SL are 1,75% ISP + 4% corn starch, 4% lentil flour + 2.18% corn starch, 6% starch + 1.27% corn starch, 8% lentil flour + 0.36% corn starch, and 4% lentil flour + 2.18% corn starch – no nitrite formulations, respectively, evaluated by Sri Lankan semi-trained panel

6.5.8.3 Consumer acceptability

Studies on consumer behavior between different cultures found that innate factors have often been implicated in the genesis of food preferences (Prescott, 1998). As for most products, preferences for meat products are unique to different countries or cultures (Munoz, 1998). In the present study, sensory evaluation was conducted to assess the effect of the incorporation of lentil flour in sausages on their sensory attributes and how does the acceptability of these products differ among Canadian and Sri Lankan consumers. This evaluation was carried out using three consumer groups; Canadian consumers (CA), Sri Lankan consumers who live in Canada (SL-CA) and Sri Lankan consumers who live in Sri Lanka (SL) and each consumer group consisted of 60 panelists. The mean liking ratings and mean intensity ratings for the sensory attributes are presented in Tables 6.10 and 6.11, respectively.

In terms of external and internal color all consumer groups rated their liking similarly giving the lowest score to 4%LF-NO2 which did not contain sodium nitrite in its formulation (Table 6.10). The external color acceptability of the samples containing sodium nitrite were rated as "like moderately" whereas no-nitrite formulation was given a below average score (dislike slightly) by Sri Lankan consumers (SL-CA and SL groups). The Canadian consumers (CA group) rated the no nitrite formulation as "like slightly". Similar trend was observed in the ranking of internal color acceptability by all 3 consumer groups and no nitrite formulation obtained a lower average score. These results indicated that consumers prefer the cured pink color in sausages, even though there is an increasing consumer demand for the products with reduced synthetic additives.

CA and SL groups did not find differences among treatments for cohesiveness and all products were ranked as "slightly to moderately" cohesive (Table 6.11). These results were consistent with the results of the instrumental cohesiveness measurement, which did not detect significant differences between treatments, suggesting that the increasing level of lentil flour had no significant impact on cohesiveness. The cohesiveness given by ISP and corn starch were similar to the lentil flour with the addition level up to 8% together with corn starch. In contrast, SL-CA group noted slightly higher cohesiveness in 4%LF compared to 8%LF and 4%LF-NO2 treatments. In terms of tenderness, the CA and CA-SL groups did not distinguish significant differences among the sausages extended with lentil flour and their tenderness was comparable to the formulation containing soy protein. However, the SL consumer group gave slightly different ratings and 6%LF was given the lowest score (slightly tender). The insignificant differences in the textural properties among the lentil flour added sausages and ISP+CS may be related to the similar starch content in the formulations. It is documented that proteins influences the textural properties of comminuted meat products (Arntfield and Maskus, 2011). In the present study, around 2-3% differences in protein content was found across the formulations and it seemed that this difference was not able to make a perceptible change in the texture of MSC sausages. Overall, consumers had a moderate liking to the overall texture of sausages. Minor differences in juiciness intensity (Table 6.10) were perceived by the SL-CA group, but not by the CA and SL groups, corresponding to the small differences observed in the moisture content of different sausage products (Table 6.5). However, none of the consumer panels found significant differences across formulations for the acceptability of juiciness. All consumer panels provided scores within the "liking" range of the hedonic scale for the juiciness acceptability for all products. ISP is widely used in meat products due to its

Table 6.10 Consumer acceptability ratings of sausages formulated with lentil flour evaluated in Canada and Sri Lanka (n = 60 per group)

Sensory attribute	Consumer group	Treatment					
		ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2	SEM[1]
External color Acceptability[y, z]	CA	4.98[a]	5.30[a]	5.15[a]	5.20[a]	4.21[b]	0.127
	SL-CA	5.32[a]	5.20[a]	5.13[a]	4.62[b]	3.73[c]	0.126
	SL	5.15[a]	4.83[ab]	4.67[ab]	4.48[b]	2.58[c]	0.150
Internal color Acceptability[y]	CA	4.77[a]	4.77[a]	4.88[a]	4.65[a]	2.83[b]	0.144
	SL-CA	5.10[a]	5.25[a]	5.00[ab]	4.55[b]	3.30[c]	0.129
	SL	5.02[ab]	5.13[a]	4.72[ab]	4.62[b]	2.98[c]	0.141
Overall texture acceptability[z]	CA[NS]	4.57	4.48	4.82	4.60	4.28	0.136
	SL-CA	4.76[a]	5.03[a]	5.07[a]	4.67[ab]	4.23[b]	0.132
	SL	4.90[a]	4.83[a]	4.50[ab]	4.92[a]	4.30[b]	0.148
Juiciness acceptability	CA[NS]	4.71	4.78	4.93	4.48	4.53	0.143
	SL-CA[NS]	4.62	4.90	5.00	4.73	4.50	0.142
	SL[NS]	4.63	4.75	4.77	5.03	4.61	0.141
Flavor acceptability[y]	CA[NS]	4.58	4.47	4.73	4.51	4.35	0.131
	SL-CA	4.77[ab]	5.00[a]	5.03[a]	4.88[a]	4.37[b]	0.132
	SL[NS]	4.95	4.85	5.63	5.00	4.55	0.34
Overall acceptability[y, z]	CA	4.37[ab]	4.50[a]	4.65[a]	4.41[ab]	4.04[b]	0.138
	SL-CA	4.82[a]	5.03[a]	5.00[a]	4.55[a]	3.95[b]	0.132
	SL	4.93[a]	4.83[a]	4.57[a]	4.87[a]	4.00[b]	0.128

Values are presented as means
[a,b]Means within the same row with different superscripts are significantly different (p<0.05)
[NS]No significant difference among treatments within the row
[1]Standard error of mean
[y and z] Panel effect and Treatment x Panel interaction effect is significant, respectively (p<0.05)
No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite
CA: Canadian consumers, SL-CA: Sri Lankan consumers in Sri Lanka, SL: Sri Lankan consumers in Sri Lanka
External color acceptability: 6 = Like very much, 1 = Dislike very much
Internal color acceptability: 6 = Like very much, 1 = Dislike very much
Overall texture acceptability: 6 = Like very much, 1 = Dislike very much
Juiciness acceptability: 6 = Like very much, 1 = Dislike very much
Flavor acceptability: 6 = Like very much, 1 = Dislike very much
Overall acceptability: 6 = Like very much, 1 = Dislike very much

Table 6.11 Attribute ratings of sausages formulated with lentil flour evaluated by consumers in Canada and Sri Lanka (n = 60 per group)

Sensory attribute	Consumer group	Treatment					
		ISP+CS	4%LF	6%LF	8%LF	4%LF-NO2	SEM[1]
Cohesiveness[y]	CA[NS]	4.93	4.85	4.72	4.87	4.68	0.136
	SL-CA	4.60 ^{ab}	4.77^a	4.63^{ab}	4.27^b	4.47^{ab}	0.157
	SL[NS]	4.39	4.57	4.30	4.45	4.32	0.146
Tenderness[y]	CA[NS]	4.65	4.67	4.80	4.35	4.75	0.136
	SL-CA[NS]	4.65	4.37	4.62	4.18	4.70	0.145
	SL	4.19^b	4.83^a	4.08^b	4.50^{ab}	4.32^{ab}	0.151
Juiciness	CA	4.65	4.73	4.93	4.65	4.90	0.135
	SL-CA	4.68^{ab}	4.87^a	4.68^{ab}	4.38^b	4.90^a	0.122
	SL[NS]	4.81	4.65	4.58	4.52	4.70	0.126
Flavor intensity[y]	CA[NS]	4.10	4.20	4.33	4.37	4.00	0.135
	SL-CA[NS]	4.53	4.38	4.53	4.37	4.43	0.142
	SL[NS]	4.51	4.37	4.55	4.67	4.43	0.131
Foreign flavor intensity[y]	CA[NS]	4.48	4.67	4.43	4.38	4.27	0.161
	SL-CA[NS]	4.48	4.57	4.40	4.12	4.17	0.167
	SL[NS]	2.90	3.13	2.88	2.87	3.13	0.18

Values are presented as means
[a,b]Means within the same row with different superscripts are significantly different (p<0.05)
[NS] No significant difference among treatments within the row
[1]Standard error of mean
[y]Panel effect is significant (p<0.05)
No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite
[y and z] Panel effect and Treatment x Panel interaction effect significant, respectively
CA: Canadian consumers, SL-CA: Sri Lankan consumers in Sri Lanka, SL: Sri Lankan consumers in Sri Lanka
Cohesiveness: 6 = Very cohesive, 1 = Very crumbly
Tenderness: 6 = Very tender, 1 = Very tough
Juiciness: 6 = Very juicy, 1 = Very dry
 Flavor intensity: 6 = Very intense, 1 = Very bland
Foreign flavor intensity: 6 = Very bland, 1 = Very intense

functions as an emulsifier and stabilizer and its capacity to increase water holding capacity (Macedo-Silva et al., 2001; Ulu, 2004). In the present study, the lentil flour was found to add comparable sensory characteristics in MSC sausages to those containing ISP.

A major disadvantage of legume flours as a food ingredient is the strong foreign flavor associated with the products. These products could impart grassy and beany flavor or bitter astringent flavor to the meat products (Asgar et al., 2010). It is worth noting that all consumer groups have ranked all formulations within the "bland" range for the foreign flavor intensity. There was no significant difference in the scores among the sausages containing lentil flour and they were comparable to the flavor of ISP+CS. All consumer groups provided hedonic scores between "like moderately" to "like very much" for the flavor acceptability and the level of lentil flour added did not affect the flavor liking scores except for SL-CA panel who gave lower scores to 4%LF-NO2. All consumer panels showed similar liking pattern for overall acceptability of the sausages. The acceptability of the sausages formulated with lentil flour and sodium nitrite was significantly better than that of no nitrite product (4%LF-NO2) and they obtained scores representing "like moderately". Lower overall liking score (like slightly) of the 4%LF-NO2 product might have resulted from less acceptable color caused by the exclusion of sodium nitrite from the formulation. The acceptability of the lentil flour added sausages were similar to the acceptability of the commercial formulation. These results revealed that lentil flour has great potential to be used as a binder in MSC sausages without compromising their sensory acceptability.

The statistical analysis of results reveled that, treatment effect was significant on seven attributes whereas effect of consumer group was significant for eight attributes out of the 11 attributes evaluated. Consumer groups gave different mean scores for external color acceptability, cohesiveness, flavor acceptability, foreign flavor intensity and overall acceptability. The Treatment x Consumer group interaction was significant for four attributes (external color acceptability, tenderness, overall texture, acceptability, and overall acceptability); thus, trends in consumer preference for these attributes differ among consumer groups. These differences observed in the scoring patterns between consumer groups might be attributed to the differences with the familiarity for lentil. The choice of Canadian and Sri Lankan consumer panelists in the present study was to evaluate the acceptability of the developed products among the consumers who have different familiarity with lentil. Lentil is an integral component in the Sri Lankan diet thus, they are very familiar with lentil consumption. The decorticated red lentils are usually the common type

of lentil and are consumed as a side dish (curry) with a rice-based meal. The popularity of lentil among Sri Lankans is due to the high nutritional value, high affordability and cooking convenience compared to other pulses (Anoma et al., 2014). A study conducted by Govindasamy et al. (2014) reported that among the lentil consumers in Sri Lanka, 58% had at least one lentil meal per day. A high proportion (25%) of the respondents consumed lentil twice per day, 29% consumed lentil once a day and about 3% consumed lentil for all three meals. Around 42% of the respondents did not eat the lentil on regular basis, but a few days in a week. However, these consumers would not be familiar with lentil flour as it has not been used as a binder in Sri Lanka. In Western countries, lentil consumption is relatively limited due to traditional eating customs, lack of consumer understanding on nutritional benefits and processing techniques to make variety of products (Zhang et al., 2015). The consumers of the SL-CA group were immigrants to Canada. Having lived for several years in Canada, they might have been adopted to diverse food habits; thus, their perceptions and preferences might be different from the consumers of the SL-CA group. On the other hand, Canadian processors could benefit if this group could predict products acceptability in a potential export market.

6.5.8.2 Principal component analysis (PCA) and hierarchical cluster analysis of sausages based on consumer acceptability results

PCA followed by cluster analysis was conducted to identify the underlying principal factors among the set of sausages and sensory attributes and results are shown in Figure 6.4. Overall the first two Dimensions (principal components) explained 92% variability in the data. The first dimension was defined by texture, color, flavor and juiciness acceptability, whereas the second dimension, explaining 31% variation was associated with texture flavor and juiciness intensity parameters. Moreover, overall acceptability and texture and flavor and color acceptability were highly correlated with each other showing that the overall acceptability of the products was determined on all sensory properties.

Figure 6.4b shows that the samples could be divided into 4 clusters. As observed in the PCA of descriptive sensory data, the product formulated without nitrite (4%LF-NO2) was positioned in the negative direction of the first dimension. Among the formulations with nitrite, the samples that differed most were the 4%LF and 8%LF. The commercial formulation ISP+CS and 6%LF clustered as one group indicating that they have relatively similar consumer acceptability.
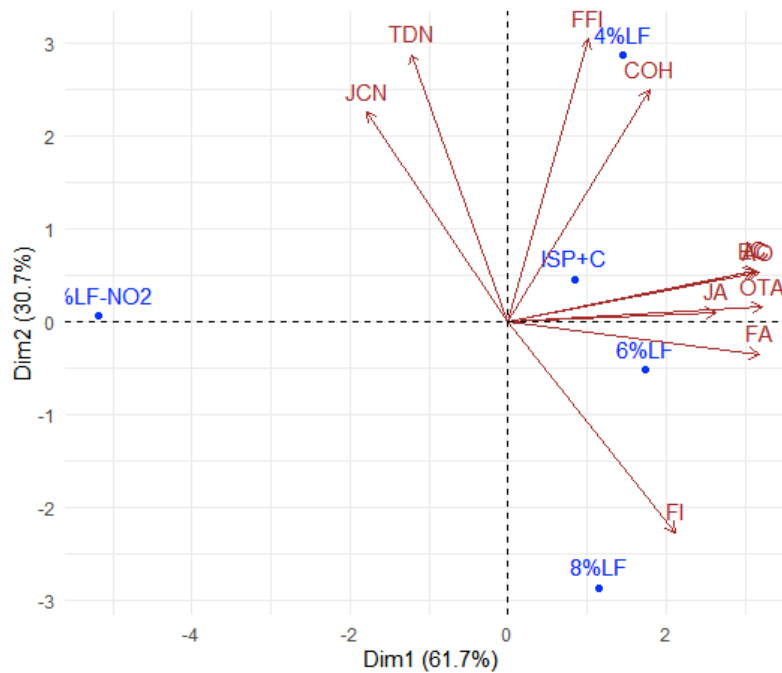
Figure 6.5 Biplot of principal component analysis (PCA) showing the multivariate variation among chicken sausages in terms of consumer sensory data

EC: external color acceptability, IC: internal color acceptability, COH: cohesiveness, TDN: tenderness OTA: Overall texture acceptability, JCN: Juiciness JA: juiciness acceptability, FI: flavor intensity, FFI: foreign flavor intensity FA: flavor acceptability, OA: overall acceptability. No-Binder: no binder, ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

However, in the PCA of descriptive sensory data, it was found that the sensory profile of the commercial formulation (ISP+CS) was much closer to the 4%LF.

Figure 6.5 shows the results of the hierarchical cluster analysis carried out to understand the clustering patterns of the sausage products based on the sensory attributes evaluated by the three consumer panels. In general, it was noted that the clustering of products showed a similar trend across the consumer panels. All consumer panels clustered 4%LF-NO2 as one group while other products were grouped into two separate clusters. It was worth noting that that all products except 6%LF evaluated by SL-CA and SL panels were grouped into same clusters. These results showed that SL-CA panel gave more similar ratings for most attributes of the products suggesting stronger predictability of acceptability scores by fellow Sri Lankan consumers
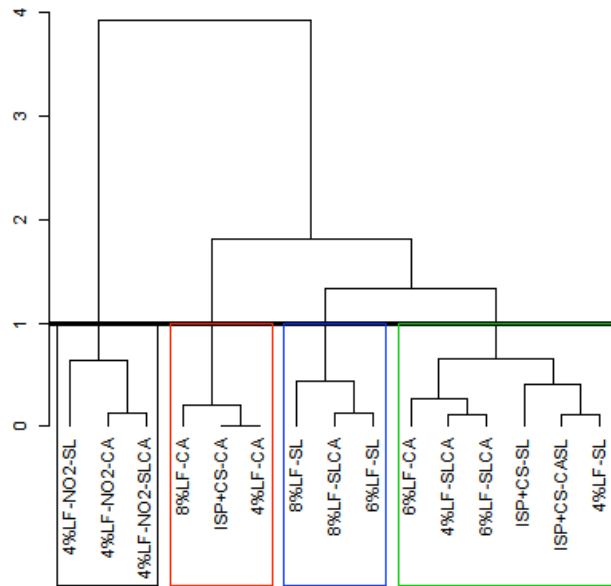
Figure 6.6 Hierarchical cluster analysis dendrogram of chicken sausages evaluated by Canadian consumers (CA), Sri Lankan consumers living in Canada (SL-CA) and Sri Lankan (SL) consumers living in Sri Lanka

ISP+CS-CA, -SLCS, and -SL: 1.75% ISP + 4% corn starch treatment evaluated by CA, SL-CA groups, respectively; 4%LF-CA, -SLCA and -SL: 4% lentil flour + 2.18% corn starch treatment evaluated by CA, SL-CA groups, respectively; 6%LF-CA, -SLCA and -SL: 6% starch + 1.27% corn starch treatment evaluated by CA, SL-CA groups, respectively; 8%LF-CA, -SLCA and -SL: 8% lentil flour + 0.36% corn treatment evaluated by CA, SL-CA groups, respectively; 4%LF-NO2-CA, -CA, and -SL: 4% lentil flour + 2.18% corn starch –no nitrite treatment evaluated by CA, SL-CA groups, respectively.

6.5.9 Consumer demographic, perceptual, and behavioral information

6.5.9.1 Socio-economic and demographic characteristic of consumers

Table 6.12 shows the socioeconomic and demographic characteristics of the consumers that participated in the study. Out of the 180 panelists, 60% were female. The male: female ratio was fairly consistent between the groups and were 38:62, 40:60, and 42:58 for CA, SL-CA and SL groups, respectively. Majority of the panelists in CA (68%) and SL (63%) groups were in the 18-29 years age category. In the SL-CA group 43% of panelists were between 30-39 years of age. A major difference observed between consumer groups was the family composition. Nearly 90% of the SL panelists have more 3 or more family members whereas approximately 50% of CA and SL-CA panelists have 2-3 and 3-4 members, respectively in their families. Education profile of the consumers shows that most of them have university level or higher qualification. The other

panelists (5 to 13%) were from the high school and below. The distribution of panelists in income categories were relatively similar across all groups and more than 50 % of the panelists fell in the middle-income category.

6.5.9.2 Importance of product attributes when buying meat products

Product attributes are key factors affecting the food purchasing decision (Batra and Sinha, 2000; Kupiec and Revell, 2001). Research on consumer behavior indicates that consumers perceive a product as a set of characteristics such as price, convenience, variety, and their decision to purchase a particular product depends primarily on a combination of these characteristics (Juric and Worsley, 1998; Ali et al., 2010). Therefore, understanding which product attributes are important to consumers when buying meat products is useful in developing new meat products. Table 6.13 illustrates the significance of different product features when buying sausages. The majority of the respondents in both countries indicated that price is an important parameter in the choice of buying sausages. The mean rating for price ranged between 4.1 and 4.5 (moderately important). In agreement, a survey conducted by Ali et al. (2010) evaluating the buying behavior of consumers for food products explained that price was an extremely important product feature affecting the consumers' buying decision. This suggested that cost of production is a critical criterion to be considered in manufacturing sausages.

Incorporation of binders or extenders is a strategy to reduce the formulation costs of comminuted meat products. Extenders commonly used in sausage formulations are characterized by high protein content and are generally either dried milk or soybean products (Aberle et al., 2012). Other common binders used are cereal flours. Cereal flours are high in starch but relatively low in protein. Results of the present study showed that lentil flour could also serve as an alternative source of high protein meat binder/extender to reduce the cost of production while maintaining or improving the total protein content. Plant proteins are now regarded as biologically active components in addition to their role as essential macronutrients. The partial replacement of animal foods with legumes is claimed to improve overall nutritional status (Tiwari et al., 2011). Present survey also showed that protein content of the products was an important product characteristic affecting buying decision. Majority of the respondents in SL-CA and CA groups rated that protein content is extremely important for them in their buying decision. Pulses are a good source of protein, providing balanced amino acid profiles. Thus, lentil flour

Table 6.12 Socio-economic and demographic characteristic of consumers (n = 60 per group)

Variable	Frequency %		
	CA	SL-CA	SL
Sample size	60	60	60
Gender	38	40	42
Male	62	60	58
Female			
Family size (%)			
1 to 2 members	55	35	9
3 to 4 members	33	57	48
>5 members	12	8	43
Age composition (%)			
18-29 years	68	17	63
30-39 years	15	43	5
40-49 years	7	17	18
50-60 years	10	23	13
Education background (%)			
High school and below	13	7	5
Some university/College (Diploma)	28	13	15
Graduate	43	32	53
Postgraduate	15	48	27
Annual household income			
$20,000 (< Rs 24000 in SL)	22	8	2
$20000-59000 (Rs 24000 – 72000 in SL)	38	37	40
$60000-99000 (Rs 72000 – 1200000 in SL)	15	30	28
$100,000 (> Rs 1200000 in SL)	25	25	30

CA: Canadian consumers, SL-CA: Sri Lankan consumers in Canada, SL: Sri Lankan consumers in Sri Lanka

Table 6.13 Consumer response on importance of product attributes when buying meat products (n = 60 per group)

Product attribute	Consumer response			
	CA	SL-CA	SL	SEM[1]
Price[NS]	4.5	4.4	4.1	0.16
Species	3.7[b]	4.9[a]	5.3[a]	0.18
Content of mechanically separated meat	2.5[b]	3.2[ab]	3.4[a]	0.20
Protein	3.9[a]	4.4[a]	3.2[b]	0.18
Fat	3.8[b]	4.6[a]	3.4[b]	0.19
Fiber	3.0[b]	3.8[a]	3.2[ab]	0.20
Salt	4.2[ab]	4.7[a]	3.9[b]	0.18
Preservatives	3.6[b]	4.8[a]	4.4[a]	0.20
Colors	3.0[b]	4.7[a]	4.2[a]	0.19
Pre-cooked	3.0[b]	4.0[a]	4.0[a]	0.20

Scale: 1 = not important, 6 = extremely important
CA: Canadian consumers, SL-CA: Sri Lankan consumers in Canada, SL: Sri Lankan consumers in Sri Lanka

would be a better binder for meat products over the cereal flours. The mean values of consumers' response indicated that species of meat was another important product feature particularly for the Sri Lankan consumers.

Religion, age, gender, household size, education level, knowledge, and health concerns are among the factors affecting the consumption of meat and meat products (Silva et al., 2010; Alahakoon et al., 2016; Schmid et al., 2017). In Sri Lanka, the most popular meat type is chicken. According to a study conducted by De Silva et al. (2010), chicken was the most preferred type of meat followed by mutton, beef and pork. Moreover, consumption of chicken was found to be not considerably affected by ethno-religious beliefs compared to that of pork and beef in Sri Lanka (Alahakoon et al., 2016). As a result, chicken products continue to grow and build their popularity as a staple component of the diet in Sri Lanka. Similarly, chicken consumption in Canada has risen by almost 9 kg per capita since 1999 and was the most consumed meat in 2018 amounting to 34 kg per capita (Bedford, 2019). On the other hand, beef and pork consumption has decreased in recent years and per capita consumption of beef and pork were 18 and 16 kg, respectively in 2018 (Bedford, 2019). This indicates that both countries have increasing potential for chicken-based products.

The increasing production of chicken would yield more mechanically separated chicken meat available for the manufacturing of meat products. Mechanically separated meat is cheaper relative to regular meat, hence, can reduce the cost of production. Our experiments reveled that MSC was a viable source of meat for the production of sausages. Of the product attributes evaluated the content of mechanically separated meat in the formulation was regarded as the least important property for all consumer groups which indicates that the use of mechanically separated meat might have minimal effect on consumer acceptability. The mean values of consumer responses indicated that fat, fiber and salt content as well as the artificial preservatives and colors are of high importance when buying meat products and Sri Lankan consumers were more concerned with preservatives than Canadian consumers. This emphasizes the benefits of using lentil seed components which exhibit antioxidant properties to reduce the use of artificial preservatives in meat products to meet consumer preferences.

In the present survey, the consumers' knowledge and opinion regarding the use of nitrite in comminuted meat products were also evaluated (Table 6.14). Majority of the consumer panelists in CA (62%), SL-CA (58%) and SL (85%) were aware that nitrite is added in some meat products to give their characteristic pink color and that the exclusion of nitrite results in reduced pink color in the products. However, it should be noted that majority of the consumers in SL-CA and SL consumer groups were willing to buy nitrite free products even at the expense of lower color acceptability while the CA consumer group was less concerned about nitrites being used in meat products.

Table 6.14 Consumer awareness and willingness to pay for nitrite free products (n = 60 per group)

Consumer awareness and willingness to pay for nitrite free products	Frequency (%)					
	CA		SL-CA		SL	
	Yes	No	Yes	No	Yes	No
Are you aware that nitrites are added in some meat products to give their pink color? Yes/No	62	38	58	42	85	15
Are you aware that the nitrite added in the product is listed under ingredients? Yes/No	53	47	60	40	75	25
If nitrite free meat product is available in the market, what would be your purchasing choice.						
I will buy it even at the expense of lower color acceptability.	32		63		47	
I will buy it only if it still has pink color.	8		8		25	
I will buy it only if it is cheaper than the sausages with nitrites.	15		7		8	
I will buy any product without considering the presence or absence of added nitrites.	45		20		20	

CA: Canadian consumers, SL-CA: Sri Lankan consumers in Canada, SL: Sri Lankan consumers in Sri Lanka

6.5.9.3 Consumer beliefs and behavioral data

Table 6.15 shows the indicators of consumer beliefs and lifestyle in regard to health and nutrition. The mean values of the consumer responses indicated that the majority of respondents considered themselves health conscious, and exercised regularly, read nutritional labels regularly, and were willing to pay more for more nutritious and healthier products at the expense of lower sensory quality. It was noted that majority of the consumers did completely agreed with the statement 'I believe that the incorporation of lentil flour in meat products makes them more nutritious and healthier'. Consumer health concerns and positive attitudes towards the incorporation of lentil in meat products indicates potential marketability of the products containing lentil even at reduced sensory characteristics.

Table 6.15 Consumer beliefs and behavioral data (n = 60 per group)

Statement	Agreement level			
	CA	SL-CA	SL	SEM[1]
I consider myself very health conscious^{NS}	4.6	4.7	4.4	0.19
I exercise on a regular basis.	4.7^a	3.6^b	3.2^b	0.26
I regularly read nutritional labels on the food I purchase^{NS}	4.5	4.2	4.2	0.22
I will pay more for a food product if it is more nutritious than a cheaper alternative^{NS}	4.2	4.7	4.2	0.24
I will opt for healthier version of a food product, even at the expense of lower sensory quality^{NS}	3.6	4.1	3.8	0.23
Price is the most important factor I consider when I buy meat products	3.8^a	3.4^{ab}	3.3^b	0.27
I believe that eating whole flour is healthier than eating refined starch	4.6^b	5.4^a	5.1^{ab}	0.22
I know that lentil is a rich source of protein and fiber ^{NS}	5.4	5.7	5.4	0.17
I believe that the incorporation of lentil flour in meat products makes them more nutritious and healthier ^{NS}	4.9	5.5	5.2	0.17

Scale: 1 = Completely disagree, 6 = Completely agree

CA: Canadian consumers, SL-CA: Sri Lankan consumers in Canada, SL: Sri Lankan consumers in Sri Lanka

6.5.9.4 Purchase intent

Purchase intention measures the consumers' willingness to buy a product. There were no distinct differences for the purchase intent pattern for each formulation among the three consumer groups. Therefore, the overall purchase intent calculated from the pooled data from all consumer groups are presented (Figure 6.7). Across all formulations, the commercial formulation (ISP+CS) was given the largest proportion (21%) of the 'definitely would buy it' purchase score. It was worth noting that, 4%LF and 6%LF were given very similar response (19%) to the commercial formulation (ISP+CS). Moreover, the sum of 'definitely would buy it' and 'probably buy it' scores were nearly 60% for the formulations 4%LF, 6%LF, and 8%LF and commercial formulation (ISP+CS) suggesting that the lentil flour incorporated sausages would have substantial purchase potential.
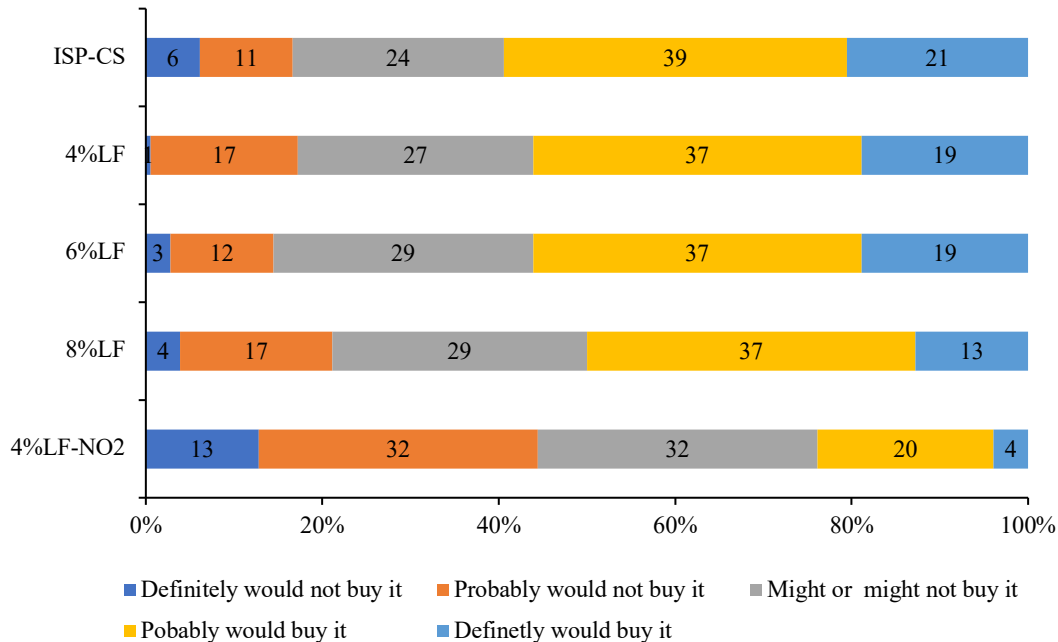
Figure 6.7 Frequency of consumer purchase intent scores for sausages (n = 180)

ISP+CS: 1.75% ISP + 4% corn starch, 4%LF: 4% lentil flour + 2.18% corn starch, 6%LF: 6% starch + 1.27% corn starch, 8%LF: 8% lentil flour + 0.36% corn starch, 4%LF-NO2: 4% lentil flour + 2.18% corn starch – no nitrite

All products received low 'definitely would not buy it' ratings and the lowest use of this category was reported for the 4%LF formulation. Among all formulations, 4%LF-NO2 formulated without nitrite received relatively lower 'definitely would buy it' and 'probably buy it' scores which could be related to its lower color acceptability. About 24% to 32% of the panellists indicated 'might or might not buy it' response for all formulations including the no nitrite formulation. This may suggest that the no nitrite formulation may even have potential market. As the consumer survey data indicated, the no nitrite products might have higher marketability among the consumers who prefer to buy products with low artificial preservatives added. Perhaps improving the color would help increase the marketability of these products.

Many variables influence the purchase intent of the consumers while choosing a product, and their decision depends on internal and external variables (Keller, 2001). Previous studies suggested that external variables such as demographics and geographics have a significant influence on purchase intention (Younus et al., 2015). However, in the present study strong correlations were not found between purchase intent and demographic variables.

212

6.6 Conclusions

This study suggests that IR treated lentil flour can be successfully used in chicken sausages and other meat formulations as a binder and extender. The inclusion of lentil flour resulted in increased water holding properties compared to the no-binder control. Lentil flour had minimal effect on textural properties of chicken sausages, and they were similar to the commercial products formulated with isolated soy protein and modified corn starch. The antioxidant efficacy of lentil flour was comparable to sodium nitrite. Therefore, lentil flour could be used in nitrite-free meat systems to provide antioxidant properties. The sensory profiles of sausages and consumer acceptance among Canadian and Sri Lankan consumers were overall similar and exclusion of nitrite results in reduced color acceptability. There were high similarities in the judgment of product acceptability between SL-CA and SL panels; thus, Sri Lankan consumers living in Canada could be used to predict the acceptability of the products in Sri Lanka. Both Canadian and Sri Lankan consumers' hedonic ratings of the sausages formulated with lentil flour were not drastically different from those of commercial products extended with ISP and corn starch. Consequently, these results suggest that IR treated lentil flour could be successfully used as a binder up to 8% level in chicken sausage formulations.

7. GENERAL DISCUSSION

Lentil is grown around the world and contributes around 7% of the global pulse production (Tiwari and Singh, 2012). Worldwide, total lentil production has increased in the last two decades, and the majority of lentil is grown in Canada. Given the increasing production and still low consumption of lentil, the prospects for further expanding pulse processing and utilization appear bright. Lentil is a nutritionally rich crop that could be successfully utilized as a food ingredient or a base for new product development.

Besides being an excellent source of macronutrients; protein and carbohydrates, lentil also contains plant secondary metabolites, including phenolic compounds that are increasingly being recognized for their potential health benefits (Tiwari and Singh, 2012). Phenolic compounds with antioxidant properties have been reported to prevent chronic degenerative diseases, including diabetes, cardiovascular diseases, cancer, autoimmune, and inflammatory diseases (Scalbert et al., 2005). Some studies showed that lentil had the highest total phenolic content compared to six other common pulses (Xu and Chang, 2007), and a greater proportion of these compounds are concentrated in the seed coat (Li, 2017). Therefore, this work was undertaken to expand our understanding of the phenolic profiles and antioxidant properties of lentil seed.

The solubility of phenolic compounds depends mainly on the chemical nature of the compounds and their polarity (Ajila, 2011). Hence, a variety of solvents have been studied for the extraction of phenolic compounds and the antioxidant activities of these phenolics were found to be related to type of solvent used (Chavan et al., 2001). Water and aqueous mixtures of ethanol, methanol or acetone are among the commonly used solvents to extract phenolics from plant material (Sun and Ho, 2005; Ajila et al., 2011). In the present study, the solubility of lentil seed coat phenolics in water and 70% aqueous ethanol was studied. A significant quantity of phenolics was found to be soluble in both solvents. The total phenolic content (TPC) of the water and aqueous ethanol extracts ranged between 41.63 mg GAE/g and 50.46 mg GAE/g of seed coat. Total extractable phenolic content was significantly affected by the type of solvent and slightly

214

higher levels (<12% difference) were found in aqueous ethanol. These results are in good agreement with those reported by Li (2017), who observed higher concentrations of lentil seed coat phenolics in 70% aqueous ethanol extracts in comparison to water. On the contrary, Oomah et al. (2011) noted that water extracted more phenolics from lentil seed coat than 80% aqueous ethanol. In addition, Khokhar and Magnusdottir (2002) also found that water to be the best solvent to extract tea phenolics compared with 70% ethanol and 80% methanol. This discrepancy was most likely due to the varietal and methodology differences between the studies. It was noteworthy, although the TPC was higher in 70% aqueous ethanol, a large difference in extractability between solvents was not observed (<12%). These results revealed that water can be a viable solvent for extracting phenolic compounds from lentil seed coat. Besides, water extraction is preferred as it is closely related to the actual phenomenon taking place in food systems where the primary solvent is water.

There are different lentil cultivars with different seed coat colors. This diversity in seed coat color phenotypes may indicate specific phenolic profiles among different genotypes. Therefore, Study II was performed to broaden the knowledge on phenolic profiles and antioxidant efficacy of water-soluble phenolics in lentil seed coat from different cultivars, including regular and zero tannin as there were no previous reports available. Identification and quantification of the phenolic composition and their antioxidant potential could help select cultivars with higher levels of bioactivity. The seven cultivars included in the present work consisted of green (CDC Greenland and CDC Greenstar), gray (CDC Maxim and CDC SB-3), and brown (CDC Robin) normal tannin and transparent (ZT-4) and gray translucent (6205-ZT) zero tannin seed coat phenotypes. Results showed that cultivar had significant impact on the phenolic composition of seed coat water extracts (WE). TPC of normal tannin and zero tannin seed coats ranged from 35.88 to 39.72 mg GAE/g and 5.01 to 5.92 GAE/g, respectively. The highest total flavonoid content (mg CE/g) and condensed tannin content (28.07 mg CE/g) were observed in CDC Greenland (green seed coat). TPC showed significant positive correlation with TFC (r = 0.95) and CTC (r = 0.95), which showed that flavonoids and condensed tannin are the main phenolic groups in lentil seed coat. This observation was consistent with that of Zhang et al. (2015) who also demonstrated strong positive correlations between TPC and TFC of whole lentil.

Concentrations of individual phenolic compounds of seed coat were determined using LC-MS. To the best of my knowledge this is the first study reporting the water-soluble phenolic composition of lentil seed coat. Kaempferol tetraglycoside, catechin-3-glucoside and procyanidin

B3 were the dominant phenolic compounds found in normal tannin seed coat which contributed around 73% - 81% of the total phenolics. A total of thirty phenolic compounds belonging to the subclasses of hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, flavonols, flavones, flavan-3-ols, and procyanidins were detected using LC-MS. Dueñas et al. (2002) studied the phenolic composition of lentil seed coat based on the compounds extracted in 80% aqueous methanol extracts. In general, the composition of phenolics in other studies are different than what was observed in the present study. These differences could be attributable to many factors, including the type of solvent, cultivar, and analytical techniques used. Nevertheless, this emphasizes the fact that the solubility, hence the bioactivity of phenolic compounds, may vary depending on the type of medium in which seed coat was applied. In this context, the findings of the current work are will be very beneficial in understanding the composition of phenolics that will be available for biological systems which have aqueous medium.

The LC-MS technique used in this determination might have limitations in the estimation of phenolic composition due to the incomplete quantification of all peaks and possible interferences by other compounds and limitation of the standards (Zhang et al., 2015). Interactions between phenolic compounds and small peptides or oligosaccharides could also result in underestimation of phenolic compounds (Saulnier et al., 1999). So, there could be other phenolic compounds in water extracts that were not identified in this experiment.

Among the phenolic compounds observed in zero tannin genotypes, one compound kaempferol tetraglycoside accounted for 79% - 89% of TPC which was very different from normal tannin varieties. The other phenolic compounds found were mostly the phenolic acids. As expected, condensed tannins were not detected.

The three replicate samples used in this study were obtained from 3 different seed lots. It was important to note that the variability in the phenolic contents among the replicates was limited (coefficient of variance <10%) suggesting high reproducibility of the results. This was a promising observation showing the applicability of seed coat in industrial use. Another promising observation was the high storage stability of phenolics in seed coat. The comparison between fresh seeds versus seeds stored for three years from cultivar CDC Maxim did not show marked differences in the phenolic compounds except for a few. Across the 30 phenolic compounds analyzed, the compounds which differed between these two samples with statistical significance were phenolic acids (3,4-dihydroxybenzoic acid, gallic acid), resveratrol-3-*β*-mono-*D*-glucoside, kaempferol-3-O-

glucoside, quercetin-3,4'-di-*O*-glucoside, (+)-catechin, taxifolin (dihydroquercetin) and procyanidin B1. On the contrary, Mirali (2017) demonstrated significant changes in the concentrations of 27 flavan-3-ols and proanthocyanidin oligomers in lentils, which were subjected to long term storage (7-14 years). Comparing the present work with the analysis by Mirali (2017), the storage conditions and storage duration were different between the two studies. This may explain why there were differences in the findings between studies. In the present study, only one cultivar (CDC Maxim) was included in this investigation; thus, future research would be necessary for better understanding the stability of lentil seed coat phenolics under different storage conditions. Moreover, the present work did not focus on studying the storage stability of either of ground seed coat or water extracts. They might be more susceptible to deteriorative reactions due to the increased surface area; thus, this may constitute an objective of further studies to verify the suitability of seed coat and its water extracts to develop as a food ingredient.

Background colors of seed coats studied in the present experiment were green, gray, and brown. Pearson correlation coefficients were calculated in order to find out the strength and direction of linear relationships between CIE color attributes (L*, a* and b*) and phenolic contents. Only L* value, which measures the lightness or darkness, established a moderate relationship (r = -0.53 to -0.65) with TPC, TFC, and CTC and the antioxidant parameters. In agreement, Xu et al. (2007) reported that dark-colored pulses possess higher phenolic content and antioxidant activities. The a* and b* did not show a significant correlation with either phenolic compounds or antioxidant properties. These findings indicated that these color parameters would not be a powerful indicator of antioxidant activity and phenolic concentrations. However, the sample size of seven cultivars used in this study might not be adequate to draw a valid conclusion on the linear relationships among these parameters.

Antioxidant activity is the fundamental bioactivity of phenolic compounds and the primary mechanism through which they exert health promotion effects (Shahidi and Neczk, 1995; Shahidi et al., 2006). As well, due to these antioxidant properties, plant phenolics have captured interest as a natural antioxidant in food applications. Antioxidant properties of phenolic compounds are known to be due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Rice-Even et al., 1996). The hydroxyl and carboxyl groups in phenolic compounds are also capable of binding metal ions, particularly iron and copper (Michalak, 2006). Therefore, the phenolic compounds function as metal ion chelators, preventing

metal catalyzed formation of free radical species (Salah et al., 1995; Carocho et al., 2014). Because no single antioxidant assay can accurately reflect all antioxidant mechanisms or all antioxidant compounds in a complex system, DPPH and ABTS free radical scavenging assays, ferrous ion chelation assay and phospholipid peroxidation assay were used in this study to estimate the antioxidant properties of seed coat water extracts. All antioxidant assays showed a similar trend, and the cultivar and phenolic concentration had a significant impact on the antioxidant activity. Significant differences were also noted among the cultivars at similar TPC concentrations, and these differences might be attributed to the variations in the composition of phenolics in the extracts. The WE of CDC Greenland (green seed coat), which exhibited superior activity in all antioxidant assays in comparison to the other cultivars, comprised the highest content of procyanidin B1 and catechin-3-glucoside. Procyanidin B1 and catechin-3-glucoside belong to the phenolic subclasses procyanidins and flavan-3-ols, respectively, which are known as highly antioxidative. Moreover, the greatest CTC and TFC were noted for CDC Greenland. This observation was in accordance with that of Beninger and Hosfirld (2003), who studied the antioxidant activity of flavonoid compounds, such as anthocyanins, quercetin glycosides, and proanthocyanidins (condensed tannin) present in the methanol seed coat extracts from 10 colored common bean genotypes. They reported that all extracts have antioxidant activity; however, the highest activity was obtained with extracts rich in condensed tannin.

The present work focused mainly on water extracts of seed coat; however, water may miss the extraction of less polar or high molecular weight phenolic compounds that may be useful for applications in high lipid mediums. Also, the extraction method had an influence on the extraction of phenolics. The repeated extraction used in the present study would not likely be feasible commercially. Therefore, other techniques such as ultrasound- assisted extraction, microwave-assisted extraction and super critical fluid extraction could be examined to increase the phenolic extraction rate or yield.

All antioxidant assays were well-correlated (r > 0.93) indicating that these techniques have excellent consistency in estimating antioxidant activity of seed coat water extracts. The strong activity displayed in all *in vitro* antioxidant assays revealed that the antioxidant mechanisms of water extracts include quenching of free radicals to terminate the radical chain reaction, chelation of transition metal ions. Moreover, the strong positive correlations found between phenolic contents (TPC, TFC and CTC) and antioxidant capacity (r > 0.93) confirm the strong contribution

of phenolics to the antioxidant capacity of water extracts. These results were supported by several other studies which also demonstrated high correlations between antioxidant activity and phenolic content of different plant extracts (Yoo et al., 2008; Kong et al., 2010; Zhang et al., 2011, Zhang et al., 2015).

An important observation was that the antioxidant capacity of WE of zero tannin seed coat were more than six times lower than that of normal tannin seed coat even at similar phenolic concentrations. This may emphasize the fact that antioxidant potential is determined by the type or composition of phenolics in the extracts. In WE of normal tannin cultivars, about 80% of total phenolics consisted of flavonoids; kaempferol tetraglycoside, catechin-o-glucoside, and procyanidins while zero tannin WE were rich in kaempferol. In support of the current findings, Zhang et al. (2015) noted that antioxidant activity of pure catechin was 2 to 3 times greater than that of kaempferol as measured by DPPH radical scavenging ability, ferric reducing antioxidant power and oxygen radical absorption capacity. These observations suggest that the combination of kaempferol tetraglycoside, catechin-3-glucoside and procyanidin have better antioxidant properties than a system containing mostly the kaempferol tetraglycoside which could mainly be due to the differences in the composition and position of reactive OH groups in the system. As well there could be synergistic effects among different phenolic compounds which enhances the antioxidant efficacy of water extracts. Moreover, the activity of different phenolic compounds may vary depending on their affinity to different free radicals available (Minatel et al., 2017). Future research on investigating synergistic or antagonistic effects of different combinations of phenolic compounds might expand the explanation for this observation.

Distribution of phenolic compounds between seed components are different. The level of total phenolics, soluble in water or ethanolic solvents, were much higher in the seed coat than in the cotyledon (Dunaz et al., 2002; Li, 2017), thus, different seed coat components exhibited varying antioxidative activity which was directly proportional to the concentrations of phenolics. Results of current study supported these previous findings. The TPC, TFC and CTC of the flour extracts were 2.14 mg GAE/g, 0.72 mg CE/g, and 1.12 CE/g, respectively. The corresponding values for the seed coat were 36.46 mg GAE/g, 5.14 mg CE/g, and 28.07 CE/g, respectively. These results clearly demonstrated that phenolic content of lentil flour was far below than that for seed coat. TPC of IR lentil flour used in the present study were slightly higher than the TPC found in IR treated pure cotyledon (1.86 mg GAE/g) and whole seed (1.98 mg GAE/g) reported by Li (2017). The

antioxidant activity of flour extracts noted for the DPPH, ABTS free radical scavenging assays, ferrous ion chelation assay and phospholipid peroxidation assay were 47%, 50.94%, 54.23% and 30.45%, respectively which were around half that of the seed coat. The degree of dehulling would therefore have a strong impact on antioxidant quality of flours.

The strong antioxidant activity exhibited by lentil especially the seed coat in *in-vitro* chemical assays revealed the promising potential of lentil seed coat as a source of natural antioxidant for food applications. This work expanded understanding the antioxidant efficacy of lentil seed components in controlling lipid oxidation in chicken meat systems. The model meat products for this analysis were prepared from mechanically separated chicken (MSC). MSC serves as an ideal system for studying the efficacy of antioxidants because of its greater susceptibility to oxidation. The higher susceptibility to oxidation is due to the high phospholipids and hemoglobin concentrations, greater aeration and large surface area exposed to oxygen resulting from cellular disruption (Moerck and Ball, 1974; Lee et al., 1975; Froning 1981; Dawson and Gartner, 1983; Froning and McKee, 2001). Although several studies reported the antioxidant potential of lentil in beef no any information was available regarding its efficacy in chicken meat systems.

The present study elucidated the impact of lentil seed coat and extracts on the lipid oxidation of MSC during cooking and refrigerated and frozen storage. It should be emphasized that the MSC treated with seed coat components (water extracts, aqueous ethanol extracts and ground seed coat) had significantly lower TBARS compared to the control samples showing their strong antioxidant capacity against lipid oxidation caused by cooking. The TBARS values for the samples treated with antioxidant compounds ranged from 0.21 to 0.45 mg MDA/kg meat, while the control sample had a value of 1.96 mg MDA / kg meat. These results clearly exhibited that the application of antioxidant compounds had inhibited the lipid oxidation in MSC. The fresh MSC was fairly stable to oxidation during refrigerated storage of seven days and application of seed coat extracts did not show significant impact on oxidative stability. However, it was noted that, the samples treated with ground seed coat showed significantly higher overall TBARS values compared to the control samples (p<0.05). This effect was not observed for the cooked MSC, thus this prooxidant effect exhibited by ground seed coat was hypothesized to be linked to the activity of oxidative enzymes such as lipoxygenase. Li (2017) demonstrated that lentil seed coat has some lipoxygenase activity of up to 0.02×10^3 units/mg of its water extracts. Lipoxygenase was found to initiate lipid oxidation in food products by catalyzing the unsaturated fatty acids containing *cis,cis*-1,4-

pentadiene causing more rapid lipid oxidation as a result (Eriksson, 1982). Li (2017) compared the antioxidant effects of raw and IR treated lentil flour in beef burgers and found greater levels of TBARS in samples treated with raw lentil flour compared to heat-treated lentil flour samples. Therefore, seed coat from heat treated lentil might be more appropriate for the applications with fresh MSC products. A further novel finding of this work was that the seed coat extracts did not show any prooxidant effects as that of seed coat when applied in uncooked MSC. This might be an indication that under the extraction conditions used in the current experiment, prooxidant compounds have not been integrated into the extracts or inactivated during the pasteurization step. Thus, the application of seed coat extracts appeared be beneficial over the application of ground seed coat.

One of the greatest interests in investigating the antioxidant potential of lentil seed components was to evaluate their applicability as a natural and clean label antioxidant strategy. Therefore, the antioxidant efficacy of lentil seed coat components was evaluated in comparison to other food grade antioxidants to confirm their applicability in meat products. Study one demonstrated the antioxidant potential of seed coat components (ground seed coat and extracts) in comparison to tocopherol, sodium ascorbate, Herbalox® (rosemary oleoresin), and catechin in a MSC model meat system. It was worth noting that the antioxidant capacity of seed coat extracts was found to be comparable or superior to that of the other antioxidant compounds. The antioxidant properties of these reference compounds are well documented and have been reported to inhibit lipid oxidation in different types of meat products. For example, a study conducted by McCarthy et al. (2001) displayed greater antioxidant activities of rosemary, tea catechins, and α-tocopherol in comparison to synthetic antioxidants; BHT/BHA in raw and cooked pork patties. Hence, based on these observations lentil seed coat components may also be effective in such other meat systems and would be able to replace synthetic antioxidants such as BHT. As lentil seed coat is a byproduct, the starting material is economical, and the extraction process is simple at least on a small scale.

Alkaline phosphates are another group of additives that are widely used in meat and poultry products due to their water holding and antioxidant properties. The phosphates are synthetic compounds, therefore, have negative association with the natural and clean label trend. This is the first report showing the applicability of pulse ingredients to replace synthetic phosphates in meat products. Based on the results, it was evident that the addition of the combinations of lentil flour

and seed coat water extract or ground seed coat was similarly effective as the addition of STPP in controlling lipid oxidation and improving water holding capacity of the product. In this meat system, lentil flour worked to replace the water holding and texture properties while the antioxidant compounds in seed coat replaced the antioxidant properties of STPP. Further study with more oxidized meat such as frozen MSC is warranted to confirm its efficacy in meat systems with different degrees of oxidation status. The previous research conducted in this field have focused only on the replacement of water holding properties of phosphates. For example, egg-shell calcium powder, functional carbohydrates such as carrageenan, alginic, acid, guar gum, and chitosan and porcine plasma and rice starch are among the ingredients studied (Hsu and Chung, 2001; Resconi et al., 2015; Bae et al., 2017). These compounds were capable of replacing the properties that enhance the water holding capacity and texture, but not the antioxidant properties. The advantage of lentil seed coat components was that they were capable of delivering the techno-functional properties of phosphates enhancing both texture stability and oxidative stability. While this study did not show any color effects due relatively low myoglobin content, in beef, color changes during storage were also delayed (Pathiratne, 2014; Li, 2017).

In this experiment, lentil SC and WE were added at two levels to provide phenolics at 300 ppm and 500 ppm concentrations. All these treatments showed antioxidant effects similar to STPP (0.3%) and no significant differences were observed between SC and WE treatments at both concentrations (300 ppm and 500 ppm) of TPC. This was probably due to that both treatments (SC or WE) contained the same concentrations of TPC. These results were in good agreement with the finding of study I (Section 3.5.4.7) which showed similar antioxidant properties of SC and WE when added in cooked MSC model system. Moreover, changing the concentration of total phenolics (300 ppm or 500 ppm) in either SC or WE treatments had no significant effect on the TBARS values. The sum of the total phenolics in 300 ppm and 500 ppm of SC or WE treatments which were coming from lentil flour and SC or WE were 428 ppm and 528 ppm, respectively. In study one it was observed that the antioxidant activity of WE as measure in *in vitro* chemical assays were increasing gradually up to around 300 ppm of TPC and then nearly plateaued with further increase in TPC up to 600 ppm. This showed that there was no significant impact on the antioxidant capacity when the TPC of the system was increasing above 300 ppm. So, this could be the reason for the similar antioxidant properties observed between the bologna treatments with 300 ppm and 500 ppm SC/WE. Based on these results it was clear that adding extra TPC at 300 ppm (from SC

or WE) in bologna extended with IR lentil flour was sufficient to replace the antioxidant properties exerted by 0.3% of STPP. It is worth noting that these results were in the absence of nitrite in the system; thus, it would be useful to examine if any synergistic effects of both.

Nitrite is an another additive widely used in cured meat products and this is the first report showing the efficacy of lentil four to replace the antioxidant properties of nitrite. During refrigerated and frozen storage, the chicken sausages produced with IR lentil flour with or without nitrite showed very similar TBARS (<2 mg MDA/kg), proving that exclusion of nitrite from sausage formulations containing IR lentil flour (4%) had no significant impact on their oxidative stability. Excluding nitrite, however, led in decreased sausage color acceptability. Although, the no nitrite products obtained lower color acceptability, the consumer survey conducted with 180 consumers revealed that more than 50 percent of the consumers were willing to buy the products free of added nitrite even at the expense of reduced sensory properties showing its potential marketability. Perhaps, improving the color would help increase the marketability of these products. However, other compounds may need to be added to replace all the functions of nitrite especially the antimicrobial properties.

Meat products to which pulses are added are becoming popular. However, there are few studies on the functionality of IR lentil flour as a meat binder in emulsion type meat products. Therefore, this study was carried out to evaluate the efficacy of IR lentil four as a binder in wiener type sausage. In meat products, flour protein and starch interact with meat protein to form a complex three-dimensional network. In this matrix flour components enhance the water and fat retention in the system (Sanjeewa et al., 2010). As hypothesized, the incorporation of lentil flour reduced the purge loss and expressible moisture in sausages. Lentil flour as a binder was involved in strong structure formation, hence, flour addition resulted in firmer texture compared to the no binder control. Further, adding lentil flour appeared to increase the protein content of the product. The binding properties of lentil flour was compared with a commercial formulation which contained isolated soy protein (ISP) and modified corn starch. Significant differences were not evident between formulations for the physicochemical, water holding and texture properties, showing that the functionality of lentil flour was similar to the ISP and corn starch. In addition, this study demonstrated the potential of making a low-cost sausage solely from MSC. Generally, MSC contains a relatively high amount of collagen and less myofibrillar proteins, and these can negatively influence the overall functionality of meat proteins by decreasing ability to retain water

during processing and storage (Viuda-Martos et al., 2012). The meat company partner in Sri Lanka had not made sausages of this type with only MSC before which is a very economical ingredient. While innovations to improve food products are promising, sensory characteristics remain the key factor shaping consumer preference and purchase decision. In the current study, sensory evaluation was carried out with Canadian and Sri Lankan consumers to evaluate the acceptability of the products formulated with lentil flour. Overall, results showed that lentil flour had very limited effect on sensory attributes of sausages. The increasing level of lentil flour from 4% to 8% slightly increased the perception of texture properties; hardness, cohesiveness, chewiness and graininess. Generally, incorporation of legume flours was found to impart foreign flavor. However, it was worth noting that none of the consumer groups were able to detect differences in the foreign flavor intensity and the after taste in the sausages extended with lentil flour compared to the commercial formulation. Overall acceptability of the lentil flour incorporated sausages was similar to the commercial formulation. These results showed that IR treated lentil flour could be successfully used as a binder up to 8% level in sausage formulations without compromising their sensory acceptability.

Application of lentil seed components in bologna had significant impact on their sensory attributes. Bologna containing lentil flour had a darker colour relative to the control prepared without binders. The most significant impact on sensory properties was observed with the application of ground seed coat. It resulted in a decrease in color acceptability compared to other treatments, but the instrumental colour parameters showed no difference between bologna treatments with seed coat components. This might be due that panelists could see the specks (seed coat particles). Perhaps, grinding seed coat to much finer particle size might help reduce this effect. The particle size of seed coat used in the present work was <250 μm. The added seed coat in uncooked MSC model system (Study 1) also resulted in lower CIE a* values relative to control with no added lentil seed components. Hence, a limitation observed with the application of lentil seed coat was its effect on color perception.

Application of seed coat also had an impact on the texture and flavor of MSC bologna. Bologna with seed coat had lower perception of springiness, cohesiveness, hardness and chewiness. In parallel, the instrumental texture measurement also gave lower values for TPA cohesiveness, springiness and chewiness and torsional gelometry stress and strain at failure. These results showed that products with seed coat would have softer or mushy texture. This softer texture could possibly

be attributable to the fiber fraction in the seed coat. Xu (2017) also observed a mushier texture in bologna incorporated with pulse fiber. Moreover, the application of seed coat in bologna gave higher perception of graininess, foreign flavor and after taste. However, it was noted that color, flavor and texture was not significantly affected due to the application of seed coat water extracts in bologna. Therefore, the application of seed coat water extracts would be the better way to deliver more phenolics into meat products. It should be recalled that there were no significant differences between the two addition levels (300 ppm TPC or 500 ppm) of water extracts with respect to the oxidation stability; therefore, 300 ppm level could be the best to be continue in future applications. In this experiment liquid extract of seed coat (with the TPC concentration of 1.9 mg GAE/g) was used but that would not be practical for the industry, therefore, further tests would be necessary with the dried extracts. Moreover, the assessment of microbiological quality i.e. molds, aflatoxin etc., is necessary in the development of lentil seed coat as a value-added food ingredient to ensure their safety.

Returning to the hypotheses of this research study, the first hypothesis was "there will be differences in phenolic content and antioxidant activity between water and 70% aqueous ethanol extracts of lentil seed coat". This hypothesis was accepted as, water and 70% aqueous ethanol extracts showed significantly different phenolic concentrations. The total phenolics and flavonoids were high in aqueous ethanol (70%) extracts whereas total tartaric esters and flavonols were high in water extracts. However, there was not sufficient evidence to accept the hypothesis "antioxidant activity (ability to delay color and lipid oxidation in raw and cooked MSC) differ significantly between water and 70% aqueous ethanol extracts of seed coat". Both had significant antioxidant capacity.

Results of the second study supported accepting the hypothesis "different cultivars with different seed coat colors will have different phenolic profiles, and antioxidant capacity". Basically, the normal tannin cultivars had higher phenolic concentration and antioxidant capacity. Of the normal tannin cultivars, green seed coat (CDC Greenland and CDC Greenstar) showed higher phenolic content ($p<0.05$) and antioxidant activity compared to the gray and brown seed coat (CDC Maxim, CDC Robin, CDC SB-3). However, the magnitude of the differences observed among the normal tannin cultivars was small and would have little or no practical importance. All normal tannin cultivars studied had appreciable phenolic content with good antioxidant properties.

The hypothesis of the third study was "components of lentil seeds (flour, ground seed coat, and seed coat water extracts) will be capable of delivering water-holding properties and antioxidant properties similar to synthetic phosphates in bologna formulated with MSC without compromising their texture and sensory properties". Results provided sufficient evidence to accept this hypothesis. Overall, the application of IR treated lentil flour and water extracts (with 300 ppm of TPC) appeared to be the best combination to replace phosphates.

In the fourth study, it was hypothesized that "inclusion of lentil flour could improve the water holding, textural, antioxidant, and sensory properties of MSC sausages". Results revealed that the properties of lentil flour added sausages were mostly similar to the commercial product containing ISP and corn starch. Secondly the hypothesis "Canadian and Sri Lankan consumers will have different perceptions toward lentil incorporated products" was accepted as overall mean scores were different between groups for many attributes. However, the magnitude of differences in mean scores between groups were small, hence it could have little practical significance.

In summary, lentil seed coat was a promising source of phenolic compounds. Water and aqueous ethanolic extracts had free radical chain breaking and metal ion chelation antioxidant characteristics and demonstrated significant role in controlling lipid oxidation in MSC systems in comparison to common commercially available antioxidants. Therefore, the application of seed coat components would have the benefits of reducing or eliminating the use of synthetic antioxidants while delivering biologically active compounds. On the other hand, the seed coat is a by-product of the lentil milling industry with limited value. This study demonstrated the potential available to add value to this by-product. The seed coat, for instance, can be used as a source for the commercial extraction of bioactive compounds and use in the development of functional foods. Besides, the product development studies performed proved that lentil flour has the potential to be used in processed meat products as a plant binder. These ingredients may further be used in other food products as well.

8. OVERALL CONCLUSIONS

The overall goal of this project was to explore the potential applications of lentil seed components in meat systems. The results indicated that the seed coat has a considerable amount of phenolic compounds which are extractable in water and aqueous (70%) ethanol. The solubility of the phenolics differed slightly between the cultivars and solvents, showing higher values for cultivar CDC Greenland and aqueous (70%) ethanol. The total phenolic, flavonoid, and condensed tannin contents and antioxidant properties varied within a narrow range among the normal tannin cultivars. Compared to normal tannin cultivars, the phenolic content and antioxidant capacity of zero tannin cultivars were markedly lower (6-fold difference). LC-MS analysis detected a number of phenolic compounds, notably the phenolic acids, flavones, flavonols, flavon-3-ols, and procyanidins. Normal tannin seed coat consisted mainly of kaempferol tetraglycoside, catechin-3-glucoside, and procyanidin, while kaempferol tetraglycoside was the dominant phenolic compound in zero tannin seed coats. Seed coat extracts of normal tannin lentil possessed strong antioxidant capacity. Correlation analysis revealed that phenolics were strongly associated with antioxidant activity and that procyanidins and flavon-3-ols mainly contributed to the antioxidant activity. Seed coat extracts performed as reducing agents, free radical scavengers, chelators of pro-oxidant transition metals, and these mechanisms contribute to the overall antioxidant efficacy of seed components. Clustering analysis classified green lentil (CDC Greenland and CDC Greenstar) as having relatively more phenolics and stronger antioxidant capacity compared to the other cultivars.

The application of seed coat and extracts at 500 ppm of total phenolic concentrations demonstrated significant antioxidant effects during the cooking and storage at refrigerated (for 7 days) and frozen (for 7 months) conditions in MSC. The comparison of antioxidant efficacy of seed coat with commercial antioxidant compounds displayed promising results. The antioxidant capacity of seed coat and its extracts measured in *in vitro* assays and meat model systems were similar to (+/-)-α-tocopherol, (+)-catechin, sodium ascorbate, and Herbalox®. Concerning the

uncooked meat, neither seed coat extracts nor commercial antioxidant compounds showed significant impact on color or lipid oxidation.

The study evaluating the applicability of lentil seed components as a replacer for phosphates in non-cured bologna sausage suggested that the combination of lentil flour and seed coat water extracts are a potentially effective system to deliver the techno-functional properties of synthetic phosphates without compromising texture and sensory properties. The exclusion of STPP from the bologna formulations extended with lentil flour had limited effect on the cook loss, expressible moisture content, and purge loss and the textural properties as interpreted by TPA hardness, cohesiveness, adhesiveness, springiness, and chewiness. Moreover, the addition of infrared heated lentil flour together with seed coat or water extract of seed coat (up to 500 ppm TPC) was able to control lipid oxidation in chicken bologna as effectively as STPP (0.3%). A minor limitation observed with the application of ground seed coat in cooked non-cured meat products was the decrease in color and flavor acceptability. Reducing the seed coat particle size might help reduce the negative impacts of adding ground seed coat. The addition of water extracts had a limited impact on color and flavor attributes; hence, this would be a better way to deliver more phenolic compounds in meat products.

In chicken sausages, lentil flour resulted in increased water-holding properties compared to the no-binder formulation. Lentil flour up to 8% had limited effect on textural properties and proximate composition, and they were similar to the commercial products formulated with isolated soy protein and modified corn starch. Exclusion of nitrite resulted in reduced a* value; however, the oxidative stability was not affected and was comparable to the products with nitrite. Sensory acceptability of the sausages extended with lentil flour was similar to the commercial products, and no apparent differences in the preferences for sausages were found between Canadian and Sri Lankan consumers. Therefore, chicken sausages with good acceptability and high lipid stability could be prepared by incorporating lentil flour up to 8% level.

Overall, results of this study suggested that lentil seed coat can serve as an excellent source of antioxidants that could be used as functional ingredients to replace synthetic antioxidants in meat products and to develop nutraceutical products or health foods. The lentil flour is a candidate plant binder with antioxidant functionality for meat products.

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Appendix A. **Evaluation procedure and definitions of terms for descriptive analysis of bologna**

Stage I	*Observe the product and touch with fingers;* Evaluate for: **Color:** Asses the overall color of the product **Overall color desirability:** Look at the exterior surface and assess the degree of liking of overall color **Surface moisture:** Degree to which sample feels wet/oily on the surface **Surface smoothness:** Degree to which the surface is smooth i.e., not rough or uneven
Stage II	*Place a piece of sausage/ meat ball into mouth; bite down with front teeth* Evaluate for: **Springiness:** Degree to which a product returns to its original shape once it has been compressed between teeth. **Hardness:** Force required to compress the sample with molars or incisors **Cohesiveness:** degree to which a substance is compressed between the teeth before it breaks. **Denseness:** Compactness of cross section
Stage III	*Chew a piece with molar teeth* Evaluate for: **Chewiness:** Number of chews required to masticate the meat sample to a consistency suitable for swallowing. **Moisture release:** Amount of juice released after 10 chews **Graininess:** Degree to which sample contains small distinct particles **Saltiness:** Intensity of salt sensation **Overall flavor intensity:** Intensity of typical chicken sausage/ meat ball seasoning and chicken meat flavor present in the mouth during mastication **Foreign flavor intensity:** Amount of any atypical or off-flavors present in the mouth during mastication (If any present, please describe in comments section)
Stage IV	*Swallow sample* Evaluate for: **Ease of swallow:** Degree to which bolus can be readily swallowed **Aftertaste**: Taste remaining in the mouth after eating. **Overall acceptability:** Degree of acceptability of product

Appendix B. Scoresheet for sensory evaluation of bologna

Panelist #: ……………………………   **Date: …………………………**
Sample Code …………………………

Instructions:
Please read and understand the method of evaluation and definitions of the sensory characteristics provided in **Table 1.** Evaluate the samples in the order that the samples are arranged. For each sensory characteristic, circle **ONE** descriptor along the 8-point scale that best describe your impression. Feel free to provide any comments as well. Please take a drink of water **before beginning** and **between** samples. Unsalted crackers are also available as needed.

Sensory character	Rating							
	8	7	6	5	4	3	2	1
Stage I: *Observe the product and touch with fingers*								
Color	Red	Dark pink	Moderately pink	Slightly pink	Slightly gray brown	Moderately gray brown	Dark gray brown	Gray
External color desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Internal color desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Surface smoothness	Extremely smooth	Very smooth	Moderately smooth	Slightly smooth	Slightly Rough	Moderately rough	Very Rough	Extremely rough

Stage I: *Place a piece of bologna into mouth; bite down with front teeth*

Springiness	Extremely Elastic	Very Elastic	Moderately elastic	Slightly elastic	Slightly Rigid	Moderately rigid	Very Rigid	Extremely rigid
Hardness	Extremely Firm	Very Firm	Moderately firm	Slightly firm	Slightly Soft	Moderately Soft	Very Soft	Extremely soft
Cohesiveness	Extremely Cohesive	Very cohesive	Moderately cohesive	Slightly cohesive	Slightly crumbly	Moderately crumbly	Very crumbly	Extremely crumbly
Denseness	Extremely compact	Very compact	Moderately compact	Slightly compact	Slightly loose	Moderately loose	Very Loose	Extremely loose

Stage II: *Chew a piece of bologna with molar teeth*

Chewiness	Extremely chewy	Very Chewy	Moderately chewy	Slightly chewy	Slightly mushy	Moderately mushy	Very Mushy	Extremely mushy
Moisture release	Extremely juicy	Very Juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very Dry	Extremely Dry
Grainy	Extremely grainy	Very grainy	Moderately grainy	Slightly grainy	Slightly smooth	Moderately smooth	Very smooth	Extremely smooth
Saltiness	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately bland	Very Bland	Extremely Bland
Overall Flavor intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately bland	Very Bland	Extremely Bland

Foreign flavor intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately bland	Very Bland	Extremely Bland
Ease of swallow	Extremely easy	Very easy	Moderately easy	Slightly easy	Slightly hard	Moderately hard	Very Hard	Extremely Hard
After taste	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately bland	Very bland	Extremely Bland
Overall acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly acceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Comments:

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Appendix C: Ranking scale for sensory descriptors of sausages

Instructions: Evaluate the samples in the order that the scorecards are arranged. For each characteristic, circle a descriptor along the scales that best describes your impression. Feel free to provide any comments as well. Please take a drink of water before beginning and between samples. Unsalted crackers are also available as needed.

Sensory character	Rank							
	8	7	6	5	4	3	2	1
Stage I: *Observe the product and touch with fingers*								
External color desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Internal color desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Surface moisture	Extremely moist	Very moist	Moderately moist	Slightly moist	Slightly dry	Moderately dry	Very Dry	Extremely dry
Surface smoothness	Extremely smooth	Very smooth	Moderately smooth	Slightly smooth	Slightly rough	Moderately rough	Very rough	Extremely rough
Stage II: *Place a piece of sausage into mouth; bite down with front teeth*								
Springiness	Extremely elastic	Very elastic	Moderately elastic	Slightly rigid	Slightly rigid	Moderately rigid	Very rigid	Extremely rigid

Hardness	Extremely firm	Very firm	Moderately firm	Slightly firm	Slightly soft	Moderately Soft	Very soft	Extremely soft
Cohesiveness	Extremely cohesive	Very cohesive	Moderately cohesive	Slightly cohesive	Slightly crumbly	Moderately crumbly	Very crumbly	Extremely crumbly
Uniformity	Extremely even	Very even	Moderately even	Slightly even	Slightly uneven	Moderately uneven	Very uneven	Extremely uneven
Moisture release	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very Dry	Extremely dry
Denseness	Extremely compact	Very compact	Moderately compact	Slightly loose	Slightly loose	Moderately loose	Very loose	Extremely loose

Stage III: *Chew a piece with molar teeth*

Chewiness	Extremely chewy	Very chewy	Moderately chewy	Slightly chewy	Slightly mushy	Moderately mushy	Very mushy	Extremely mushy
Moisture release	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very Dry	Extremely dry
Grainy	Extremely grainy	Very grainy	Moderately grainy	Slightly grainy	Slightly smooth	Moderately smooth	Very smooth	Extremely smooth
Skin	Extremely distinct	Very distinct	Moderately distinct	Slightly distinct	Slightly smooth	Moderately smooth	Very smooth	Extremely smooth
Saltiness	Extremely high	Very high	Moderately high	Slightly high	Just about right	Slightly low	Moderately low	Very low

Overall Flavor intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately Bland	Very bland	Extremely bland
Foreign flavor intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately Bland	Very bland	Extremely bland

Stage IV: *Swallow the chewed sample*

Ease of swallow	Extremely easy	Very easy	Moderately easy	Slightly easy	Slightly hard	Moderately Hard	Very hard	Extremely hard
Mouth coating	Extremely high	Very high	Moderately high	Slightly high	Slightly low	Moderately Low	Very Low	Extremely low
After taste	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately Bland	Very bland	Extremely bland
Overall acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly acceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Part D: Scoresheet for Consumer Sensory Evaluation of Chicken Sausages

Panelist #: ………………….

Instruction: Please evaluate the samples in the order that the sample are presented to you from left to right. Please take a bite of cracker and a drink of water **before** and **between** samples.

1) For each sensory characteristic, check **ONE** descriptor along the 6-point scale that best describe your impression.

External color acceptability	Like very much ☐	Like moderately ☐	Like slightly ☐	Dislike slightly ☐	Dislike moderately ☐	Dislike very much ☐
Internal color acceptability	Like very much ☐	Like moderately ☐	Like slightly ☐	Dislike slightly ☐	Dislike moderately ☐	Dislike very much ☐
Texture	Very tender ☐	Moderately Tender ☐	Slightly tender ☐	Slightly tough ☐	Moderately tough ☐	Very Tough ☐
Texture acceptability	Like very much ☐	Like moderately ☐	Like slightly ☐	Dislike slightly ☐	Dislike moderately ☐	Dislike very much ☐
Juiciness	Very Juicy ☐	Moderately juicy ☐	Slightly juicy ☐	Slightly dry ☐	Moderately dry ☐	Very dry ☐
Juiciness acceptability	Like very much ☐	Like moderately ☐	Like slightly ☐	Dislike slightly ☐	Dislike moderately ☐	Dislike very much ☐
Flavor intensity	Very intense ☐	Modernly intense ☐	Slightly Intense ☐	Slightly bland ☐	Moderately bland ☐	Very bland ☐
Flavor Acceptability	Like very much ☐	Like moderately ☐	Like slightly ☐	Dislike slightly ☐	Dislike moderately ☐	Dislike very much ☐
Overall Acceptability	Like very much ☐	Like moderately ☐	Like slightly ☐	Dislike slightly ☐	Dislike moderately ☐	Dislike very much ☐

Please provide any comments :

………

………

………

………………………………………………………………………………………

2) If this product is commercially available, what would be your purchase intention?

	Definitely would buy it
	Probably would buy it
	Might or might not buy it
	Probably would not buy it
	Definitely would not buy it

- **Please review that you have answered all the questions above.**
- **You may now proceed to the next sample or**
- **If this is your last sample, you may now proceed to PART II: Consumer Survey**

Appendix E: Questionnaire for Consumer study

Panelist #:

Part II: Consumer survey

Please answer the questions below. The information will be treated with strict confidence and all information collected will not be identified with your name.

1. On average, how often do you consume sausages?

	3- 5 times per week
	1-2 times per week
	1-2 times per month
	I don't eat sausages typically

2. Have you ever purchased sausages?

	Yes
	No

3. If you are interested to buy sausages, please indicate how important the following features are to you when shopping for sausages.

	Not at all important ⟵⟶ Extremely					
	1	2	3	4	5	6
Price						
Type of meat						
Protein content						
Fat content						
Fiber content						
Salt content						
Added preservatives						
Added colors						
Pre-cooked option						

4. What color is preferred in sausages?

	Pale pink
	Dark ping
	Red
	Grey brown

5. Are you aware that nitrites are added in some meat products to give their pink color?

	Yes
	No

6. Are you aware that the nitrite added in the product is listed under ingredients?

	Yes
	No

7. Please write what do you know about the use of nitrites added in meat products?

……
……
……
……

8. If nitrite free sausage product is available in the market, what would be your purchasing choice?

	I will buy it even at the expense of lower color acceptability.
	I will buy it only if it still has pink color.
	I will buy it only if it is cheaper than the sausages with nitrites.
	I will buy any product without considering the presence or absence of added nitrites.

9. Below is a list of statements relating to food purchasing habits and lifestyle. For each, please indicate how much you agree or disagree on the scale provided.

	Completely disagree ⟷ Completely agree					
	1	2	3	4	5	6
I consider myself very health conscious.						
I exercise on a regular basis.						
I regularly read nutritional labels on the food I purchase.						
I will pay more for a food product if it is more nutritious than a cheaper alternative.						
I will opt for healthier version of a food product, even at the expense of lower sensory quality.						
Price is the most important factor I consider when I buy meat products.						
I believe that eating whole flour healthier is than eating refined starch						
I know that lentil is a rich source of protein and fiber						
I believe that the incorporation of lentil flour in meat products makes them more nutritious and healthier						

10. Demographic information
    The following questions are intended to understand the general demographics of participants. The information will be kept confidential and will only be used to understand broad trends, and not on an individual level.

    How many people live in your home including yourself?
    Enter number: _____

Which of the following categories best describes your role in grocery shopping for your household?

	Primary shopper
	Share the shopping
	Someone else is the primary shopper

Which one of the following best describes your monthly household income level before taxes?

	Under Rs. 20,000
	Rs. 20,000 - Rs. 39,000
	Rs. 40,000 - Rs. 59,000
	Rs. 60,000 - Rs. 79,000
	Rs. 80,000 - Rs. 99,000
	Over Rs. 100,000

Gender

	Male
	Female

Age category

	18 - 29 years
	30 - 39 years
	40 - 49 years
	50 - 59 years
	60 - 69 years
	Over 70 years

Education (Highest level completed)

	Some Grade School
	Some high school (Ordinary level)
	High school Graduate (Advance level)
	Some University (Diploma)
	University / College Graduate
	Graduate School

This is the END of the survey. Thank you for participating in this study. Please hand in your completed Score Sheet and Consumer Survey as you exit. Feel free to help yourself to some treats.