EFFECTS OF SEED MOISTURE AND MICRONIZING TEMPERATURE ON LENTIL FLOUR PROPERTIES AND THE STABILITIES OF COLOUR AND UNSATURATED LIPIDS OF BEEF-LENTIL SYSTEMS

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ABSTRACT

This study investigated the effect of seed moisture level of lentil and surface temperature of micronization (infrared heat treatment) on the physico-chemical and functional properties of resulting flours and how these flours affected colour and unsaturated lipid oxidation when incorporated into ground beef products. Flour from raw seed (non-tempered and non-micronized) was used as the control. Whole seeds of small green lentil (*Lens culinaris* L., var. Eston) without tempering (8% moisture) and tempered to 16% or 23% moisture was infrared heat treated (micronized) to 115, 130, 150 or 165 °C surface temperature. The decreased protein solubility (2-60%) and lipoxygenase (70-100%), peroxidase (32-100%) and trypsin inhibitory (up to 54%) activities of resulting flours indicated changes in the protein fraction due to heat-moisture treatment. Starch gelatinization was observed at the 23% moisture level and changes in pasting properties, and water and oil absorption capacities varied with treatment. The heat-moisture combinations modified properties of starch and protein to different degrees and, consequently, lentil flour functionalities. Incorporation of lentil flour as a binder in low fat (<10%) beef burgers at 6% (w/w) showed that flours from micronized lentil seeds enhanced retention of redness and suppression of lipid oxidation as indicated by Hunter a* values and thiobarbituric acid reactive substances values, respectively, in a retail display setting. Investigation of total phenolics in aqueous salt extracts of lentil flours showed a decrease in content with increased micronization temperature. The antioxidant assays showed no changes in the ferric ion reducing power or reduction of hydroxyl radical scavenging and superoxide radical scavenging activities with heat-moisture treatment. Reduction of lipoxygenase and peroxidase activities was evident in lentil flour aqueous salt extracts, and the enzyme activities were localized to seed cotyledons. The myoglobin-liposome model study showed that a flour extract from the 16% moisture and 150 °C treatment resulted in a slower rate of oxymyoglobin oxidation initiation than other treatments which had different levels of lipoxygenase and peroxidase activities. Unsaturated lipids accelerated oxymyoglobin degradation irrespective of the presence of lentil extract. The extended fresh red colour retention of ground beef due to addition of flours from micronized seed compared to that from non-micronized seed may be related to suppression of pro-oxidant activities and the activity of potential antioxidants. The putative antioxidative compounds in lentil that are available for meat components may include compounds other than lentil seed phenolics.

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1. INTRODUCTION

Lentil is increasingly being used in healthy diets in order to achieve general well-being and reduce the risk of illness and heart disease (Agriculture and Agri-Food Canada, 2010). It is used as a main part of the diet in the Mediterranean region, Middle East and Asia and often combined with rice (Yadav et al., 2007). High protein (24.3 – 30.2%) and low fat (0.7 – 3.6%) contents of lentil (Wang & Daun, 2006; Urbano et al., 2007) increase its appeal as a plant-based ingredient in value-added processing (Der, 2010). Apart from the nutritional properties, functional properties such as solubility, water binding, emulsification, foaming and gelation abilities of lentil flour play an important role when using it in food formulations and processing (Boye et al., 2010). Therefore, the characteristics of lentil flour and its functional behaviour must be understood to use it as a food ingredient (Der, 2010).

Micronization is a short time heating process where infrared radiation is applied to a product on a vibrating bed. Micronization has been used in the food industry to increase food safety, shelf stability and nutritional value as well as to decrease cooking time of starch-rich grains and seeds (Zheng et al., 1998; Arntfield et al., 2001). Changes in protein and carbohydrate fractions of lentil seed by micronization can modify its functional characteristics thus enabling the expansion of applications of lentil flour in the food industry. Pre-treatments such as tempering prior to micronization have shown positive effects on starch and protein properties (Arntfield et al., 2001; Bellido et al., 2006).

Burgers and other comminuted meat products are popular food items worldwide and there have been efforts to reduce the fat content of these products while minimizing the product cost (Der, 2010). Since lentil is high in protein and starch and low in fat, it can serve as a binder in burgers with additional functional traits such as gluten-free and low-fat. Use of flour from micronized lentil seeds as a binder in beef burgers have shown enhanced colour stability of fresh ground meat as well as consumer acceptability when flour of micronized lentil seeds (tempered to achieve 15% moisture level and micronized to 135 °C) was incorporated as a binder (Der, 2010).

Functional properties of lentil flour should be improved in order to increase its utilization in different food products. Tempering of lentil seeds to achieve different moisture levels and micronizing at different temperature levels could provide lentil flours with different functionalities, widening their utilization in food products including fresh meat systems.

1.1 Hypotheses

The hypotheses formulated in the study were:

(1) Different tempering moisture levels and different surface temperatures achieved during micronization affect the physico-chemical properties of small green lentil seed (var. *Eston*) differently. Therefore, the resulting flours have varying functionalities.

(2) Lentil flours obtained from seeds subjected to different micronization (infrared heat-moisture) treatments will cause variation in the oxidative stability of fresh meat colour and lipids.

(3) Lentil flours obtained from seeds subjected to different micronization (infrared heat-moisture) treatments will have different levels of antioxidant potential in their salt-soluble fractions.

1.2 Objectives

The objectives of the study were to:

(1) Determine the effects of different micronization (infrared heat-moisture) treatments of lentil seed on the physico-chemical and functional properties of the resulting flours.

(2) Determine the effects of different micronization (heat-moisture) treatments of lentil seed on colour and lipid oxidation stability of fresh ground beef products when lentil flour was incorporated as a binder.

(3) Study the properties of aqueous salt-soluble components of lentil flours prepared from micronized seed which could cause the stabilities of fresh meat colour and lipids.

2. LITERATURE REVIEW

2.1 Lentil: uses and market classes

Lentil (*Lens culinaris* L) is a pulse crop best adapted to grow in the cooler temperate zones of the world and in the winter season in the Mediterranean climates. Main lentil producing countries in the world include India, Canada, Turkey, Syria, Australia, Nepal and the United States, while Canada is the leading lentil exporting nation (Saskatchewan Ministry of Agriculture, 2010). Lentil is mainly used in human food. A small quantity of lentil is used for livestock feed when it is undesirable for human consumption due to degrading factors such as chipping, wrinkling or staining (Agriculture and Agri-Food Canada, 2010). Lentil is sold as whole seeds or dehulled and split into halves. Traditionally, lentil is a major component of the diet in many Middle Eastern countries and India and most commonly used in main dishes, side dishes and as sprouted grain in salads. Lentil is quick and easy to prepare since it has a shorter cooking time than other pulses and pre-soaking before cooking is not required. The cooked lentil seeds have a special earthy flavour and the split type can easily absorb flavours from spices and seasonings. As minor uses, flour from lentil is used in soups, stews and purees or mixed with cereals to make bread and cakes and also in infant foods (Sandhu & Singh, 2007; Yadav et al., 2007).

In addition to its food use, lentil has uses in traditional medicine as well. Lentil soups are known to improve digestion and are prescribed during convalescence and it is also considered as a blood purifier. Application of lentil paste to skin to treat skin disorders has been a practice in traditional medicine. Lentil is believed to alleviate peptic or duodenal ulceration and other intestinal diseases (Sandhu & Singh, 2007).

The colour of the lentil seed coat can be clear, green, tan, grey, brown or black, while the cotyledon is yellow, red or green. Red and green lentil comprises the two main market types. The green lentil types have a green seed coat and yellow cotyledons. Based on the seed size, the green lentil types are referred to as large, medium and small. The large green type has a seed weight of 60 to 70 grams per 1000 seeds and includes the varieties Laird, CDC Glamis, CDC Sovereign, CDC Grandora, CDC Plato and CDC Sedley. CDC Richlea, CDC Vantage and CDC Meteor

varieties are medium green type with a seed weight of 50 to 55 grams per 1000 seeds whereas small green lentil type is about 35 grams per 1000 seeds and include the varieties *Eston*, *CDC Viceroy* and *CDC Milestone*. Canadian red lentil which has a brown or pale green seed coat with red cotyledons could be of 30 to 40 grams per 1000 seeds and includes *Crimson*, *CDC Redcap*, *CDC Redberry*, *CDC Robin*, *CDC Blaze*, *CDC Rouleau* and *CDC Rosetown* varieties (Agriculture and Agri-Food Canada, 2010; Saskatchewan Ministry of Agriculture, 2014).

2.2 Nutritional value of lentil

As a pulse, lentil is an important source of dietary protein for a large part of the world's population (Boye et al., 2010). Regular dietary intake of pulses has been related with the lowered risk of some diseases such as diabetes, cancer and cardiovascular disease (Barbana & Boye, 2012). The energy provided by lentil is similar to that of cereals such as wheat and also other pulses such as faba beans, peas or beans (Urbano et al., 2007).

2.2.1 Macro-nutrients

Nitrogen

Average total nitrogen content of lentil is 4.25 g per 100 g of dry matter. Almost 15% of the total nitrogen is present as small peptides, nucleic acids, purine and pyrimidine bases and alkaloids and the remaining nitrogen is classified as protein nitrogen (Urbano et al., 2007). Most of the protein found within pulse seeds is located in the cotyledons in the form of storage proteins, which are categorised as albumins, globulins and glutelins based on their solubility properties and of these, globulins represent approximately 70% of the total protein (Urbano et al., 2007; Roy et al., 2010). A minor fraction of nitrogen is present in insoluble form due to the non-covalent interactions or disulfide bonds between different proteins and remains connected with the insoluble dietary fiber fraction (Urbano et al., 2007). Lentil protein is deficient in tryptophan and sulfur containing amino acids such as methionine and cysteine (Urbano et al., 2007). Urbano et al. (2007) reported that lentil contains non-protein amino acids such as taurine, gamma-amino butyric acid, hydroxyarginine and hydroxyornithine (Rozan et al., 2001; Kuo et al., 2004).

Fat

Lentil has a low fat content which can range from 0.7 to 3.6% (Urbano et al., 2007). In the fatty acid profile of lentil, unsaturated fatty acids are present in high amounts and of these oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) constitute 70 to 85% of the total fatty acids. Kumar et al. (2006) reported that saturated fatty acids are present in lesser amounts in lentil and palmitic (C16:0) is the main saturated fatty acid present at 10 to 15% of the total fatty acids (Urbano et al., 2007).

Carbohydrates

Carbohydrate content in whole lentil varies from 43 to 70% (Urbano et al., 2007). Soluble sugars such as fructose, glucose and sucrose comprise 1 to 2.5% of lentil carbohydrate while 2 to 8% are galacto-oligosaccharides such as raffinose, ciceritol, stachyose and verbascose. Starch content of lentil can vary between 35 to 63% with an amylose content of 20 to 45.5% and almost 20% of the total carbohydrates in lentil are non-starch polysaccharides (Urbano et al., 2007).

Food carbohydrates are characterized by the glycemic index (GI) which is a measure of the postprandial increase in blood glucose level. Consumption of high GI foods may increase the risk of obesity, type 2 diabetes and heart disease since these foods cause a rapid increase in blood glucose and insulin levels resulting in reactive hypoglycemia and elevated levels of serum free fatty acids (Chung et al., 2008). Pulse starches, including lentil, have lower GI than cereal or tuber starches and the consumption of pulses is therefore related to reduced incidence and prevalence of diabetes and heart diseases (Chung et al., 2008). The reduced bioavailability of pulse starches due to high levels of amylose, large amount of viscous, soluble dietary fiber and strong interaction between amylose chains can influence the rate and extent of pulse starch digestibility resulting in slow and moderate postprandial glucose and insulin responses, respectively (Hoover & Zhou, 2003). According to the rate of glucose release and absorption in the gastrointestinal tract, starch is classified into rapidly digestible starch (RDS), which causes a sudden increase in blood glucose level after ingestion, slowly digestible starch (SDS) that is digested completely in the small intestine and resistant starch (RS) which cannot be digested in the small intestine. Legume starches contain high amounts of SDS which causes slow increase of blood glucose levels and a moderate effect on GI increase (Chung et al., 2009; Lehmann & Robin, 2007).

Dietary fiber

Dietary fiber promotes beneficial physiological effects including laxation and reduction of blood glucose and cholesterol (AACC Report, 2001). Dietary fiber is classified as insoluble and soluble fiber and these have unique physiological functions and nutritional benefits (Tosh & Yada, 2010). Legumes contain high contents of both insoluble and soluble fibers (Tosh & Yada, 2010). Total dietary fiber content of lentil ranges from 9.7 to 24.1% (Urbano et al., 2007). Cellulose, hemicellulose and lignin are insoluble fibers whereas oligosaccharides, pectin and β-glucan are soluble fibers which help to lower blood cholesterol and control blood glucose levels (Tosh & Yada, 2010).

2.2.2 Micro-nutrients

Minerals and vitamins

Minerals play important roles in physiological functions in the human body and their deficiencies can have significant negative impacts (Karakoy et al., 2012). Lentil is an important source of dietary essential minerals including potassium, phosphorous, calcium, magnesium, sodium, iron, zinc, copper and manganese (Urbano et al., 2007). However, the bioavailability of some minerals such as iron could be adversely affected by natural chelating agents and anti-nutrients such as phytic acid, tannins and oxalate present in pulses which interfere with nutrient utilization (Urbano et al., 2007; Faris et al., 2013). Lentil also contains trace elements such as aluminium, chromium, cobalt and selenium (Urbano et al., 2007). Selenium content in lentil varies according to the type of soil and lentil cultivar grown. In the province of Saskatchewan, harvested lentil seeds contain 425-673 µg of selenium per 100 g of dry lentil, which is more than 80% of the recommended dietary allowance (Faris et al., 2013). Most of the minerals of lentil are present in the cotyledons (Urbano et al., 2007).

Lentil is also rich in vitamins such as thiamine, riboflavin, niacin, B6 and folic acid. However, similar to most other legume species lentil also contains only small amounts of vitamin C, carotene and retinol (Urbano et al., 2007). In addition, vitamins E and K contents in lentil are also low (Faris et al., 2013).

2.2.3 Antinutrients

Lentil contains antinutritional factors which limit its nutritional acceptability and availability (Barbana & Boye, 2013). The accumulation of antinutritional compounds within the pulse seeds plays a protective role by helping the plant complete its life cycle under adverse conditions such as predation from parasites, insects, fungi and herbivores (Duranti, 2006). Antinutritional compounds disrupt the digestion process when raw seed or flour is consumed by monogastrics making them unpalatable, but ruminants are not affected since they have host-specific microorganisms capable of digesting pulse seed or flour (Roy et al., 2010). Phenolic compounds, phytic acid, oligosaccharides and saponins are examples of non-protein antinutritional compounds, whereas lectins or agglutinins, trypsin inhibitors, chymotrypsin inhibitors, antifungal peptides and ribosome-inactivating proteins are some of the protein antinutritional compounds found in lentil (Roy et al., 2010; Barbana & Boye, 2013). Trypsin and chymotrypsin inhibitors in lentil can decrease the effectiveness of pancreatic enzymes and interfere with the digestion of protein which can cause an enlargement of the pancreas (Urbano et al., 2012). Lectins are another type of antinutritional compounds found in raw lentil seeds or flour which can bind to mono- or oligosaccharides and cause deleterious effects in humans such as growth suppression, diarrhea, bloating, vomiting and red blood cell agglutination (Roy et al., 2010). Phytic acid forms complexes with proteins and minerals decreasing the availability of these nutrients for absorption (Cheryan, 1980). Saponins, another non-protein antinutritional compound found in lentil in low contents (Ruiz et al., 1997), have the ability to haemolyze red blood cells (Urbano et al., 2012). However, research findings have indicated that some of the antinutritional compounds have health benefits. Certain polyphenolic compounds and saponins have anticarcinogentic properties (Barbana & Boye, 2013).

2.3 Enzymes

Lipoxygenase

Lipoxygenases are a class of non-heme iron-containing proteins found in both plants and animals (Gardner, 2001). In plants, lipoxygenases is found mainly in vegetative tissues, but also accumulate in various seeds, especially in leguminous seeds and catalyse the oxidation of polyunsaturated fatty acids with *cis, cis*-1,4- pentadiene structures, such as linoleic (C18:2) and linolenic (C18:3) acids forming conjugated diene hydroperoxides (Loiseau et al., 2001). Plant

lipoxygenases are also capable of oxidizing polyunsaturated fatty acids such as arachidonic acid in either esterified or free form (Gardner, 2001). Although lipoxygenase activity is important for the plant's defense against pathogens, there are negative aspects of the enzyme in foods since lipoxygenase-mediated conversion of polyunsaturated fatty acids to aldehydes and alcohols is a major contributor to the off-flavour in legume protein products (Kermasha & Metche, 1987; Gardner, 2001). Based on results of a study on lipoxygenase activity of legume seeds, Chang & McCurdy (1985) reported that lentil, soybean and cowpea had the highest levels of lipoxygenase activity (over 2000 units per mg of flour) among the fourteen legume seeds studied.

Peroxidases

Peroxidases are another group of enzymes which can contribute to deteriorative changes in flavour, texture, colour and nutrition of both raw and processed foods. Studies done on legume seeds such as peas, green beans and pinto beans have shown that peroxidase activity is responsible for off-flavour production (Kermasha & Metche, 1988). Studies have shown that peroxidases are the most heat–stable enzymes. McEldoon & Dordick (1996) demonstrated that soybean peroxidase has an extremely high melting temperature of 90.5 °C, at pH 8.0 in the presence of 1 mM $CaCl_2$. Results of another study on soybean seed coat peroxidase by Ghaemmaghami et al. (2010) showed that the enzyme activity was 2.5 times higher at an elevated temperature of 65 °C compared to the activity at room temperature and 95% of the activity was retained for 30 min at 75 °C. Under certain conditions of limited heat treatments, peroxidases can regain activity during storage resulting in loss of flavour or development of off-flavour (Kermasha & Metche, 1988; Yemenicioglu et al., 1998).

2.4 Antioxidant activity of lentil

Lentil contains antioxidant compounds such as phenolic compounds which prevent oxidation in foods as well as protect against oxidative damage in the human body (Aguilera et al., 2010; Gharachorloo et al., 2012). Phenolic compounds are radical scavengers and inhibit lipid peroxidation by binding with free radicals generated in lipid peroxidation, therefore the presence of these compounds is a good indicator of potential antioxidant activity (Aguilera et al., 2010). Most of the phenolic compounds in lentil are concentrated in the hull and therefore lentil hulls have superior antioxidant activity compared to whole seeds or cotyledons (Oomah et al., 2011).

2.5 Processing of lentil

Before using legumes as ingredients in food preparations, they undergo several processing steps including cleaning, drying, sorting, splitting, milling and fractionating and other steps like dehulling, puffing, roasting and grinding based on the pulse and its intended use (USA Dry Pea and Lentil Council, 2010). Processing can increase the utilization and consumption of pulses and also enhance the functional characteristics (Maskus, 2010). Minimizing the loss of nutritional value and maximizing the protein quality of the pulses are among the main goals in processing because the protein quality of pulses is important in achieving the desired texture and quality of the food (USA Dry Pea and Lentil Council, 2010). Functional properties such as water and fat absorption, foaming and emulsification properties can be affected by several factors such as pulse type, seed pre-treatment and milling procedure (Maskus, 2010).

Size and shape of lentil seed, appearance of the seed coat, colour, uniformity of appearance and cotyledon colour are important aspects for consumer preferences which need to be considered in processing lentil for consumption. In lentil primary processing, unwanted organic and inorganic materials are removed using screens and air-flow mechanisms and the remaining seeds are separated into required quality classes of lentil according to diameter, thickness, density and colour (Vandenberg, 2009).

2.5.1 Dehulling and milling of lentil

In secondary processing of lentil, decortication or dehulling, which is the removal of seed coat from cotyledons, followed by polishing and splitting of the seeds are done. Further milling, grinding or fractionation of the whole or decorticated lentil seeds are done in the next processing stage, enabling the use of lentil in processed food products (Vandenberg, 2009). Refined cotyledons with good appearance, texture and cooking qualities are produced during the dehulling process which makes efficient digestion and utilisation of lentil in the body. The dehulling is done by loosening the seed coat and then removing it. Loosening of the seed coat of lentil seed can be done by drying or soaking in water or a combination of both. Puffing is another method practiced in dehulling which involves subjecting the seeds to high temperature for a short time, adding water and allowing it to absorb overnight and finally roasting to make the cotyledons expand and split the seed coat. Studies have shown that puffing can be improved by addition of hardening agents such as calcium phosphate, egg white, gums, calcium or sodium

caseinate. If the seeds are to be split, subsequently they proceed to a splitting machine (USA Dry Pea and Lentil Council, 2010). De-hulling and splitting into halves are mainly done for red lentil (Canadian Grain Commission, 2013).

Milling is a crucial step in processing of legumes which causes the reduction of the particle size. Milling of lentil is done mainly by two types of techniques; impact milling and direct-pressure milling. In impact milling, the particle is fractured by a rotating assembly comprising of blunt or hammer type blades, which apply a blunt force across a wide area. Hammer mills, pin mills, cage mills and turbo mills are examples of mills used in impact milling. In direct-pressure milling, two rotating bars or one rotating bar and a stationary phase produce the milling action and the particle is crushed or pinched between the two surfaces. Examples of this kind of mills include roller mills, cracking mills and oscillator mills (USA Dry Pea and Lentil Council, 2010).

Milling into different particle size ranges has various effects on chemical and physical properties of the resulting flour (USA Dry Pea and Lentil Council, 2010). Ker et al. (2010) reported that use of different milling conditions has effects on water absorption, solids lost, protein solubility and thermal properties of cowpea flour and differences in chemical and functional properties in differently milled flours influence how the flours perform when used in food products. There are remarkable differences between red lentil flours milled from hammer mill, pin mill, roller mill and stone mill in terms of particle size distribution, composition, colour, pasting and functional properties. Understanding of flour quality produced from different milling processes and technologies would give an understanding into which flours and flour streams work best in different food products as well as an indication of the optimum levels of flour that can be used in various food products (CIGI, 2013).

2.5.2 Micronization

Micronization is an infrared heating process which offers the benefit of lower costs resulting from the high efficiency of radiation heating than the conventional conduction or convection technologies (Wray, 1999; Bellido et al., 2006). Infrared radiation transfers thermal energy in the form of electromagnetic waves and by exposing an object to infrared radiation the heat energy is absorbed by the material (Krishnamurthy et al., 2008). The wavelength range employed in infrared heating is from 1800 to 3400 *nm* (Fasina et al., 2001). Micronization has

been applied in drying of different materials such as food, coatings, adhesives, ink, paperboard and textiles (Emami et al., 2010). In biological materials, the penetration of infrared radiation into the material causes the water molecules to vibrate at a frequency of 60,000 -150,000 MHz causing a rapid internal heating and increase in water vapour pressure inside and subsequent swelling and rupturing of the material (Fasina et al., 2001). Infrared drying has been used in the food industry for legumes, cereals, vegetables, pasta, meat and fish (Emami et al., 2010). The use of infrared radiation technology for dehydrating foods has several advantages including reduction of drying time, alternate energy source, increased energy efficiency, uniform temperature in the product while drying, better quality finished products and clean working environment (Krishnamurthy et al., 2008). Infrared heating can also be effectively used for enzyme and pathogen inactivation. However, the penetration power of infrared radiation is limited and therefore infrared heating is considered as a surface treatment (Krishnamurthy et al., 2008).

Micronization helps to overcome the slow cooking time of legumes which is a major drawback and a contributing factor for their under-utilization (Wray, 1999). Cooking time of legumes is the time needed for the seeds to attain a texture which is acceptable for consumption (Arntfield et al., 2001). Studies have shown that micronization considerably reduced the cooking times of legumes such as cowpea, lentil and split peas, thus extending their utilisation (Mwangwela et al., 2007a). By measuring the moisture uptake of micronized and non-micronized lentil seeds during boiling, Cenkowski and Sosulski (1997) demonstrated that micronization caused a significant decrease in cooking time. The changes in the cell structure of lentil caused by micronization improve the water uptake during cooking thereby decreasing the normal cooking time (Wray, 1999). Similar reductions in cooking time of micronized legumes have been reported for split peas, *Laird* lentil and *Bechuna* white cowpea which showed 30, 50 and 44% reduction, respectively, after micronization to 153 °C (Mwangwela et al., 2007b). Even though the exact mechanism by which the cooking time of pulses is reduced by micronization is not clear, various studies on physicochemical changes during micronization have shown that properties such as starch gelatinization, reduced protein solubility, and increased pectin solubility and seed porosity are responsible for the changes in cooking time (Bellido et al., 2006).

In addition to improving the cooking qualities, changes brought about by micronization in pasting properties, water absorption, foaming and gelling capacities enhance the utilization of flours from micronized legume seeds in food systems (Mwangwela et al., 2007a). Moreover,

antinutritional compounds present in legumes can be eliminated or significantly reduced by micronization. As reported by Fasina et al. (2001), Kouzeh-Kanani et al. (1981) had shown that urease and trypsin inhibitor activity of full-fat soybean were reduced to acceptable levels when infrared heated to 124 °C.

The final temperature is a critical parameter which determines the effectiveness of the micronization as a precooking treatment for legumes (Mwangwela et al., 2007b). Arntfield et al. (2001) demonstrated that lentil seeds micronized to 170 °C with a final moisture level of 7% (w/w) had significantly harder texture than lentil micronized to 138 °C leading to increased cooking time, which is a negative impact of the process. Similar observations were seen in the study by Mwangwela et al. (2007b) where micronization of cowpea seeds to 170 °C required a longer cooking time than micronization treatments of 130 and 153 °C. The increase of cooking time after micronization is attributed to the low amounts of water available in the seeds during processing as shown by studies on pinto beans, chickpeas and yellow peas (Bellido et al., 2006).

2.5.2.1 Micronization pre-treatments

Traditional preparation of many legumes involves soaking or tempering to ensure penetration of water into the seeds which shortens the cooking time by accelerating starch gelatinization and protein denaturation (Arntfield et al., 1997; Bellido et al., 2006). The composition of the soaking solution is critical since tempering with water alone may result in release of magnesium and calcium ions leading to increased hardness resulting in longer cooking times, and hence aqueous salt solutions containing carbonates, polyphosphates or EDTA have been employed (Arntfield et al., 1997; 2001). However, studies have shown that at tempering levels such as 20%, water alone was as effective as any salt solutions and at higher tempering levels such as 40%, salts were more effective in reducing cooking time but all salts do not give good quality products because some salts such as carbonates leave brown deposits on seeds (Arntfield et al., 1997).

In addition to reducing the cooking time, soaking improves the nutritional quality of micronized legumes as the seeds which had been soaked had significantly lower levels of trypsin inhibitory activity and oligosaccharides but the starch content remained unchanged (Fasina et al., 2001). In micronization, the time, temperature and moisture conditions determine the extent of

the physico-chemical changes taking place in the legume seeds and tempering or conditioning the seeds to a specific moisture contents is usually done prior to micronization (Scanlon et al., 2005).

Tempering that results in an excess amount of water is not suitable for micronization since it increases the amount of water that must be evaporated during the heat treatment (Bellido et al., 2006). Superheated steam is generated from the seed moisture subjected to high temperature heat of micronization, altering the lentil seed properties resulting in physico-chemical changes in starch and proteins (Scanlon et al., 2005). Moreover, chemical changes such as the Maillard reaction are also sensitive to the prevailing moisture and temperature conditions during thermal treatment (Scanlon et al., 2005). In studies done with lentil, it had been shown that higher moisture levels prior to micronization resulted in increased levels of starch gelatinization (Arntfield et al., 1997). Cenkowski and Sosulski (1996) demonstrated the effect of different tempering moisture levels on cooking time of lentil. Lentil seeds tempered to achieve moisture levels of 25.8 and 38.6% (w/w) and micronized to a final moisture level of 18%, required 15 and 10 min of cooking times, respectively, in comparison to the 30 min cooking time for the control. Results of this study also showed that micronization served to gelatinize 45 to 65% of the starch in the lentil seed and cooking of micronized seeds for 5 min served to gelatinize most of the remaining starch. In order to achieve the desired physical and chemical properties of lentil and other legumes, it is necessary to find out the required temperature-moisture combinations (Scanlon et al., 2005).

2.6 Applications of lentil in food industry

Unhealthy body weight-related conditions such as obesity, diabetes and heart diseases prevalent in many countries of the world have prompted commercial food manufacturers to pursue new, more healthful products. Food developers are looking for ways to make value-added products with health benefit attributes using legumes such as peas, lentil and chickpea without compromising the taste. Incorporation of lentil flour into recipes and formulations enriches the protein and fiber contents as well as giving a pleasant appearance and positive blending and mixing characteristics. Besides the nutritional benefits, legume flours offer an excellent alternative for people who have a sensitivity to gluten. They can also provide new and unique flavours and are especially good at thickening liquid mixtures such as sauces or soups. Moreover,

precooked lentil flour shows extended stability and shelf life comparable to that of wheat flour and is microbiologically safe (USA Dry Pea and Lentil Council, 2010).

2.6.1 Lentil based ingredients

The effect of processing treatments on functional properties of pulse flour had been studied widely in order to understand the utilization of pulses as ingredients in food formulations. Modifications by enzyme or heat treatments have been studied as methods to modify the properties of pulse ingredients (Maskas, 2010). Ground pulse flour can be divided into fiber, protein and starch enriched components which are unique in their composition and exhibit functional characteristics such as water and fat binding, gelling, emulsifying and foaming (Pulse Canada, 2013). Legume starch is used to modify the texture of food products. In addition, legume starch can be used in preparation of low-fat foods where non-gelling, pre-gelatinized starch replaces the lipid in the food (USA Dry Pea and Lentil Council, 2010). As a result of their high amylopectin content, legume starches show a restricted swelling and an increased overall stability during processing which make them suitable for use in a variety of food products (USA Dry Pea and Lentil Council, 2010).

Nutrition bars or snacks, one of the popular legume based food applications offer a convenient, healthy food option to the consumer while serving as non-dairy and non-gluten foods (USA Dry Pea and Lentil Council, 2010). Development of gluten free, 100% pulse-based cracker snacks using nine commercially available pulse flours and fractions, including red and green lentil flours, was done by Han et al. (2010). The quality of the final products was evaluated for texture and colour profile by sensory panels and consumer acceptability and nutritional analyses to complete the product evaluation. The physical characteristics, compositional and nutritional profiles of the crackers were similar to the existing gluten free cracker products currently available in Canadian and the US markets and were rated highly in consumer acceptance testing showing that lentil based crackers, among the others have well developed appeal to consumers. In another study by Ryland et al. (2010), development of a nutritious and acceptable snack bar using micronized flaked lentils was carried out. *Eston* lentil tempered to 16.5% moisture level and micronized to a temperature of 126 °C and final moisture level of 8% were flaked to 1.35 ± 0.5 mm size and used in six snack bar formulations exhibiting a wide range of flavour and texture characteristics while partially replacing oats. Results of this study showed that the snack bars

formulated with lentil contained 8-10% more folate, providing about 10% of the recommended daily amount in a 30 g serving compared to those made from oats alone. Moreover, lentil snack bars contained more dietary fiber, protein and iron. Studies on the use of lentil in extruded snacks have also been reported. Crisp expanded wafers produced from extruded pellets of flour blends of lentil and corn had optimal consumer acceptability when the blends contained 40-80% lentil flour (Hardacre et al., 2006).

Processed lentil flour and dehydrated lentil granules are also being used in baked goods (USA Dry Pea and Lentil Council, 2010). Dalgetty et al. (2006) showed that hulls or cotyledon fiber from lentil can be successfully incorporated into bread flour to increase the total fiber content and improve the moistness of bread without significantly increasing crumb firmness during storage. Shahzadi et al. (2005) concluded that blending lentil flour with wheat flour improved rheological and sensory properties of *chapatti.*

2.7 Fresh meat colour

The colour of meat is a principal factor that influences consumer's purchase decision for product selection or rejection (Khliji et al., 2010). Therefore, fresh meat discolouration can cause substantial loss in terms of economic value (Faustman & Phillips, 2001). Many researchers have investigated the colour of meat as a main quality attribute which reflects the freshness and wholesomeness of meat, especially beef (Mohamed et al., 2008). Loss of the desirable cherry-red appearance of meat with subsequent replacement by reddish brown or brown colour is a natural process resulting from intrinsic and extrinsic factors (Faustman & Phillips, 2001).

2.7.1 Factors affecting fresh meat colour

The final colour of meat is affected by several factors such as animal species and genetics, nutritional background of the animal, post-mortem changes in muscle, for instance the changes in pH and temperature decline, inter- and intramuscular effects, post-mortem storage temperatures and time, and many other variables encountered during processing including antimicrobial interventions, packaging, display and lighting (American Meat Science Association, 2012). The factors affecting beef quality have been broadly categorised into intrinsic factors or extrinsic factors (Mohamed et al., 2008). Intrinsic factors include breed, gender, age, muscle, carcass weight and fatness. Muscles having a high proportion of red oxidative myofibers contain more

myoglobin and the concentration of myoglobin in muscles increases as the animals age (Faustman & Phillips, 2001). Extrinsic factors affecting the meat quality are related to environmental conditions and post-mortem factors. Muscle tissue properties and meat quality are affected by post-mortem technological factors such as electrical stimulation, carcass suspension techniques, chilling rate, ageing, packaging, freezing and further processing (Mohamed et al., 2008). Application of antimicrobial agents such as acetic and lactic acid has shown to negatively affect ground beef colour (Mancini & Hunt, 2005). In a study by Realini et al. (2004), it was shown that post-mortem addition of vitamin C to ground beef was effective in delaying the red colour deterioration in grain or grass produced meat. Vitamin C is known to preserve red meat colour and possesses anti oxidative properties against lipid oxidation depending on the concentration (Schaefer et al., 1995).

Animal diet is an important factor affecting the colour of the produced meat (Mohamed et al., 2008). Results of studies done by Yang et al. (2002) and O'Sullivan et al. (2003) on the effect of grass and concentrate feeding of cattle on beef colour showed that higher redness, as depicted by Hunter 'a' values, was found for grass feeding than with concentrate feeding. Dietary vitamin E supplementation also resulted in higher 'a' values than in non-supplemented animals as reported by O'Grady et al. (1998). Vitamin E increases the lipid stability of the muscle, which could improve the colour stability (Mancini & Hunt, 2005). Several studies have shown the role of dietary vitamin E supplementation in improving the antioxidant to pro-oxidant balance in muscle and extending the shelf life of beef (Mohamed et al., 2008).

The common enzymes normally present in muscle foods such as catalase, superoxide dismutase and glutathione peroxidase may work as antioxidants and are involving in reducing oxidation of meat and keeping the colour stability (Mohamed et al., 2008).

2.7.2. Myoglobin chemistry

Myoglobin, a heme protein found in skeletal muscle, provides the red colour associated with meat which affects its appearance (Faustman & Phillips, 2001). Myoglobin is a water soluble protein containing 8 α-helices linked by short non-helical sections. Myoglobin contains a heme prosthetic group located within the protein's hydrophobic pocket (Mancini & Hunt, 2005). There are six bonds associated with this iron atom; four connect iron to the heme ring, the 5th attaches to the proximal histidine-93, and the 6th site is available to reversibly bind ligands

including diatomic oxygen, carbon monoxide, water, and nitric oxide. The ligand present at the 6th coordination site and the valence state of iron determine meat color via four chemical forms of myoglobin, deoxymyoglobin, oxymyoglobin, carboxymyoglobin and metmyoglobin (American Meat Science Association, 2012).

Deoxymyoglobin contains ferrous (Fe^{2+}) iron with a vacant 6^{th} coordination site and results in a dark purplish-red or purplish-pink colour typical of the interior colour of fresh meat and that in vacuum packages. Oxygenation of deoxymyoglobin forms a bright-red colour via the formation of oxymyoglobin. Oxymyoglobin has diatomic oxygen attached to the 6^{th} coordination site of ferrous (Fe^{2+}) iron. Carboxymyoglobin formation occurs when carbon monoxide attaches to the vacant 6^{th} position, producing a stable bright-red color when the environment is devoid of oxygen. Atmospheres containing oxygen will result in the conversion of carboxymyoglobin to either oxymyoglobin or metmyoglobin. Metmyoglobin contains ferric iron (Fe^{3+}) and it is the oxidized tan to brown coloured form of myoglobin. Typically, metmyoglobin forms easily at low concentrations of oxygen and water is the ligand at the 6^{th} position of the iron in metmyoglobin (American Meat Science Association, 2012).

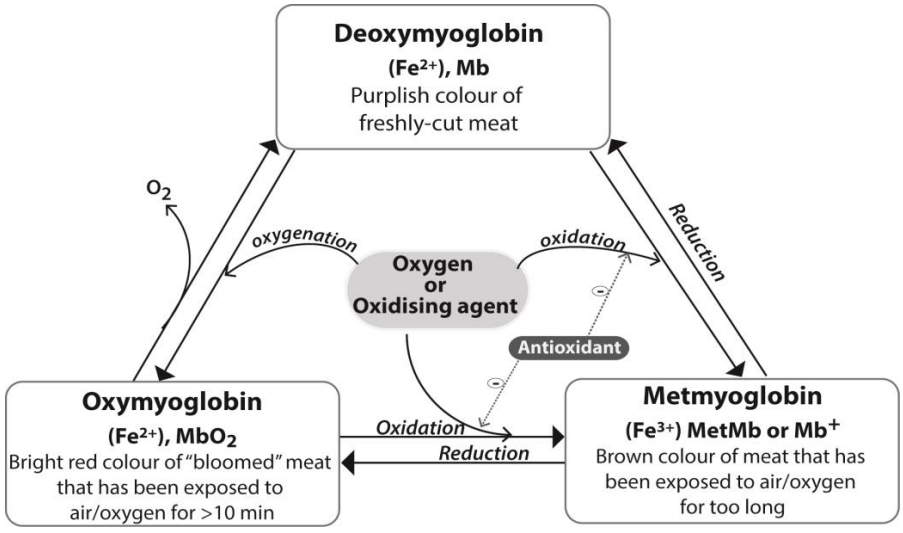

Figure 2.1 Different forms of myoglobin, oxidation state of iron in heme group and visible colour of pigment form. Straight dotted arrows indicate the oxidation steps that exogenous or endogenous antioxidant can interfere and slow down brown metmyoglobin formation. Adapted from Mancini & Hunt (2005).

2.7.3 Lipid oxidation

Lipid oxidation is a main factor which limits the quality and acceptability of meats leading to discolouration, drip losses, off-odour and off-flavour development, texture defects and the production of potentially toxic compounds (Chaijan, 2008). Meats displayed under aerobic conditions or in high-oxygen modified atmosphere packaging are susceptible to lipid oxidation due to the presence of the factors necessary for this process such as unsaturated fatty acids, oxygen and chemical species that accelerate oxidation (Faustman et al., 2010). Lipid oxidation is a chain reaction which involves the production of free radicals and consists of initiation, propagation and termination reactions (Chaijan, 2008). Intrinsic and extrinsic factors, such as the concentration of prooxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, oxygen consumption reactions and the fatty acid composition of the meat can influence the lipid oxidation process (Chaijan, 2008). Meat from non-ruminants contains higher content of unsaturated fatty acids and displays more rapid lipid oxidation than that of ruminants. Muscles with greater proportions of red fibers contain more iron and phospholipid than muscles containing predominantly white fibers and, therefore more susceptible for lipid oxidation. Lipid oxidation is higher in ground meat than whole cuts because the grinding process incorporates oxygen, mixes reactive components and increases surface area as a result of particle size reduction (Faustman et al., 2010). Other factors such as enzymatic and non-enzymatic reducing systems, increasing temperature and time can accelerate lipid oxidation (Chaijan, 2008).

2.7.4 Myoglobin and lipid oxidation relationship

The discolouration of fresh meat is determined by the relative concentrations of the three redox forms of myoglobin; deoxymyoglobin, oxymyoglobin and metmyoglobin (Faustman & Phillips, 2001). The products generated in the biochemical reactions responsible for myoglobin and lipid oxidation can further accelerate these two oxidation processes in a reciprocal manner (Faustman et al., 2010). Several studies have reported that the meat discolouration is enhanced by lipid oxidation (Faustman et al., 2010). Significant support for an interaction between these two processes has been provided by research done on the effect of α-tocopherol on lipid oxidation which showed that α-tocopherol not only delayed lipid oxidation, but also delayed beef discolouration (Faustman, 2004). Zakrys et al. (2008) investigated the quality parameters in beef packaged under modified atmosphere packaging at different oxygen levels. The results of this

study demonstrated that changes in oxymyoglobin and redness represented by Hunter a* values were dependent on lipid oxidation and showed a strong negative correlation with thiobarbituric acid reactive substances. According to McKenna et al. (2005), muscles with greater colour stability were characterized by less oxygen consumption and less lipid oxidation. Model system studies by Yin & Faustman (1994) and O'Grady et al. (2001) have also demonstrated the role of lipid oxidation on myoglobin oxidation (Faustman et al., 2010).

Reactive intermediates are produced during oxidation of oxymyoglobin to metmyoglobin and these are capable of enhancing further oxidation of oxymyoglobin and unsaturated fatty acids. Specifically, superoxide anion is formed, which dismutases to hydrogen peroxide (Faustman et al., 2010). Hydrogen peroxide further reacts with iron to produce hydroxyl radical which has the ability to penetrate into the hydrophobic lipid region facilitating lipid oxidation (Chaijan, 2008). Hydrogen peroxide can react with metmyoglobin generated in this oxidation sequence to form ferrylmyoglobin, an activated metmyoglobin complex capable of enhancing lipid oxidation (Faustman et al., 2010). A wide range of aldehyde products formed in lipid oxidation can induce the oxidation of oxymyoglobin (Chaijan, 2007). Faustman et al. (1999) reported that oxidation products of monounsaturated fatty acids such as hexenal, heptenal, octenal and nonenal enhanced metmyoglobin formation from oxymyoglobin more rapidly than their saturated counterparts. The effect of 4-hydroxynonenal, a secondary product of linoleic acid oxidation, on acceleration of oxymyoglobin oxidation is widely studied (Faustman et al., 2010). Moreover, aldehydes alter the redox stability of myoglobin by increasing oxymyoglobin oxidation, decreasing the metmyoglobin reduction via enzymatic process, and enhance the prooxidant activity of metmyoglobin (Chaijan, 2008).

The relationship of colour to lipid oxidation remains an active research area and there are studies that measured lipid oxidation and myoglobin oxidation in meat which demonstrated that the two processes are not linked (Faustman et al., 2010). For example, Hayes et al. (2009) demonstrated that addition of the antioxidant sesamol (solubilized in distilled water) to porcine and bovine meat systems (homogenized in 0.12 M KCl/ 5 mM Histidine at pH 5.5) led to decreased lipid oxidation but enhanced oxymyoglobin oxidation. Faustman et al. (2010) reported that study by McBride et al. (2007) showed rosemary extracts minimized lipid oxidation but had no effect on preservation of redness in fresh ground beef. The extent of lipid oxidation is proportional to the concentration of oxygen present and minimal in low partial oxygen pressure

conditions. High oxygen concentration favours oxymyoglobin redox stability whereas lipid oxidation would occur readily in this condition. Therefore, there is no strong interaction between lipid and myoglobin under conditions when the oxygen concentrations is very high or very low (Faustman et al., 2010).

2.8 Use of legume seed flours in meat products

Substitution of meat with non-meat ingredients is an important strategy for reducing production costs in the meat industry in addition to the trend in reduction of fat in meat products which has drawn great attention in recent years for health reasons (Gramantina et al., 2012). Non-meat additives are used in meat products as fillers, extenders or binders which can improve or maintain the nutritional qualities of end products while lowering the ingredient cost (Modi et al., 2003). Plant and animal proteins are used in meat products in order to perform basic functions such as fat emulsification, water retention and formation of structure of meat products (Dzudie et al., 2002). Functional and bioactive properties of proteins from legume seeds have been widely studied and they can make an attractive alternative to wheat flour as a meat binder for replacement of a portion of the proteins in low-fat meat production (Gramantina et al., 2012).

2.8.1 Fresh meat products

Use of legume flours in fresh meat products such as burgers has been reported in the literature. Modi et al. (2003) conducted studies on the use of four types of legume flours in buffalo hamburger formulations. Roasted (150 °C for 5 min) and unroasted flours from green gram, black gram, Bengal gram and soy were incorporated at 8% (w/w) level and the chemical composition and physical properties of the products were studied after production and storage. The formulations with roasted flours showed lower fat absorption on frying and lower lipid oxidation as indicated by the values of thiobarbituric acid reactive substances (TBARS), compared to the unroasted flours. Burgers with any of these binders were organoleptically acceptable even after storage at -16 °C for 4 months. However, burgers with black gram flour had better sensory quality attributes in addition to the highest yield, lowest shrinkage and lowest fat absorption compared to other legumes. Kassem & Emara (2010) demonstrated that incorporation of textured soy significantly decreased the overall acceptability of burgers displaying that the characteristic beany flavour of soybean limits its uses despite many advantages. Der (2010)

studied the cooking properties, colour, texture, oxidative and sensory properties of low fat beef burgers incorporated with flours from micronized dehulled green and red lentil as binders at 6 and 12% (w/w) levels. Increasing binder addition to low-fat beef burgers increased cooking yield and minimized dimensional shrinkage upon cooking. Raw burgers containing flour from micronized lentil displayed significantly greater retention of redness from days 1 to 5 of storage at 4 °C. The TBARS of burgers containing flour from micronized lentil were also significantly lower compared with those containing non-micronized lentil after 9 to 11 weeks of frozen storage. Consumer panel analysis showed higher acceptability for burgers containing 6% flour from micronized lentil or 6% toasted wheat crumb compared with those containing non-micronized lentil flour or no binder. This study results showed that micronization of lentil lowered or eliminated off-flavour development in burgers compared to those containing non-micronized lentil flour and this reduction in off-flavour development was attributed to the decrease of lipoxygenase observed upon micronization of lentil seed.

2.8.2 Processed meat products

Addition of legume flours and legume proteins in processed meat products has also been evaluated by the meat industry. Verma et al. (1985) evaluated the nutritional quality of pork sausages incorporated with chickpea flour and reported that net protein utilization, biological value and protein efficiency ratio were unaffected by replacing 30% of meat protein with chickpea flour. In a similar study by Dzudie et al. (2010), incorporation of common bean flour showed increased water holding capacity and decreased cooking losses. Sanjeewa et al. (2010) studied the suitability of chickpea grown in Western Canada in a low-fat (fat <5%) emulsion-type meat product as a model and concluded that inclusion of chickpea flour improved the textural and sensory properties of the product. Serdaroglu (2005) studied the effect of extending meatballs with flours of black eye bean, chickpea and lentil at 10% (w/w) levels and showed that black eye bean and lentil resulted in greater cooking yield, fat and moisture retention and all meatballs containing legume flours had high acceptability in sensory evaluations.

There are some studies which showed unfavourable effects caused by the addition of legume flours to meat products. For example, Verma et al. (1984) used chickpea flour in a protein to protein basis in mutton, beef and pork sausages and showed that substitution levels above 30% showed less acceptability in pork and beef sausages. In all sausages, incorporation of chickpea

flour led to increased cooking losses, softer textures and discolouration of raw sausages which became more prominent during storage at 0 °C. Chaudhray & Ledward (1988) showed that incorporation of black gram flour in sausages resulted in higher TBARS values, paler colour and these sausages were less acceptable than the controls. During storage, the substituted sausages discoloured more rapidly than the controls and microbial growth became evident sooner. However, this study also concluded that moist heat treatment of the flour prior to incorporation in the batter eliminated these defects and the products were equally acceptable as the controls.

From this information it is clear that lentil is a nutritious and healthy food ingredient rich in carbohydrate, protein and micro-nutrients. Therefore, lentil can be incorporated into various food products where functionalities of carbohydrates and proteins are required. However, functionalities of native or raw lentil flour may not be desirable for all food products. Accordingly, varying processing strategies that affect seed constituents may help to create variations in functionalities in lentil flour. This research was conducted as two studies to test hypotheses formulated to meet objectives outlined in the introduction section:

Study 1: Effects of tempering moisture and micronization temperature of seeds on the physico-chemical and functional properties of lentil flour.

Study 2: Performance of flour from micronized lentil seeds in fresh ground beef; study on stability of product colour and lipids and the factors in flour affecting the stability.

3. STUDY 1: EFFECTS OF TEMPERING MOISTURE AND MICRONIZATION TEMPERATURE OF SEEDS ON THE PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF LENTIL FLOUR

3.1 Abstract

The effects of the seed moisture level of lentil seed and micronization temperature on the physical, chemical, functional and thermal properties of resulting flour were investigated in this study. Three tempering moisture levels and four micronizing temperatures were employed for green lentil var. *Eston* seed. The three tempering conditions were; non-tempered (natural moisture level of 8%) and tempered to achieve 16 and 23% seed moisture. Four micronization temperatures were tested; 115, 130, 150 and 165 ºC at the seed surface achieved at the end of the treatment. Seeds were milled to obtain flours and flour from non-tempered and non-micronized lentil seed was used as the control. The process was replicated two times using the same source of seed. A significant ($p<0.05$) increase in the water holding capacity of resulting lentil flours was observed at tempering levels of 16 and 23% seed moisture and micronizing temperatures above 130 ºC. Higher oil absorption capacity was observed for flours from 16 and 23% seed moisture levels than micronization at 8% moisture. Starch gelatinization was observed only when seeds were micronized at 23% seed moisture level and 18-25% gelatinized starch was found depending on temperature of the treatment. Resistant starch percentage and protein dispersibility index of flours decreased with all micronizing temperatures and tempering levels. Endogenous enzyme activities of lentil flour decreased with increasing micronizing temperatures as indicated by reduced lipoxygenase and peroxidase activities. The trypsin inhibitory activity was reduced with micronization. Differential scanning calorimetry results did not show changes in peak denaturation temperatures for starch and protein except in the flours from micronization treatments above 150 °C. However, decrease in the enthalpy values (8-90%) was observed for both starch and protein peaks. From Rapid Visco Analyzer results, very low final viscosity values were observed for the flours of 23% tempered seed and micronized to 150 or 165 °C.

3.2 Introduction

Research studies have shown that the consumption of pulses have potential health benefits such as reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol (Hu, 2003; Philanto & Korhonen, 2003; Tharanathan & Mahadevamma, 2003; Jacobs & Gallaher, 2004). Lentil is a good source of carbohydrates, proteins, vitamins and minerals and its low fat content makes lentil a good selection for consideration as part of a healthy diet (Barbana & Boye, 2012). Due to the promising functional and nutritional properties as well as the growing consumer preference for plant-based foods, there has been a growing interest in value-added processing of lentil for the development of new food ingredients (Barbana & Boye, 2012). In order to consider the potential of lentil as a food ingredient, it is necessary to evaluate the characteristics of lentil components and functional properties (Maskaus, 2010; Der, 2010).

The functional and physico-chemical properties of lentil can further be enhanced by processing which can optimize its applications in food. Micronization, a dry heating process in which infrared radiation is applied to a product on a vibrating bed, has been employed in the food industry to increase food safety, shelf stability and nutritional value and to decrease cooking times (Der, 2010). The seed micronization temperatures and pre-treatments used may affect the properties of resulting lentil flour. Changes in physico-chemical properties of resulting lentil flour may create variation in functional properties that could be appealing to the food processing industry.

Based on the hypothesis that different tempering moisture levels and different surface temperatures of lentil seeds achieved during micronization affect physico-chemical and functional properties of resulting flours differently, this study was carried out with the objective of evaluating the physico-chemical and functional characteristics of lentil flours obtained from seeds at three moisture levels (8% seed moisture and tempered to achieve 16 and 23% seed moisture levels) and micronized to reach four surface temperatures (115, 130, 150 and 165 °C).

3.3 Materials and methods

3.3.1 Lentil seeds

Small green lentil seeds with the hull on (var. *Eston*) from the 2009 crop year and grown near Moose Jaw, Saskatchewan, Canada was obtained from InfraReady Products Ltd., Saskatoon,

SK. The experimental design consisted of three tempering conditions (8% or original seed moisture, 15 or 25% targeted final moisture levels) and four micronizing temperatures (115, 130, 150 or 165 °C seed surface temperature). Two replicates of tempering and micronization were done at two different times (6 months apart) using the same source of seeds. Each replicate consisted of twelve micronized and one non-micronized (raw) lentil sample. Sample size was 3 kg from each treatment.

3.3.2 Pre-treatments and micronization of lentil seeds

Tempering

Tempering of lentil seeds was done by adding a predetermined amount of de-ionized water according to the method AACC 26-95 (1995) and allowing to equilibrate. The following formula was used to calculate the required water content.

$$W = [L (Moisture_T – Moisture_O)] / (100 - Moisture_O) \quad (3.1)$$

Where, W = Weight of water required (grams), L = Weight of lentil seeds (grams), $Moisture_T$ = Moisture content required at tempering (%), and $Moisture_O$ = Moisture content of seeds before tempering (%).

Water was added to lentil seeds in polythene bags and the sealed bags were shaken manually for even distribution of water. The seeds were allowed to absorb moisture and temper at ambient temperature to achieve the desired moisture content (4-8 h depending on the desired final seed moisture level). Moisture content of lentil seeds before and after tempering was determined using AACC 44-17.01 (2003) method.

Heat treatment

Lentil seeds (tempered or not) were heat treated using micronizing technology available at InfraReady Products (1998) Ltd., Saskatoon, SK. (Micronizer from the Micronizing Company UK Ltd, Sufolk, UK and Model A 156379-B0 FMC Syntron ® vibrating conveyor and feeder from Bulk Handling Equipment, Homer City, PA, USA). The instrument consisted of a moving vibrating bed above which the infrared lamps were positioned (Figure 3.1). Lentil seeds were fed onto the moving vibrating bed and infrared heat treated (micronized) to reach surface temperatures of 115, 130, 150 or 165 ºC. Surface temperature of seeds was measured using a

hand-held IR Temp Gun thermometer (Oakton, Vernon Hills, IL). The micronized seeds were collected into a container placed at the end of the vibrating bed and directly processed as detailed in 3.3.3.

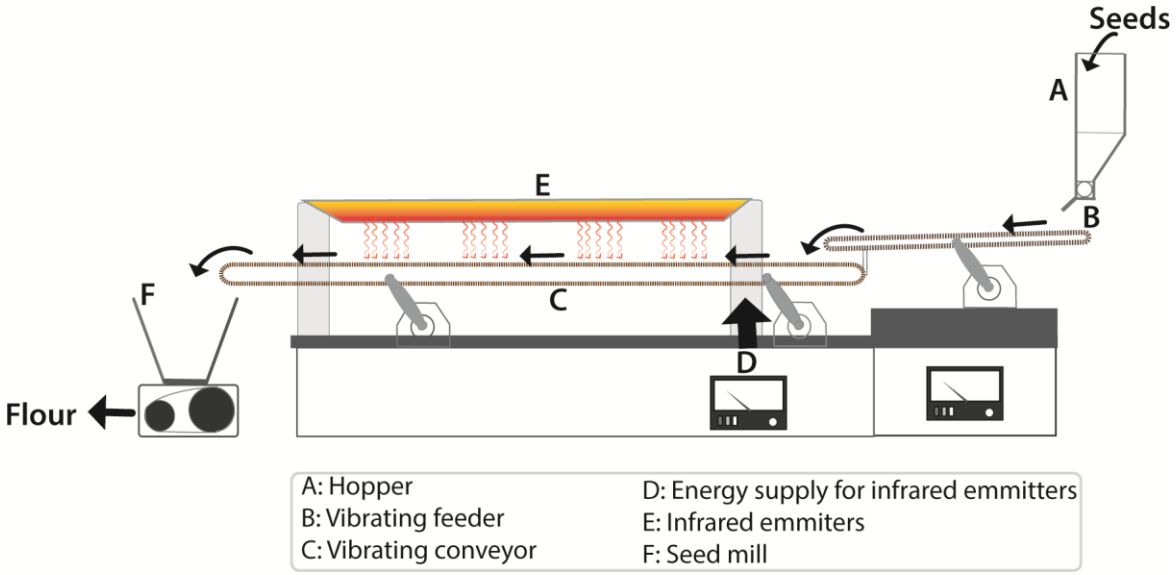

Figure 3.1 Schematic diagram of the laboratory-scale infrared system. Adapted from Fasina et al., (2001).

3.3.3 Milling of lentil seeds

Heat treated and non-treated seeds were flaked using a roller mill (Apollo Machine Products, Saskatoon, SK) and then a kitchen mill (Model 91, Blentech, Orem, USA) to pass through a 0.5 mm screen. Flour samples obtained were vacuum packaged in polyethylene bags and stored at 4 °C away from direct light, heat and moisture.

3.3.4 Physico-chemical properties of lentil flours

The following tests were carried out with the flour samples from micronized and raw lentil seeds. For each replicate of samples, at least 2 technical repeats were done. Analyses were done within 6 months after preparation of flours.

3.3.4.1 pH, moisture, crude fat, crude protein and total ash

pH

The AOAC Method 943.02 (1990) was employed to measure pH of flour slurries. Each flour sample (10.0 g) was blended with water (100 mL) for one min and the pH of the slurry was

measured at 22 °C using a pH meter (Model 915, Fisher Scientific, Nepean, ON) equipped with a pH/ATC Ag/AgCl electrode.

Moisture

Moisture contents of flour samples were determined according to AOAC Method 925.10 (1990). Each flour sample (2.0 g) was weighed into a pre-weighed aluminum pan, dried for 10 h at 105 °C in a drying oven, cooled in a desiccator and the weight was recorded. The process of drying, cooling and weighing was repeated at one hour intervals until a constant weight was obtained. The moisture percentage was calculated from the weight loss between the initial and final weights.

Crude fat

Crude fat content of flours were determined according to AACC Method 30-25 (1995). Fat from flour samples (2-5 g) were extracted with petroleum ether using soxhlet extraction system (Gerhardt Soxtherm 406 six place system with Multistat Controller, Northants, UK).

Crude protein

The AACC Method 46-11 (1995) was employed to determine the crude protein content. Each flour sample (1.0 g) was digested by the Kjeldahl method followed by distillation and titration to determine the total nitrogen content of the sample. A conversion factor of 6.25 was used to obtain crude protein content from total nitrogen.

Total ash

Total ash content was determined by AOAC Method 923.03 (1990). Each flour sample (3-5 g) was weighed into a crucible pre dried for 1 h at 550 ºC and ashed at 550 ºC until a grey mass was formed (2-3 h) in a muffle furnace. Ash content was calculated by reweighing the crucible with the ash after cooling in a desiccator.

3.3.4.2 Total starch, gelatinized starch and resistant starch

Total starch

The total starch content of lentil flours was determined by AACC Method 76.13 using a test kit from Megazyme International Ireland Ltd. In the assay, starch dextrins were quantitatively hydrolysed to glucose by amyloglucosidase and the glucose produced was measured after reaction with glucose oxidase/ peroxidase reagent. Briefly, 100 mg of the flour sample was weighed into a glass tube, dispersed with aqueous ethanol (0.2 mL, 80% v/v) and stirred on a vortex mixer. The tube was placed in a boiling water bath for 5 min after adding 2 mL of dimethyl sulfoxide. To this solution, 3 mL of thermostable α-amylase (300 U) in 4-morpholinepropanesulfonic acid buffer (MOPS, 50 mM, pH 7.0) was added, stirred vigorously by vortexing and incubated in a boiling water bath for 5 min. The solution was then incubated at 50 °C in a water bath for 30 min after addition of 4 mL of acetate buffer (200 mM, pH 4.5) followed by 0.1 mL of amylogucosidase reagent. The tube was centrifuged at 1500 *g* for 10 min using Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA). Supernatant (1.0 mL) was mixed with distilled water (9.0 mL). Aliquots of the diluted solution (0.1 mL) were transferred to glass tubes in duplicate. Glucose determination reagent (GOPOD) (3 mL) was added to each tube including glucose standards and reagent blanks. Tubes were incubated at 50 °C for 20 min. The absorbance of sample and glucose standard was measured against reagent blank at 510 nm using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The total starch content of the sample was calculated using the following equation:

$$\text{Starch},\% = \Delta E \times F \times 100 \times 10 \times 1/1000 \times 100/W \times 162/180 \qquad (3.2)$$

Where: ΔE = absorbance (reaction) read against the reagent blank, F = 100 (μg of glucose) divided by absorbance for 100 μg of glucose (conversion from absorbance to μg), 100 = volume correction (0.1 mL taken from 10 mL), 10 = dilution factor, 1/1000 = conversion from μg to mg, 100/W = factor to express "starch" as a percentage of flour weight, W = weight in mg ("as is" basis) of flour analyzed, and 162/180 = adjustment from free glucose to anhydro glucose (as occurs in starch).

Gelatinized starch

Gelatinized starch content was determined according to the method described by Emami et al. (2010). Each flour sample (20 mg) was weighed into a 50 mL polypropylene centrifuge tube and 5 mL of ethanol (80%, v/v) was added, mixed by vortexing and incubated at 40 °C in a water bath for 10 min. The mixture was centrifuged at 1500×*g* for 10 min using Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA) and the supernatant containing the free sugars was discarded. The samples were washed twice with ethanol (10 mL) and the starch pellets were dried by evaporating the ethanol. Amyloglucosidase enzyme solution (25 mL) was added to the dried starch pellets dispersed in water (5 mL), mixed and incubated for 30 min in a shaking water bath. Enzymatic reaction was stopped by adding trichloroacetic acid (25% w/v, 2 mL) and the sample was centrifuged at 1500×*g* for 5 min using Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA). Supernatant (0.1 mL) was mixed with *o*-toludine reagent (3 mL) and incubated in a boiling water bath for 10 min. Absorbance of the solution was measured at 630 nm using the UV/visible spectrophotometer with a standard series of glucose solutions (containing 25, 50, 75 and 100 µg glucose) treated similarly as the sample solution. The starch content digested by amyloglucosidase (D) was calculated from the glucose content on a dry basis as glucose content × 0.9. A correction factor (k) was calculated by determining the ungelatinized starch content cleaved by amyloglucosidase. The following equation was used to calculate the gelatinized starch percentage.

$$G = [(D - k)/TS] \times 100 \quad (3.3)$$

Where, G is the gelatinized starch (% dry basis), TS is the total starch (% dry basis), D is the starch digested by amyloglucosidase (% dry basis) and k is the correction factor.

Resistant starch

Resistant starch content was determined according to the method described by Goni et al. (1996). Each flour sample (100 mg) was mixed with KCl-HCl buffer (0.2 M, 10 mL, pH 1.5) in a 50 mL centrifuge tube. Proteins in the sample were removed by addition of 0.2 mL pepsin solution (1 g pepsin in 10 mL KCl-HCl buffer, pH 1.5) and holding in a constant shaking water bath at 40 ºC for 60 min. Digestible starch was hydrolyzed by addition of α-amylase solution and incubating at 37 ºC for 16 h in a constant shaking water bath. The residue containing resistant

starch was solubilized with 2 M KOH (3 mL) and subjected to enzymatic hydrolysis by amyloglucosidase. The released glucose was determined by measuring the absorbance at 500 nm after reaction of the sample solution (0.5 mL) with glucose oxidase-peroxidase reagent (1 mL) against a reagent blank using a glucose standard curve (10-60 ppm). The resistant starch content was calculated as mg of glucose × 0.9.

3.3.4.3 Colour

Colour of lentil flours was measured using Hunterlab MiniScan XE Colorimeter (Hunter Association Laboratory, Inc., Reston, VA) using the illuminant A and 10º observer. Flour samples were filled into transparent plastic petri dishes (6 cm diameter) and covered with the lid allowing the flour to pack tightly before measurements were taken. Instrument was standardized using the black and white tiles and the pink tile which had values of 76.4 for L* (lightness), 25.4 for a* (redness) and 17.6 for b* (yellowness) was used to check instrument performance during colour measurements.

3.3.4.4 Particle size distribution

Particle size distribution of dry flour samples was determined using Malvern Scirocco 2000 Mastersizer (Malvern Instruments Inc., Westborough, MA, USA) according to the protocol of Canadian International Grain Institute (CIGI), Winnipeg MB, Canada.

3.3.5 Functional properties of lentil flours

3.3.5.1 Protein dispersibility index

Protein dispersibility index (PDI) of flour samples were determined by AOCS Method Ba 10-65 with slight modifications. Each flour sample (10.00 g) was mixed with distilled water (150 mL) at 25 ºC and blended at a speed of 8500 rpm for 10 min using the polytron (PT 3100, Kinematica AG, Switzerland). The slurry was centrifuged at 1500×*g* for 10 min using Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA) and a portion of the supernatant was pipetted quantitatively for determination of water dispersible nitrogen by the Kjeldahl method. The protein dispersibility index was calculated using the following formula using crude protein values:

$$\text{PDI} = \frac{\%\ \text{water dispersible protein}}{\%\ \text{total protein}} \times 100 \quad (3.4)$$

3.3.5.2 Water holding capacity

Water holding capacity (WHC) was determined by AACC Method 56-30.01. Initially, approximate WHC of the flour sample was determined and the value obtained was used to calculate the weight of the sample required to determine the actual WHC. The WHC of flours was presented on dry weight basis.

3.3.5.3 Oil absorption capacity

Oil absorption capacity (OAC) was determined using the method described by Ghavidel and Prakash (2006). Each flour sample (1.0 g) was mixed with corn oil (5 mL) in a pre-weighed 50 mL centrifuge tube and stirred for 1 min using a stirring rod. After allowing the flour oil mixture to equilibrate at ambient temperature for 30 min, the mixture was centrifuged at 5000×*g* at 27 ºC for 25 min using Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA). The oil layer on the top of the sample was decanted, the residual oil was drained by keeping the tube at an angle of 45° for 1 h and the tube was reweighed. OAC of flours was presented on dry weight basis.

3.3.6 Thermal and pasting properties of lentil flours

3.3.6.1 Thermal properties by Differential Scanning Calorimetry (DSC)

Thermal properties of flours were determined using the method described by Chung et al. (2008). A differential scanning calorimeter calibrated with indium (Model Q 2000 TA Instruments, New Castle, DE) was used to obtain parameters of thermal denaturation of lentil flour constituents. Each flour sample (3.33 mg on dry weight basis) was weighed into the alodine coated aluminium pan, deionized water (10 µL) was added using a micro syringe to form a slurry of 75% moisture and the pan and lid was sealed hermetically and samples were equilibrated at ambient temperature overnight. The sealed pans were subjected to a temperature increase from 5 to 180 ºC at a heating rate of 10 ºC per minute. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and enthalpy (ΔH) were determined from the endothermic peaks of the thermogram. Samples were analyzed in triplicate.

3.3.6.2 Pasting properties by Rapid Visco Analyzer

Pasting properties of flour samples were determined by AACC Method 76-21.01 using a Rapid Visco Analyzer (Newport Scientific Pty Ltd., Warriewood, NSW, Australia). Each flour sample (3.5 g corrected for 14% moisture content) was weighed into the canister containing deionized water (25 mL corrected for moisture content) using Table II of the method. The stirrer was placed into the canister and the sample was mixed with the water by vigorously jogging the blade of the stirrer up and down until no lumps adhered to the paddle. The stirrer and canister assembly was firmly inserted into the paddle coupling immediately and the measurement cycle was initiated with the standard profile 1. Performance of the instrument was checked periodically using test starch. Values for peak viscosity, trough, breakdown, final viscosity, and setback, peak time and pasting temperature (°C) were obtained from the viscograms. All analyses were carried out in triplicate.

3.3.7 Enzyme activity of lentil flours

3.3.7.1 Lipoxygenase activity

Lipoxygenase activity of the flour samples were determined by the method described by Chang and McCurdy (1985). Extracts of flour samples were prepared by mixing the flour with 0.05 M phosphate buffer (pH 6.9) at a 1:10 (w/v) ratio using a magnetic stirrer at 4 °C for 2 h. The slurry was centrifuged at 11,500×g for 30 min at 4 ºC using a Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA). The resultant supernatant was used for lipoxygenase assay after filtering and diluting as required. Linoleic acid (0.946 mM, Sigma Aldrich) was used as the substrate and generation of lipid oxidation products due to the enzyme activity was measured as absorbance value of assay mixture at 234 nm using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) each min for 30 min. The absorbance values were plotted against reaction time. Lipoxygenase activity of the flour extract was calculated from the slope of the linear region of the curve considering that one unit of lipoxygenase activity was equivalent to an increase in absorbance at 234 nm by 0.001 units per minute.

3.3.7.2 Peroxidase activity

Peroxidase activity of the flour samples was determined by the method described by Yemenicioglu et al. (1998) with minor modifications. Each flour sample (5 g) was extracted with 15 mL of 0.1 M phosphate buffer (pH 7.0) by stirring with a magnetic stirrer for 2 h and the slurry was centrifuged at 11,500×*g* for 15 min at 4 ºC using Sorvall RC-6 Plus centrifuge (Thermo Scientific, Asheville, NC, USA) and resultant supernatant was filtered and used for peroxidase activity measurement. The extract (0.1 mL) was mixed with 0.1 M phosphate buffer (3 mL), 20 mM guaiacol solution (0.05 mL) and 12.3 mM hydrogen peroxide solution (0.03 mL) in a cuvette, placed in the spectrophotometer quickly and the absorbance increase of the mixture was measured at 420 nm for 5 min against a reagent blank. Enzyme activity was calculated from the linear portion of the plot of time versus absorbance values.

3.3.8 Trypsin inhibitory activity

Trypsin inhibitory activity of lentil flour samples was determined according to the AACC Method 22-40.01 using benzoyl-DL arginine-*p*-nitroanilide hydrochloride (BAPNA) as the substrate with slight modifications which included the preparation of a sample blank with each sample. A crude extract was prepared by stirring each flour sample (1 g) with 0.01N NaOH solution (50 mL) for 3 h and filtering. Diluted sample solutions (0, 0.6, 1.0, 1.4 and 1.8 mL) were pipetted into glass tubes in duplicate and the total volume was adjusted to 2.0 mL. A 2.0 mL of trypsin solution containing 2 mg of trypsin (from bovine pancreas, activity ≥ 10,000 BAEE units/ mg protein, Sigma Aldrich) in 100 mL of 1 mM HCl was added, mixed and kept in a water bath at 37 °C. Substrate solution (5.0 mL) which was equilibrated at 37 °C was added to the sample containing enzyme solution mixture and then incubated at the same temperature. The reaction was stopped after 10 min by adding 1.0 mL of 30% (v/v) acetic acid. Sample blanks were prepared by pipetting 0, 0.6, 1.0, 1.4, 1.8 and 2.0 mL of the diluted sample extract into tubes, adjusting the volume to 2.0 mL with water, adding 5.0 mL of substrate solution and incubating at 37 °C for 10 min. Then 1.0 mL of acetic acid solution followed by 2.0 mL of trypsin solution was added. Absorbance of each sample solution and sample blank was measured at 410 nm using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) against a reagent blank. The absorbance value for each sample blank solution was subtracted from the value of the sample solution and plotted against sample volume. Trypsin

inhibitory activity was determined using the intercept of the graph and expressed as Trypsin Inhibitory Units (TIU) per mg of protein.

3.4 Statistical analysis

Each seed treatment combination for 3 kg of lentil was replicated two times. The mean and standard deviation of each measurement for the two replications were calculated. Treatment means were compared using the mixed procedure of SAS Institute Inc., Cary, NC, USA (2008). Mean separation was done using the least significant difference (LSD) procedure. The level of significance was set at $p < 0.05$.

3.5 Results and discussion

Physico-chemical characteristics of lentil flours

Lentil flour slurries had pH values between 6.4 and 6.5 (Table 3.1) and the seed tempering moisture level or micronization temperature showed no effect on the pH values. Moisture contents of the lentil flours ranged between 2.7 and 13.5% (Table 3.1). There was a decrease in final moisture content of the flours as the seed micronization temperature increased. Added water in the seed tempering step prior to micronization was lost due to the infrared heat treatment and an increasing moisture loss was observed with increasing temperature achieved during micronization of seeds. A higher retention of moisture was observed for flours from 16 and 23% tempering and 115 and 130 °C temperatures of micronized treatments than the other treatments. Reduction of free water level was observed for the flours obtained from seeds micronized above 150 °C. Also it should be noted that, tempering level of 23% and micronization below 130 °C tend to increase moisture level of the resulting flours. However, the moisture level of 13.5% is close to the typical moisture levels of dry cereal flours such as wheat (13.2%), rye (13.7%) or rice (13.1%) which are commonly used in the food industry (Institute of Food Science and Technology, 2013). Cenkowski and Sosulski (1997) observed a final moisture level of 18% for large green lentil seeds, tempered to 39% moisture prior to micronization to a temperature of 140 °C for 55 s.

Protein, fat and ash contents of the lentil flour samples were within the range of values reported in the literature for flours from untreated lentil seeds. Ma et al. (2011) reported 24.4% protein, 0.8% fat and 2.3% ash contents for green lentil with hulls. Tempering moisture level or

the micronization temperature of the seeds did not affect the total protein or ash content of flours but there was a slight increase in lipid content for some micronization treatments, most likely due to the change in cell matrix permeability leading to better release and extractability of lipid and lipid soluble constituents.

Table 3.1 The pH, moisture and proximate composition (% on dry weight basis) of lentil flours from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures[1,2].

Seed treatment	pH	%Moisture	%Protein	%Fat	%Ash
Non-micronized	6.4 ± 0.0	8.1 ± 0.7^{bc}	26.6 ± 0.0	1.1 ± 0.3^{bc}	2.6 ± 0.0
Non-tempered					
Micronized to 115 °C	6.4 ± 0.1	6.2 ± 0.2^{cd}	25.9 ± 0.4	1.3 ± 0.3^{abc}	2.6 ± 0.0
Micronized to 130 °C	6.4 ± 0.1	6.1 ± 0.1^{cd}	26.3 ± 0.4	1.2 ± 0.2^{bc}	2.6 ± 0.0
Micronized to 150 °C	6.5 ± 0.0	4.8 ± 0.2^{de}	26.7 ± 0.7	1.6 ± 0.4^{a}	2.6 ± 0.0
Micronized to 165 °C	6.5 ± 0.1	3.4 ± 0.7^{e}	26.8 ± 0.6	1.3 ± 0.0^{abc}	2.5 ± 0.0
16% tempered					
Micronized to 115 °C	6.4 ± 0.0	9.2 ± 0.8^{b}	26.5 ± 0.1	1.1 ± 0.1^{bc}	2.6 ± 0.0
Micronized to 130 °C	6.4 ± 0.0	8.2 ± 0.3^{bc}	26.5 ± 0.7	1.1 ± 0.0^{bc}	2.6 ± 0.1
Micronized to 150 °C	6.5 ± 0.0	6.3 ± 0.4^{cd}	26.5 ± 0.1	1.3 ± 0.2^{abc}	2.7 ± 0.0
Micronized to 165 °C	6.5 ± 0.1	3.0 ± 1.7^{e}	26.6 ± 0.6	1.3 ± 0.0^{abc}	2.6 ± 0.1
23% tempered					
Micronized to 115 °C	6.5 ± 0.0	13.5 ± 1.2^{a}	26.4 ± 0.6	1.0 ± 0.0^{c}	2.6 ± 0.1
Micronized to 130 °C	6.5 ± 0.1	13.2 ± 1.6^{a}	26.8 ± 1.0	1.4 ± 0.2^{abc}	2.7 ± 0.0
Micronized to 150 °C	6.5 ± 0.1	3.8 ± 2.6^{de}	26.5 ± 0.8	1.5 ± 0.3^{ab}	2.7 ± 0.0
Micronized to 165 °C	6.4 ± 0.0	2.7 ± 1.1^{e}	26.4 ± 1.5	1.5 ± 0.2^{ab}	2.7 ± 0.0

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).

Surface colour of the lentil seeds became darker with the increasing temperature of micronization. Similar observations were made by Arntfield et al. (2001) where non-dehulled green lentil micronized to 138 and 170 °C resulted in seeds which were darker, more red and less yellow as the micronizing temperature increased. This effect was higher at low moisture and high temperature conditions. The seed coats had a wrinkled appearance in some of the seeds tempered to 16 and 23% moisture levels and micronized to 115 and 130 °C temperatures. Der (2010) also observed a spotting effect on the surface of the micronized lentil seeds. These visual changes could be due to the uneven moisture distribution in the seeds prior to micronization.

When micronized lentil seeds were ground into flour, flours from non-tempered and 16% tempered seeds did not show a significant difference in redness and yellowness from the raw lentil flour at all micronizing temperatures, as indicated by the Hunter a* and b* values, respectively (Table 3.2). The lightness values (Hunter L*) were also not significantly different, except for flours from non-tempered 115 and 130 °C treatments which were lighter in colour compared to the raw lentil flour. However, the flours from micronized seeds with 23% seed moisture level showed significantly higher darkness, redness and yellowness as indicated by the lower Hunter L* and higher a* and b* values, respectively. All three colour parameters were significantly correlated with the tempering moisture level ($p<0.05$). Lightness (Hunter L*) was negatively correlated with the moisture level ($r = -0.78$) whereas the correlation between moisture level and redness (Hunter a*) or yellowness (Hunter b*) were positive ($r = 0.73$ and 0.81, respectively). The darkening effect in lentil due to micronization may be attributed to Maillard browning, a reaction involving reducing sugars and free amino groups of protein (Der, 2010).

The absorption of infrared radiation into a solid food differs with moisture levels and as a result, the change of colour can be different (Krishnamurthy et al., 2008; Der, 2010). Therefore, the differences in colour in the seeds and flour samples can be due to the different extents of the Maillard reaction products. Arntfield et al. (1997) also observed that there was a significant decrease in lightness of the lentil seeds as tempering level increased and the redness was significantly higher for lentil tempered to 33% compared to those at 25% moisture and suggested that browning occurred at higher tempering moisture.

Tempering lentil seeds to achieve moisture contents of 17 to 45% and micronizing to return to initial moisture content of 13%, Scanlon et al. (2005) demonstrated that the porosity (measured and calculated by moisture diffusivity and density changes that occurred) of the micronized lentil increased with increasing tempering moisture up to approximately 25%. This may be due to the void creation induced by evaporating the moisture of the water-imbibed seeds. However, porosity decreased at higher tempering moisture contents (above 25%), most likely due to filling of voids by amylose from partially gelatinized starch granules. Drier and more porous seed structure resulting from micronization may increase the effect of milling resulting in finer particle sizes (Der, 2010). Particle size distribution of the lentil flours in the current study are shown in Table 3.3. The particle size is given in µm and the three columns show the percentages (10, 50 and 90) of the particles which are below the value (in µm) given in the particular row.

Table 3.2 Hunter L* a* b* values of lentil flours from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures[1,2].

Seed treatment	L* (Lightness)	a* (redness)	b*(yellowness)
Non-micronized	84.3 ± 0.3^{cd}	3.9 ± 0.1^{def}	20.7 ± 0.1^{bcde}
Non-tempered			
Micronized to 115 °C	85.8 ± 0.3^{ab}	3.5 ± 0.1^{ef}	19.2 ± 0.4^{ef}
Micronized to 130 °C	86.2 ± 0.0^{a}	3.4 ± 0.1^{f}	19.3 ± 0.4^{ef}
Micronized to 150 °C	84.2 ± 0.1^{cd}	3.6 ± 0.1^{ef}	19.5 ± 0.2^{def}
Micronized to 165 °C	84.4 ± 0.3^{cd}	3.5 ± 0.3^{ef}	18.8 ± 0.9^{f}
16% tempered			
Micronized to 115 °C	85.1 ± 0.4^{abc}	3.5 ± 0.1^{ef}	20.6 ± 0.6^{bcde}
Micronized to 130 °C	84.7 ± 0.4^{bcd}	3.6 ± 0.0^{ef}	20.9 ± 0.1^{bcd}
Micronized to 150 °C	84.2 ± 1.0^{cd}	3.7 ± 0.0^{ef}	20.2 ± 0.6^{cdef}
Micronized to 165 °C	83.7 ± 1.0^{d}	4.0 ± 0.1^{cde}	20.1 ± 0.5^{cdef}
23% tempered			
Micronized to 115 °C	81.2 ± 1.1^{e}	4.3 ± 0.3^{bcd}	24.1 ± 1.3^{a}
Micronized to 130 °C	79.9 ± 0.3^{f}	4.4 ± 0.1^{bc}	25.1 ± 0.9^{a}
Micronized to 150 °C	81.6 ± 0.4^{e}	4.8 ± 0.2^{b}	21.6 ± 1.7^{bc}
Micronized to 165 °C	80.7 ± 0.8^{ef}	5.6 ± 0.8^{a}	22.0 ± 0.2^{b}

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).

In general, 90% of particles in the micronized seed flours had particle size smaller than non-micronized seed flour, except for the flours from 23% tempered treatment micronized to 115 and 130 °C temperatures (Table 3.3). Of the micronized flours, flours from these two treatments had the highest moisture contents (13.5 and 13.2%, respectively) which meant the seeds from these two treatments after micronization had high contents of water during size reduction step or milling. The higher levels of moisture of the micronized seeds from these treatments may have affected the ability of the seed to fracture during preparation of flour than the seeds with lower moisture levels.

The total starch content of the flours obtained in this study was between 43.2 and 44.1% (Table 3.4). Analysis of flours of selected treatments showed total starch content remained at a constant level for all flours. According to the quality data for western Canadian green lentil from the Canadian Grain Commission (2008), the total starch contents for small green lentil, including *Eston* variety used in this study, ranged from 41.9 to 44.6%. The lentil flours used in the study

had total starch contents typical for the small green lentil and seed micronization treatments had no effect on the total starch level of resulting flours.

Table 3.3 Particle size distribution of lentil flours from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures.

Seed treatment	Particle size (µm)		
	10%[1]	**50%**	**90%**
Non-micronized	11.7	122.0	380.7
Non-tempered			
Micronized to 115 °C	9.4	42.6	322.0
Micronized to 130 °C	8.9	44.6	356.0
Micronized to 150 °C	8.3	36.4	311.2
Micronized to 165 °C	8.5	32.4	303.4
16% tempered			
Micronized to 115 °C	11.3	58.4	368.7
Micronized to 130 °C	12.1	58.7	328.5
Micronized to 150 °C	11.3	49.0	316.1
Micronized to 165 °C	10.1	44.3	271.7
23% tempered			
Micronized to 115 °C	22.2	130.6	601.6
Micronized to 130 °C	32.6	136.2	568.0
Micronized to 150 °C	12.9	57.1	246.9
Micronized to 165 °C	11.5	62.4	328.3

[1] Percentage indicates x % of particle population is < y µm in size.

During micronization, starch gelatinization had occurred only in the 23% tempering treatment and not in non-tempered or 16% tempered seeds (Table 3.4). Arntfield et al. (1997) had observed that tempering moisture level had significant effect on starch gelatinization of lentil as well as the residual seed moisture following micronization. In this particular study, tempering levels of 25, 29 and 33% moisture gave increased levels of gelatinized starch which were 42, 52 and 70%, respectively, when all treated seeds were micronized to a final seed moisture level of 12%. Moreover, the level of gelatinized starch was significantly lower when seeds were micronized to a final moisture level of 7%. In a similar study by Fasina et al. (2001) in which the effect of infrared heating on the properties of five legume seeds (10% moisture prior to micronization) including lentil were studied, less than 10% gelatinized starch content was

observed for all legume seeds. Starch gelatinization is an order-disorder phase transition involving diffusion of water into the starch granules, hydration and swelling of granules, uptake of heat, loss of birefringence and crystallinity, and amylose leaching out of the granule (Kaur & Sandhu, 2010). Therefore, it is necessary to have high moisture content in the seed during the gelatinization process since moisture is required for the removal of amylose and swelling of the granule during starch gelatinization (Arntfield et al., 1997). Except for the 23% tempering moisture level, the moisture-temperature combinations used in the present study were not sufficient to gelatinize starch to a detectable level.

Table 3.4 Total starch (TS), gelatinized starch (GS) and resistant starch (RS) of lentil flours (% on dry weight basis) from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures[1,2].

Seed treatment	% TS	% GS	% RS[3]
Non-micronized	44.1 ± 0.9	NA	15.4 ± 0.4[a]
Non-tempered			
Micronized to 115 °C	ND	0	10.2 ± 0.2[c]
Micronized to 130 °C	43.2 ± 0.6	0	10.4 ± 0.4[c]
Micronized to 150 °C	ND	0	9.9 ± 0.5[d]
Micronized to 165 °C	ND	0	8.5 ± 0.1[e]
16% tempered			
Micronized to 115 °C	ND	0	11.0 ± 0.5[b]
Micronized to 130 °C	43.6 ± 0.9	0	10.4 ± 0.1[c]
Micronized to 150 °C	ND	0	9.8 ± 0.1[d]
Micronized to 165 °C	ND	0	9.5 ± 0.8[d]
23% tempered			
Micronized to 115 °C	ND	21.1 ± 0.7[b]	6.8 ± 0.2[f]
Micronized to 130 °C	43.6 ± 0.9	23.6 ± 0.6[a]	5.0 ± 0.0[g]
Micronized to 150 °C	ND	24.8 ± 0.1[a]	5.0 ± 0.1[g]
Micronized to 165 °C	ND	17.7 ± 0.3[c]	4.6 ± 0.2[h]

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).
[3]RS values are given as % of the flours.
ND is not determined, NA is not applicable.

There was a significant decrease in the resistant starch content of lentil flours with the increasing tempering level and micronizing temperature as shown in Table 3.4. Studies on resistant starch contents of lentil flour from micronized seeds are not available in the literature.

However, Garcia-Alonso et al. (1998) reported raw lentil, chickpea and beans had higher resistant starch contents (16-21%) than retrograded or cooked ones. This was attributed to the fact that no gelatinization had occurred in raw legumes. In another study, Aguilera et al. (2009) demonstrated that the resistant starch content of chickpea and lentil decreased by 65% and 49%, respectively, compared to the raw flours as a result of thermal treatment of seeds (boiling for 30 or 60 min, respectively, followed by dehydration in a forced-air tunnel at 75±3 °C for 6 h). Chung et al. (2006) reported that resistant starch contents in gelatinized and partially gelatinized rice starches were lower than that in unprocessed rice starch. Emami et al. (2010) showed that micronization reduced the resistant starch contents in three barley types (normal, high amylose and waxy barley) and the degree of starch gelatinization achieved in the micronized samples had a significant relationship with this reduction. The lowest resistant starch contents observed for the flours obtained from micronization of 23% tempered seeds in this study can be attributed to the starch gelatinization that occurred as a result of micronization at this tempering level. The reduction in resistant starch contents in lentil flours from micronized seeds is an undesirable effect of micronization since resistant starch is reported to reduce glycemic index, high cholesterol level in blood and the risk of colorectal cancer (Emami et al., 2010).

Functional properties

Protein solubility in an aqueous medium is an important criterion that affects the nutritional value and also the utilization of legume flours in terms of functional properties such as foaming, emulsification and gelation which are related to soluble proteins (Zheng et al., 1998; Mwangwela et al., 2007). Denaturation of proteins is caused by heat processing resulting in reduced solubility (Zheng et al., 1998). Compared to non-micronized seed flour, Protein dispersibility index (PDI) values of lentil flour decreased for all micronization treatments (Table 3.5) except for the flour from non-tempered seed micronized to 115 °C. Dispersed protein in the neutral pH as used for determining PDI is very much dependant on the solubility of the lentil flour components. Increase in tempering moisture level in combination with increasing micronizing temperature caused decrease in PDI indicating lack of solubilization of proteins due to aggregation or denaturation in the seeds that had high tempering moisture levels and micronized to higher temperatures. Micronization temperature showed a significant negative correlation with the PDI values ($r = -0.87$, $p<0.05$). Decreased protein solubility due to

micronization has been reported for cowpea micronized to 130 and 170 °C (Mwangwela et al., 2007), navy and black beans micronized to 112 and 117 °C, respectively (Bellido et al., 2006), and pinto and kidney beans micronized to 140 °C (Fasina et al., 2001).

The changes in protein structure during heating may affect solubility properties of a protein. Cross linking between protein molecules during heating may cause the formation of aggregates rendering the protein insoluble (Ma et al., 2011). Intramolecular hydrogen bonds and non-polar bonds can be cleaved and reformed during heating and subsequent cooling making proteins less soluble due to changes in conformation (Ma et al., 2011). The high moisture contents during micronization make the proteins more susceptible to denaturation and aggregation causing decreased solubility values (Arntfield et al., 1998).

Table 3.5 Protein dispersibility index (PDI), water holding capacity (WHC) and oil absorption capacity (OAC) of lentil flours from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures[1,2].

Seed treatment	PDI	WHC (mL/g)	OAC (g/g)
Non-micronized	43.6 ± 0.5^{a}	0.9 ± 0.0^{d}	0.8 ± 0.1^{de}
Non-tempered			
Micronized to 115 °C	42.9 ± 1.0^{a}	1.0 ± 0.1^{cd}	0.8 ± 0.0^{de}
Micronized to 130 °C	41.0 ± 1.0^{ab}	0.9 ± 0.1^{cd}	0.8 ± 0.0^{e}
Micronized to 150 °C	32.4 ± 2.0^{d}	1.1 ± 0.1^{cd}	0.8 ± 0.0^{cde}
Micronized to 165 °C	21.6 ± 3.2^{f}	1.2 ± 0.2^{c}	0.8 ± 0.0^{de}
16% tempered			
Micronized to 115 °C	38.5 ± 2.0^{bc}	1.0 ± 0.2^{cd}	1.0 ± 0.0^{b}
Micronized to 130 °C	35.0 ± 3.5^{cd}	1.1 ± 0.2^{cd}	1.1 ± 0.0^{b}
Micronized to 150 °C	21.5 ± 3.9^{f}	1.4 ± 0.4^{b}	0.9 ± 0.0^{c}
Micronized to 165 °C	17.5 ± 0.7^{g}	1.7 ± 0.2^{b}	0.8 ± 0.0^{de}
23% tempered			
Micronized to 115 °C	35.6 ± 2.2^{cd}	1.5 ± 0.3^{b}	1.4 ± 0.1^{a}
Micronized to 130 °C	25.6 ± 4.3^{e}	2.2 ± 0.3^{a}	1.4 ± 0.1^{a}
Micronized to 150 °C	17.2 ± 2.9^{g}	2.4 ± 0.0^{a}	0.9 ± 0.0^{cd}
Micronized to 165 °C	16.8 ± 1.6^{g}	2.3 ± 0.0^{a}	1.0 ± 0.1^{b}

[1]Values are means ± standard deviation.

[2]Means with different superscripts within each column are significantly different ($p<0.05$).

Water holding capacity is the ability to physically hold water and is a very important functional property required in flours for many food applications (Ma et al., 2011). The water retention ability of the flours was improved with tempering level and micronization temperature as indicated by the results of water holding capacity tests (Table 3.5).

Lentil flours from 16% seed tempered treatments micronized to 150 and 165 °C and the 23% seed tempered treatment micronized to 115, 130, 150 and 165 °C had significantly ($p<0.05$) higher water holding capacities than the raw and other tempering and micronization temperature treatments indicating that more structural changes to starch and protein had taken place at higher temperatures as well as at high tempering moisture levels. The changes in macromolecular structures of starch and proteins of the lentil flour that occurred due to hydration and heat may have affected the physico-chemical parameters that led to greater water holding ability. High water holding capacity of flours could make them good ingredients in bakery applications as it can improve the handling characteristics of the dough (Ma et al., 2011). Thermal treatments such as roasting and boiling of chickpea and lentil have been shown to increase water holding capacities of the flours. Ma et al. (2011) reported that there was a 146% increase in water holding capacity of flour obtained from dehulled green lentil after boiling. Several studies have shown that water holding capacity has increased due to micronization. Fasina et al. (2001) demonstrated that micronization (<10% moisture, 140 °C) of pinto and black beans, lentil and green pea resulted in increased water absorption capacity, and attributed this change to protein denaturation. Mwangwela (2007) reported that flour from cowpea seeds micronized to 170 °C showed higher water holding capacity than the flour from seeds micronized to 130 °C. The structural changes of the macro- and micro molecules in the seed particles brought about by heat treatment allow greater porosity and fluid entrapment. In addition, amino acid residues exposed as a result of protein denaturation cause greater water binding properties (Ma et al., 2011). Gelatinization of starch and the swelling of crude fiber (cell wall and seed coat components) during heating can also contribute to increased water holding capacity (Aguilera et al., 2009).

Oil absorption capacity (OAC) is a measure of the physical entrapment of oil in the flour matrix (Ghavidel & Prakash, 2006). It is an important functional property of oil-added food products which influences mouth feel and retention of flavour molecules (Ma et al., 2011). The oil absorption capacity values did not show a remarkable difference due to micronization except for the 23% tempered 115 and 130 °C micronizing treatments which showed an increase in the

values (Table 3.5). This observation indicates that high tempering moisture level in combination with micronization temperatures of 115 and 130 °C actually facilitates more oil absorption or binding of non-polar hydrophobic components compared to flours from the seeds tempered to same level of moisture and micronized to temperatures above 130 °C. The OAC indicated an opposite trend to the water holding capacity.

Differential scanning calorimetry (DSC) has been widely used to study starch gelatinization and protein denaturation (Henshaw et al., 2003). The phase transition process is represented by the rapid scan from room temperature to a certain above-boiling temperature while the DSC enthalpy represents the net sum of all endothermic processes taking place in substrate macromolecules during heating (Ratnayake et al., 2009). According to DSC results, starch gelatinization and thermal denaturation of proteins of the lentil seed flours was evident with micronization. Starch (44.1%, on dry weight basis; Table 3.4) and protein (26.6%, on dry weight basis; Table 3.1) are the most abundant macromolecules of lentil flour. Lipids are present in very small amounts in lentil flour (1.2%). Lentil flour from non-micronized seeds showed two recognizable endothermic peaks attributing to starch (peak 1, peak temperature 66.8 °C, confirmed from literature data (Chung et al., 2008) and a second peak with a peak temperature of 85.5 °C as shown in Figure 3.2.

The second peak could be attributed to protein denaturation. The peak temperature values observed for protein denaturation in the present study is towards the lower values in the range of data found in the literature. Ahmed et al. (2009) observed peak denaturation temperatures of 110 to 135 °C for lentil flour slurries and attributed this to thermal denaturation of globulin. Kaur et al. (2007) reported peak denaturation temperatures between 97.5 and 99.1 °C for protein isolates from different lentil cultivars. The low denaturation temperatures observed for proteins in the present study could be due to the heterogeneous nature of the lentil flours.

When the enthalpy values corresponding to starch gelatinization (peak 1) were calculated, there was a significant decrease in enthalpy for flours of 16% tempered and micronized seeds (8-51%), and 23% tempered and micronized seeds (62-94%), compared to the flour from non-micronized seeds. However, the unchanged enthalpy values of the flours from non-tempered, micronized seeds once again confirmed the fact that higher moisture is necessary for the starch gelatinization. Thermal peaks corresponding to protein denaturation were observed for flours from non-tempered seeds at all micronization temperatures. For the flours from tempered seeds,

well resolved peaks for protein denaturation were observed only for 115 and 130 °C temperature treatments from 16% tempered seeds. Inability to observe a clear protein denaturation event in flours treated above 130 °C could be due to the near complete protein denaturation that occurred at high moisture and high temperature conditions.

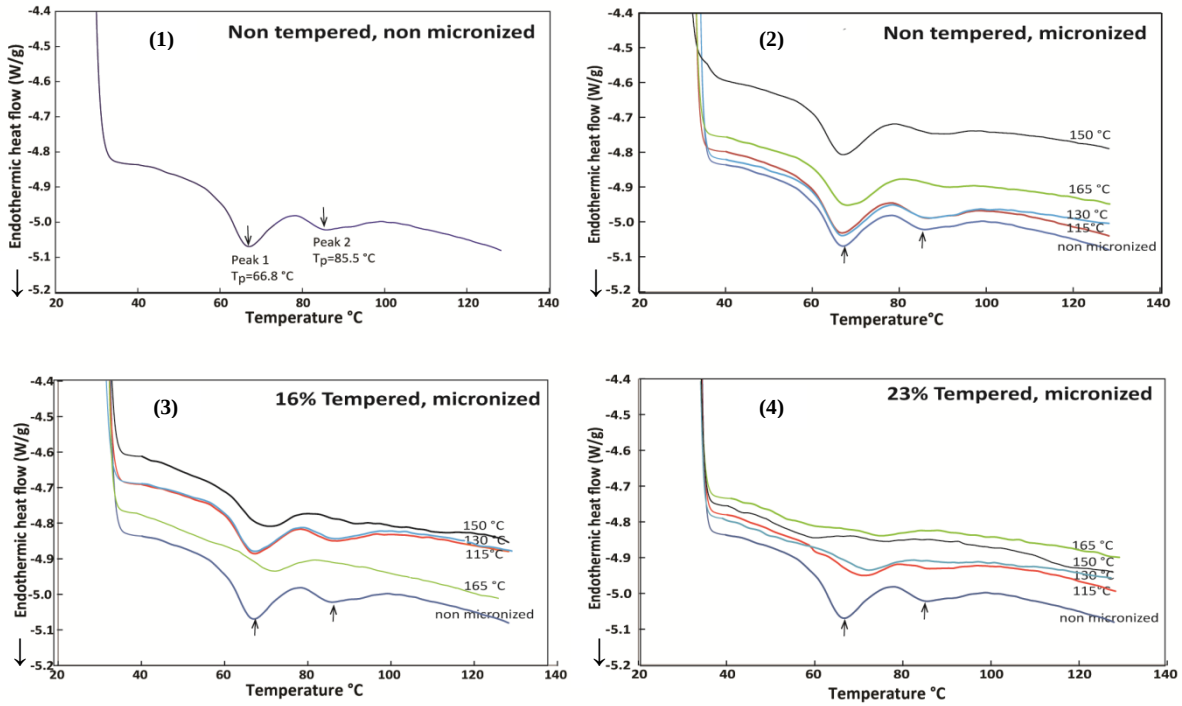

Figure 3.2 DSC thermograms of lentil flour samples (1) Raw (non-micronized), (2) Flours from non-tempered, micronized seeds (3) Flours from micronized seeds tempered to 16% moisture, (4) Flours from micronized seeds tempered to 23% moisture, Arrows inside the graph indicate identifiable endothermic peaks for treated flours.

The pasting properties of flours indicated by the rapid visco analyzer (RVA) results in Table 3.6 showed changes in peak viscosity, final viscosity, pasting temperature and peak time of the flours due to micronization of the seeds.

Peak viscosity indicates the water-binding capacity of starch or mixture and pasting temperature provides an indication of the minimum temperature required to cook a given sample. Final viscosity is the most commonly used parameter to define the quality of a particular sample as it indicates the ability of the material to form a viscous paste or gel after cooking and cooling (RVA, Application and Method, Perten Instruments, USA, 2011). The peak viscosity values was significantly higher ($p<0.05$) for the flour samples of non-tempered seeds micronized to 115 and 130 °C compared to flour of non-micronized seeds. A significant decrease ($p<0.05$) in peak

viscosity of flour was observed for non-tempered seeds micronized to 165 °C, and 16% and 23% tempered seeds micronized to 150 and 165 °C compared to non-micronized lentil flour.

Table 3.6 Pasting properties obtained by Rapid visco analyzer (RVA) for lentil flours from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures[1,2].

Seed treatment	Peak viscosity (RVU)	Final viscosity (RVU)	Peak time (minutes)	Pasting temperature (°C)
Non-micronized	85.9 ± 2.1^{bc}	129.1 ± 1.7^{bc}	5.2 ± 0.3^{fg}	72.3 ± 0.0^{efg}
Non-tempered				
Micronized to 115 °C	104.2 ± 4.2^{a}	139.0 ± 3.7^{ab}	4.7 ± 0.0^{g}	71.2 ± 0.9^{fg}
Micronized to 130 °C	104.3 ± 4.6^{a}	147.5 ± 12.4^{a}	5.1 ± 0.5^{fg}	70.8 ± 0.3^{gh}
Micronized to 150 °C	89.3 ± 3.2^{b}	127.0 ± 7.3^{cd}	6.2 ± 0.8^{de}	72.6 ± 0.0^{ef}
Micronized to 165 °C	57.8 ± 8.5^{d}	89.3 ± 9.1^{e}	7.4 ± 0.2^{ab}	74.6 ± 0.6^{cd}
16% tempered				
Micronized to 115 °C	91.8 ± 0.9^{b}	139.5 ± 1.7^{ab}	5.9 ± 0.3^{ef}	71.0 ± 0.6^{fgh}
Micronized to 130 °C	79.6 ± 2.7^{c}	117.4 ± 1.4^{d}	6.5 ± 0.2^{cde}	71.3 ± 0.3^{fg}
Micronized to 150 °C	50.0 ± 1.3^{e}	82.3 ± 0.4^{e}	7.8 ± 0.3^{a}	72.1 ± 0.3^{efg}
Micronized to 165 °C	37.3 ± 1.1^{f}	71.1 ± 1.1^{f}	6.6 ± 0.5^{cd}	80.3 ± 0.6^{a}
23% tempered				
Micronized to 115 °C	91.9 ± 0.9^{b}	136.0 ± 0.9^{bc}	6.6 ± 0.1^{cde}	69.6 ± 0.9^{h}
Micronized to 130 ° C	88.8 ± 0.1^{b}	137.3 ± 0.4^{abc}	6.8 ± 0.5^{bcd}	73.2 ± 1.4^{de}
Micronized to 150 °C	37.4 ± 3.5^{f}	59.6 ± 2.5^{g}	6.9 ± 0.4^{bc}	76.1 ± 0.9^{bc}
Micronized to 165 °C	26.4 ± 1.8^{h}	48.1 ± 0.7^{h}	6.9 ± 0.1^{bc}	77.5 ± 1.1^{b}

[1]Values are means ± standard deviation.

[2]Means with different superscripts within each column are significantly different ($p<0.05$).

The peak viscosity values observed for higher tempering levels and micronization temperatures cannot be compared with the increased water binding capacities of the flours observed at higher tempering and temperature levels mainly because the WHC values were obtained at ambient temperature. Heating of starch in an aqueous environment results in breakdown of starch granules as a result of imbibing water and swelling, and formation of a continuous gel from the amylose released (RVA, Application and Method, Perten Instruments, USA, 2011). Gelatinized starch is already present in lentil flours from 23% tempered seeds (18 to 25% of the flour, Table 3.4) and the flours from micronization temperatures of 115 and 130 °C resulted in high viscous pastes as indicated by the final viscosity values. Very low final viscosity values observed for the flours

from seeds tempered to 23% moisture and micronized to 150 or 165 °C temperatures indicate that these treatments resulted in much less viscous pastes. Therefore, final viscosities of lentil flours are not directly related with the gelatinized starch contents obtained in this study, but more closely followed micronization temperature. Although starch is the major component which controls the pasting properties, temperature induced changes in non-starch polysaccharides and proteins also contribute to the gelling and pasting properties by way of swelling, denaturation and unfolding (Kaur & Sandhu, 2010). Mwangwela et al. (2007) reported similar results to the present study where the increasing micronization temperatures (41% moisture, 130 and 170 °C) resulted in decreased peak viscosities for cowpea flour.

RVA results of the present study indicate that different seed moisture and micronization temperature resulted in lentil flours with a wide range of viscosities which could be utilized in food industry.

Enzyme activities

Certain enzyme activities of lentil flours were affected by seed micronization treatments (Table 3.7). Lipoxygenase is widely distributed in plant tissues, particularly in legumes, and catalyzes the oxidation of unsaturated fatty acids and their glycerides that contain a methylene interrupted system of double bonds such as linoleic (C18:2 cis), linolenic (C18:3 cis) and arachidonic (C20:4 cis) acids (Al-Obaidy & Siddiqi, 1981). There was a significant decrease in lipoxygenase activity for lentil flours from all micronized treatments compared to flour from non-micronized seeds. Lipoxygenase activity showed a 70-80% decrease for 115 °C micronization temperature treatment at different moisture levels. All micronization temperatures above 115 °C showed more than 96% decrease in lipoxygenase activity regardless of the tempering level compared to the non-micronized treatment (Table 3.7). Henderson et al. (1991) observed that exposure of pea flour to dry heat (60 °C for 30 min) resulted in reduction of lipoxygenase activity by more than 85%. Chang & McCurdy (1985) reported lipoxygenase activity of 24 $\times 10^9$ units per gram of protein for lentil. A significant variation in reported lipoxygenase values exist which is attributed to differences in quantitative techniques involving sample preparation, enzyme extraction conditions, substrate conditions, surfactants used and assay conditions (Chang & McCurdy, 1985 and Der, 2010). Lipoxygenase activity of flours showed a negative and

significant ($p<0.05$) correlation with micronizing temperature ($r = -0.77$) while the moisture level did not show a relationship with the lipoxygenase activity.

Table 3.7 Lipoxygenase, peroxidase and trypsin inhibitory activities of lentil flours from non-micronized seeds and tempered or non-tempered seeds micronized to different surface temperatures[1,2].

Seed treatment	Lipoxygenase activity (10^5 units per g of total protein)	Trypsin inhibitory activity (TIU per mg of protein)	Peroxidase activity (units per g of protein)
Non-micronized	134.6 ± 3.0^{a}	1.3 ± 0.2^{a}	186.4 ± 3.4^{a}
Non-tempered			
Micronized to 115 °C	32.7 ± 2.4^{c}	1.4 ± 0.0^{a}	126.6 ± 8.1^{b}
Micronized to 130 °C	4.5 ± 0.9^{e}	1.3 ± 0.1^{a}	66.8 ± 9.2^{cd}
Micronized to 150 °C	0.6 ± 0.4^{e}	1.2 ± 0.0^{a}	63.9 ± 2.2^{d}
Micronized to 165 °C	0.1 ± 0.0^{e}	0.9 ± 0.0^{b}	6.6 ± 2.2^{e}
16% tempered			
Micronized to 115 °C	25.0 ± 3.6^{d}	0.9 ± 0.1^{b}	75.3 ± 1.1^{c}
Micronized to 130 °C	1.2 ± 0.6^{e}	0.8 ± 0.0^{bc}	71.0 ± 2.2^{cd}
Micronized to 150 °C	0.4 ± 0.0^{e}	0.8 ± 0.1^{b}	7.9 ± 1.1^{e}
Micronized to 165 °C	0.1 ± 0.0^{e}	0.8 ± 0.1^{d}	0
23% tempered			
Micronized to 115 °C	40.5 ± 5.6^{b}	0.9 ± 0.1^{b}	8.9 ± 0.0^{e}
Micronized to 130 °C	0.4 ± 0.0^{e}	0.8 ± 0.0^{b}	0.2 ± 0.0^{e}
Micronized to 150 °C	0.9 ± 0.0^{e}	0.8 ± 0.0^{bc}	0
Micronized to 165 °C	0.2 ± 0.0^{e}	0.6 ± 0.1^{cd}	0

[1]Values are means ± standard deviation.

[2]Means with different superscripts within each column are significantly different ($p<0.05$).

Trypsin inhibitory activity of the legume flours is regarded as an antinutritional property. Non-micronized lentil flour had an activity of 1.3 TIU per mg of protein and high tempering and high micronization temperatures of lentil seed reduced the trypsin inhibitory activities of the flours (Table 3.7). Non-tempered 165 °C heat treatment and all 16% and 23% tempered treatments showed trypsin inhibitory activities of 0.9 to 0.6 TIU per mg of protein, which are significantly lower compared to the activity of non-micronized lentil flour (1.3 TIU). Fasina et al. (2001) reported that the infrared heating significantly reduced the trypsin inhibitory activity of lentil (from 3.55 to 2.46 mg per g of sample). In the study by Ma et al. (2011) where the trypsin

inhibitory activity of pulse flours before and after thermal treatments were evaluated, more than 95% reduction was observed for roasted dehulled green lentil. Different results for trypsin inhibitory activity of lentil were reported in the literature; 3-8 (Guillamon et al., 2007), 0.2-5.1 (Savage, 1997) and 2.6 (Vidal-Valverde et al., 1993) TIU per mg of sample. The variation of the trypsin inhibitory activity of lentil has been attributed to differences among cultivars, drought and thermal stress during vegetative growth and influence of rainfall during seed development (Guillamon et al., 2007).

Peroxidase is another enzyme which can contribute to deteriorative changes in flavour, texture, colour and consequently nutritional value of foods. It is highly resistant to thermal inactivation and has the ability to regain activity during storage after limited heat treatment (Kermasha & Metche, 1998). The peroxidase activity of lentil flours was reduced due to seed micronization (Table 3.7). The non-tempered seeds retained at least 40% of peroxidase activity even when they were treated to 150 °C. However, the 16% tempered seeds had less than 5% activity at 150 °C similar to the levels observed for 23% tempered seeds treated above 115 °C or non-tempered seeds treated at 165 °C. Both moisture ($r = -0.61$, $p<0.05$) and micronizing temperature ($r = -0.61$, $p<0.05$) had a significant correlation with the peroxidase activity indicating that inactivation of peroxidases is sensitive to the tempering level and micronization temperature. Several studies have been done on soybean peroxidases. Ghamemmaghami et al. (2010) reported that soybean hull peroxidase was a highly robust enzyme which had stability and activity over a wide range of pH and elevated temperatures. There were no studies found in the literature on peroxidase activity of lentil; however, the off-flavour production in pea and green bean has been attributed to peroxidase activity (Kermasha & Metche, 1998).

3.6 Conclusions

The combination of tempering moisture level and micronization temperature of seeds significantly affected the physical, chemical and functional properties of resulting lentil flours. Changes in starch and protein properties of lentil due to micronization were evident from the changes observed in the parameters studied here. The presence of increased seed moisture levels during micronization seemed to be necessary for gelatinization of starch, which is an important consideration for the flours which are to be used in different food applications. Protein denaturation and starch structural changes as a result of micronization were obvious from DSC

studies. These changes were reflected in the size of majority of particles of the flour, final viscosity that flours achieved, ability to absorb water or oil, and the colour parameters. The denaturation of protein may have contributed to the changes in protein dispersibility index (indicates solubility). Enzyme activities, such as lipoxygenase and peroxidase, and also the trypsin inhibitory activity decreased as a result of micronization. Therefore seed tempering and micronization together can create lentil flours having a wider range of functionalities than the flour of native untreated seeds provides.

3.7 Connection to the next study

The changes in functionalities of flours from micronized lentil seeds could have beneficial effects when these flours are used as a binder in meat products such as beef burgers, particularly flours which provide high liquid absorbing ability. Plant-based binders are known to accelerate fresh meat colour deterioration of ground beef. The most important parameters for the consumer acceptability of fresh beef products such as burgers are the red colour and the flavour of the cooked product which are related to chemical state of myoglobin and unsaturated lipids of meat. In the next part of the study, lentil flours from different seed moisture-heat treatments were used as binders in beef burgers to investigate if the binders would have a favourable influence on fresh ground beef which are stored in retail display setting. Experiments to understand how micronization make positive changes in lentil flour properties were also investigated.

4. STUDY 2: PERFORMANCE OF FLOUR FROM MICRONIZED LENTIL SEEDS IN FRESH GROUND BEEF; STUDY ON STABILITY OF PRODUCT COLOUR AND LIPIDS AND THE FACTORS IN FLOUR AFFECTING THE STABILITY

4.1 Abstract

The effect of flours from micronized lentil seed on the stability of fresh meat colour and unsaturated lipids of ground beef and the properties of flours which could bring about these changes were investigated in this study. Burgers incorporated with lentil flour from micronized seeds (6% w/w) exhibited enhanced retention of redness in meat and suppression of meat lipid oxidation as indicated by Hunter Lab a* values and thiobarbituric acid reactive substances values, respectively. Addition of flours from seeds tempered to 16-23% moisture levels and micronization temperatures above 130 ºC showed a better effect than the other treatments in stabilization of red colour and unsaturated lipids even after day 4 of retail display. Flour from non-tempered seeds (8% moisture) that were micronized to 150 ºC and 165 ºC also showed a similar effect. However, flour from non-tempered seeds micronized to 115 and 130 ºC temperatures showed the least effectiveness on stabilizing fresh meat colour, oxymyoglobin level and unsaturated meat lipids. Investigation of the antioxidant potential of soluble components of lentil flour samples showed a decrease in total phenolic compounds with increasing seed tempering moisture and increasing micronization temperature, while hydroxyl radical scavenging activity, superoxide radical scavenging activity and ferric ion reducing power remained relatively stable. These results do not support the hypothesis of enhanced antioxidant activities in lentil flour due to micronization. Selected lentil flour extracts, prepared to resemble the aqueous conditions in burgers, were tested for oxymyoglobin oxidation with and without unsaturated lipids in a myoglobin-liposome model system. Addition of lentil flour caused almost 50% reduction of oxymyoglobin content when the flour extract of non-micronized lentil seeds was added. Presence of unsaturated lipids accelerated oxymyoglobin degradation with non-micronized and non-tempered seed flours while the flours of 115 °C micronization treatment showed possible acceleration of oxymyoglobin degradation compared to other temperature

treatments. The extracts of flours that showed the least change in Hunter a* value during retail storage showed the slowest rate of oxymyoglobin oxidation under the model conditions. Considering the results of antioxidant activity of the flour extracts and oxidative enzyme activities of flours in Study 1 and the performance of flours in the model oxidation systems (both meat and oxymyglobin-lipid), most likely the suppression of prooxidants (due to micronization) and presence of some antioxidant compounds may have extended the stability of oxymyoglobin and unsaturated lipids.

4.2 Introduction

In certain processed meat products, use of economical extenders, binders or fillers reduces cost of production. The meat processing industry is very careful in selecting the binders that can simultaneously improve nutritional and sensory qualities of the end-product. The reduction of fat level in meat products is another important consideration which had drawn great attention of the meat industry (Modi et al., 2003) and particularly it is becoming popular in formulating foods for healthy body weight management. High starch and protein contents and low fat content in lentil makes it a suitable binder while improving the cooking and sensory characteristics of popular meat products such as burgers, wieners and sausages (Der, 2010). Moreover, as a legume, lentil does not contain gluten protein found in some cereal flours and therefore lentil products are eligible to claim "gluten-free" on the product label.

Colour of meat is a significant sensory attribute for the perception of freshness, quality and the value at purchase (Faustman et al., 2010). Myoglobin, a globular heme protein in the muscle tissues, is responsible for the reddish colour of meat (Chaijan, 2008). Post-slaughter biochemical changes occurring in muscle tissue initiated by oxidation reactions have a key effect on the colour of fresh meat. The oxidation of central iron atom within the heme group causes discolouration, a change from red oxymyoglobin to brownish metmyoglobin (Faustman et al., 2010). Temperature, pH, metmyoglobin reducing activity, partial oxygen pressure and lipid oxidation are the factors which affect the oxidation of myoglobin (Faustman et al., 2010). Lipid oxidation and myoglobin oxidation in muscle foods take place in a concurrent manner (Chaijan, 2008). Superoxide anion and hydrogen peroxide produced during oxymyoglobin oxidation further react with iron to produce hydroxyl radicals which can penetrate into hydrophobic lipid regions facilitating lipid oxidation. In addition, the aldehyde products resulting from lipid

oxidation induce the oxidation of oxymyoglobin (Chaijan, 2008). Significant support for an interaction between lipid oxidation and discolouration has been provided by studies done with the inclusion of antioxidants. Faustman (2004) reported that α-tocopherol, a lipid soluble antioxidant, delayed lipid oxidation in fresh meat from various livestock species and also delayed beef discolouration. Plant-based ingredients used as fillers or binders in meat formulations are known to have antioxidant properties and the effectiveness of meat lipid oxidation of wheat- and soy-based products have been reported in the literature (Wanasundara & Pegg, 2007).

In addition to their high nutritional values, the health benefits of legumes such as antioxidant activity have attracted consumer acceptance which could be effective in expanding use of legumes in food (Han & Baik, 2008). When used as a binder in comminuted meat products, minor constituents present in lentil such as phenolic compounds and small molecular weight proteins can also provide beneficial effects in delaying lipid oxidation and red colour deterioration.

In lentil, phenolic compounds make a higher contribution to the observed antioxidant activity than tocopherols or ascorbate present in the seed (Fernandez-Orozco et al., 2003). Phenolic compounds are good indicators of antioxidant activity since they are radical scavengers and are also able to inhibit lipid oxidation by binding with free radicals generated during the process as well as acting as chelators of metal ions that induce oxidative processes (Han & Baik, 2008). Studies on phenolics and antioxidants of lentil have shown that most of these compounds are concentrated in the hulls (Oomah et al., 2011). Han & Baik (2008) reported that total phenolic content and antioxidant activity were affected by processing conditions.

Based on the chemical reactions involved, antioxidant capacity assays are divided into two categories: (1) hydrogen atom transfer (HAT) reaction based assays and (2) single electron transfer (ET) reaction based assays (Huang et al., 2005). HAT- and ET-based assays are intended to measure the radical (or oxidant) scavenging capacity. HAT-based methods generally are composed of a synthetic free radical generator, an oxidizable molecular probe, and an antioxidant. ET-based assays involve one redox reaction with the oxidant (which is also the probe for monitoring the reaction) as an indicator of the reaction end point (Huang et al., 2005). Total phenolics assay by Folin-Ciocalteu reagent and ferric ion reducing antioxidant power (FRAP) assay are ET-based assays. HAT- based assays include hydroxyl radical and superoxide radical scavenging activity assays.

This study was based on the two hypotheses that lentil flours obtained from seeds of different tempering moisture levels and different micronization temperatures cause a variation on oxidation stability of fresh meat colour and lipids, and lentil flours obtained from seeds of different moisture and temperature treatments have different levels of antioxidant potential in their aqueous salt extracts. Therefore, the objectives of this study were to investigate the different moisture-temperature levels of lentil seed micronization on colour and lipid oxidation stability of lentil flour incorporated ground beef products such as beef burgers, determine if micronization of lentil seeds had an effect on the total phenolic contents and antioxidant activity of the resulting flours, and study the possible path of stabilizing red colour of meat by lentil flour.

In the first part of the study, all 13 lentil flour samples prepared in Study 1 (12 micronized and 1 non-micronized seed treatments) were incorporated into fresh ground beef to form burgers. The lentil flours were stored at 4 °C in vacuum packed bags and used between 4-6 months after preparation. For comparison purpose, burgers without any binder or with toasted wheat crumbs which is used as a binder in meat industry were also prepared. In the second part, all lentil flour samples were analyzed for the content of phenolic compounds and antioxidant activity of the dilute salt soluble components. Activity of lipoxygenase and peroxidase in lentil seed coat and cotyledon was also assessed with and without heat treatment. In the third part of this study, using a liposome model, myoglobin oxidation was studied to understand the influence of water soluble components of selected (4) lentil flour on the myoglobin oxidation.

4.3 Materials and methods

4.3.1 Assessment of lentil flour in burger formulations

4.3.1.1 Raw materials

Lentil seeds, pre-treatments, micronization procedure and flour preparation were essentially the same as in Study 1 and explained in section 3.3, and flours from both replicates were used. Toasted wheat crumb (enriched bleached wheat flour, niacin, iron, thiamine mono nitrate, riboflavin, folic acid, durum flour, leavening agent, dried yeast) was obtained from Newly Weds Foods (Edmonton, AB). Beef was from bottom round of Canada AAA grade meat (Lakeside Packers, Brooks, AB), stored at -4 °C until used.

Lentil flours and wheat-based binders were incorporated at 6% (w/w) level to form beef burgers. Of the fifteen batches of beef burgers formulated, twelve batches were with lentil flours

from different tempering and micronization treatments and the remaining batches were with raw lentil flour (1), toasted wheat crumbs (1) and no-binder (1) containing extra 6% beef instead of binder which was the control. Burger preparation was replicated (2) by making burgers at different times using the flours from the two replicates prepared in micronization treatments.

4.3.1.2 Burger formulations

The formulations used in burger preparation are as in Table 4.1. Formulation batches of 2 kg with a target fat level of 10% in burgers were prepared.

Table 4.1 Formulations for burgers with different binders (% by weight)

Binder	Beef	Water	Salt	Binder	Total
No binder	88	11.1	0.9	0	100
Toasted wheat crumbs	82	11.1	0.9	6	100
Raw lentil flour	82	11.1	0.9	6	100
Lentil flour from micronized seeds (12 batches)	82	11.1	0.9	6	100

4.3.1.3 Processing of burgers

All the meat processing steps were done at -4 °C. The lean meat and the fat were separated and ground first through a 'kidney' plate and then through a 10 mm (3/8″) grinder plate. The fat content of the ground lean and fat were analyzed using the rapid fat analyzer (HFT200, Data Support Co., Encino, CA). Weight of lean meat and fat to be mixed in order to get the required final fat content of 10% was calculated using the Pearson Square method. Samples of meat were taken for proximate analysis and pH measurement. Meat was weighed out, mixed with salt, binder and ice water for 1 min using the tumbler. Mixing was done for 30 s first and then the sides of the tumbler were scraped and mixing was continued for another 30 s. The meat mixture was then passed through a 3 mm (1/8″) grind plate. The temperature of the mixture was maintained below 4 °C throughout the process. Burgers of 12 cm diameters (approximately weighing 120 g) were prepared using a Hollymatic patty machine. A random order of processing of burger formulations was carried out. Around 14 burgers were prepared from each treatment and from these, four burgers were selected randomly and cut into mini-burgers which had a diameter of 6 cm using a cookie cutter. This was to accommodate space available in the display case to hold burgers from 15 batches. One of these small burgers from each batch was placed in a

sealed polythene bag and kept in the freezer (-20 °C) for thiobarbituric acid reactive substances (TBARS) analysis and considered as day 0 sample. The 3 remaining burgers from each batch were placed on Styrofoam trays and wrapped with an oxygen permeable film (Choice Wrap, Huntsman Packaging Co., Uniontown, OH), which was reported to have a moisture vapour transmission rate of 33.9 g/100 in^2/24h, and stored in the retail display case at 4 ºC under light intensity between 1000 and 1350 lx for 5 d.

4.3.1.4 Evaluation of surface colour of burgers

Surface color of the fresh burgers in the retail display case was investigated by measuring Hunter L*, a*, b* parameters using a Hunter LabMiniScan XE Colorimeter (Hunter Association Laboratory Inc., Reston, VA) with the illuminant A and 10º observer. The instrument was standardized using the black and white tiles and the pink tile which had values of 76.4 for L* (lightness), 25.4 for a* (redness) and 17.6 for b* (yellowness) was used as the reference tile. Two readings were taken from each sample after rotating 90° clockwise between the two measurements. Surface colour measurements were carried out from 4 h after burgers were formed (day 0) and at each 24 h up to 4 days of storage under same conditions.

4.3.1.5 Evaluation of lipid oxidation products of burgers

Raw burgers kept in the retail display case were analyzed for TBARS on days 0, 2 and 4 according to the method by Bedinghaus and Ockerman (1995). Burgers stored in the freezer were also analyzed for TBARS after 4 months. Burger samples were pulverized using a kitchen style food processor for 10 s and mixed thoroughly with a plastic spatula to obtain a homogeneous mixture and continued grinding for another 10 s. The TBARS in the burgers were extracted by adding 50 mL of 20% (w/v) trichloroacetic acid (TCA) solution containing 1.6% (w/v) phosphoric acid to 5.0 g of the ground sample in stomacher bags and mixing for 2 min using the stomacher. Mixing was continued for another 1 min after addition of 50 mL of distilled water. The extract was filtered through Whatmann no. 1 filter paper and subsequently reacted with freshly prepared 0.02 M thiobarbituric acid (TBA) reagent to form pink coloured TBA-malonadialdehyde complex. The absorbance of this complex was measured at 532 nm using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). 1,1,3,3-tetramethoxypropane (TMP) was used as the standard.

4.3.2 Assessment of putative antioxidant activity of lentil flours

4.3.2.1 Preparation of flour extracts

Lentil flour extracts were prepared from the two replicated runs of flour production explained in Study 1. Lentil flour extracts were prepared to correspond to the aqueous phase that would surround the flour particles when added to fresh beef burger formulations considering the moisture content of ground beef (68.0%) and also the added moisture (11.1%) in the burger formulation. (According to this consideration, the ratio of water: lentil flour: salt was 100:8.8:1.6). Lentil flour (2.2 g) was mixed with 25 mL of water containing 0.4 g of NaCl in a centrifuge tube, kept in a shaking water bath at 23 ºC for 2 h, centrifuged at 1500×*g* for 15 min, filtered and the supernatant was collected for the assays after diluting as needed. The extracts were stored at 4 °C. All assays were done in duplicate.

4.3.2.2 Total phenolics assay

Total phenolic content was determined using the method described by Velioglu et al. (1998) with modifications. An aliquot of the diluted extract (100 µL) was mixed with Folin-Ciocalteu reagent (0.75 mL) by vortexing in a micro centrifuge tube and allowed to stand at room temperature for 5 min, followed by addition of 6% (w/v) sodium carbonate solution (0.75 mL) and incubating at ambient temperature for 90 min. Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate. Blank solutions were prepared in the same manner using 100 µL deionized water instead of the sample solutions. The absorbance of the mixture was measured at 765 nm against a reagent blank using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). The total phenolic content in lentil flour extracts were calculated using a standard curve prepared with gallic acid (concentration range 10-50 mg/mL). The total phenolic content in lentil flour aqueous extracts was expressed as gallic acid equivalents per 100 g.

4.3.2.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the method described by Zou et al. (2012). This assay measures the ferric ion reduction ability of the components in the extract at low pH. When the ferric 2,4,6-tripyridyl-*s*-triazine complex (Fe^{3+}-TPTZ) in the FRAP reagent is reduced to ferrous form (Fe^{2+}-TPTZ), an intense blue colour is developed. FRAP reagent was prepared by

mixing 10 volumes of acetate buffer (250 mM, pH 3.6), with 1 volume of 10 mM TPTZ in 40 mM HCl and with 1 volume of 20 mM $FeCl_3.6H_2O$. Briefly, diluted lentil flour extract was mixed with the FRAP reagent, incubated at 37 ºC for 8 min and the absorbance was measured at 593 nm using the spectrophotometer. A standard series was prepared using ferrous sulfate (concentration range 0.25-5 × 10^{-4} mol/L as Fe^{2+}) and the FRAP value of the sample was calculated and expressed as Fe^{2+} equivalents per 100 g of sample.

4.3.2.4 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of lentil flour was determined using the method described by Halliwell et al. (1987). The oxidation products of 2-deoxyribose by hydroxyl radicals, formed due to the reaction between hydrogen peroxide and Fe(II) (Fenton reaction), degrade into malondialdehyde which can react with TBA. In this assay, the ability of lentil flour extracts to scavenge hydroxyl radicals and thereby suppress deoxyribose breakdown was examined. Briefly, 500 µL sample solution was mixed with 100 µL 2-deoxyribose (28 mM, in KH_2PO_4-KOH buffer, pH 7.4), 200 µL ferric chloride (200 µM) and EDTA (1.04 mM) mixture (1:1 v/v), 100 µL hydrogen peroxide (1.0 mM) and 100 µL ascorbic acid (1.0 mM) in a glass vial and incubated at 37 °C for 1 h. Then, 1.0 mL TBA (1% in 50 mM NaOH) and 1.0 mL trichloroacetic acid (2.8%) solutions were added to the reaction mixture, heated at 100 °C for 20 min in a water bath. The absorbance of the resultant TBARS was measured at 532 nm against a blank (prepared by adding all the reagents except the sample, which was replaced with KH_2PO_4-KOH buffer; TBA and trichloroacetic acid added with no incubation at 37 °C) using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). All solutions were prepared fresh. Carnosine was used as the standard antioxidant at same concentration levels as the lentil flour extracts in this assay. Hydroxyl radical scavenging activity was calculated using the following equation:

$$\% \text{ Hydroxyl radical scavenging activity} = \frac{[A_C - A_S]}{A_C} \times 100 \quad (4.1)$$

Where, A_C is the absorbance of the control and A_S is the absorbance of the sample at 532 nm.

4.3.2.5 Superoxide radical scavenging activity

Superoxide radical scavenging activity of lentil flour was determined by the method described by Saint-Cricq de Gaulejac et al. (1999). The hypoxanthine-xanthine oxidase system generates superoxide radicals which reduce the nitroblue tetrazolium to a blue formasan product. In the presence of free radical scavengers, the formation of the blue formasan is proportionally reduced. Sample solution (100 µL) was added to the reaction mixture containing 2.2 mL sodium phosphate buffer (100 mM, pH 7.4), 100 µL nitro blue tetrazolium (1 mM), 100 µL xanthine oxidase (1.67 U/mL) and 500 µL hypoxanthine (5 mM), and the absorbance change was recorded for 10 min at 560 nm using the spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) against a blank. Control sample was prepared with all the reagents and sodium phosphate buffer (100 µL) instead of the sample solution and the blank was prepared by mixing sodium phosphate buffer (2.4 mL), nitro blue tetrazolium (100 µL) and hypoxanthine (500 µL). Carnosine was used as the standard antioxidant in this assay. Superoxide radical scavenging activity was calculated using the following equation:

$$\%\ \text{Superoxide radical scavenging activity} = \frac{[\Delta A_C - \Delta A_S]}{\Delta A_C} \times 100 \qquad (4.2)$$

Where, ΔA_C is the absorbance difference between 10 min and 0 min at 560 nm for the control. ΔA_S is the absorbance difference between 10 min and 0 min at 560 nm for the sample.

4.3.3 Assessment of lipid oxidation promoting enzyme activities of lentil seed components

4.3.3.1 Preparation of lentil components

For this study, untreated lentil seeds used were from the same batch as used in the other studies. First, 100 g of seeds were added to MilliQ water (2 times of the seed weight) and allowed to equilibrate for 1 h. Imbibition of this water caused the seed coat to loosen from cotyledons and made it possible to remove manually. All seed coats and cotyledons were collected separately without discarding any remaining water. They were kept under a fume hood overnight to allow ambient drying (Figure 4.1). Both seed coat and cotyledons were ground separately to prepare particles < 0.5 mm.

Figure 4.1 Images of lentil seed physical components, var. *Eston*, small green. Pictures were captured using Nikon D7000 camera attached with AF-S micro 105 mm lens.

4.3.3.2 Heat treatment of lentil flour components

Of the micronization treatments, lentil flour from non-tempered 115 °C micronization treatment showed the highest lipoxygenase and peroxidase activities. These enzyme activities were lower for lentil flour from non-tempered 150 °C micronization treatment. Therefore, these two temperatures were selected to heat treat seed coat and cotyledon fractions and assess the enzyme activities. Lentil components obtained as explained in 4.3.3.1 were heat treated by keeping in an oven pre-heated to 115 and 150 °C and monitoring the surface temperature of the lentil components using a thermocouple. They were heated for 10 min after the surface temperature reached 115 and 150 °C.

4.3.3.3 Enzyme activity of lentil flour components

The activities of lipoxygenase and peroxidase of heat treated and untreated lentil flour components were assessed according to the procedures described in sections 3.3.7.1 and 3.3.7.2, respectively.

4.3.4 Assessment of lentil flour extract activity on oxymyoglobin oxidation with and without unsaturated lipids

4.3.4.1 Lentil flour extract

Lentil flour extract was prepared as explained in section 4.3.2. For this part of the study, lentil flour selection was based on the results of first part of the Study 2 and enzyme activities of

flours (in the Study 1) to encompass variation in fresh beef colour stabilization. Lentil flour selection for this assessment was based on following observations:

- Flours of 16% tempering and 150 ºC treatment had reduced enzyme activities and provided higher colour and lipid oxidation stabilities in fresh ground beef.
- Flour of non-tempered seeds treated to 115 °C had higher enzyme activities and resulted in lower colour and lipid oxidation stability of fresh ground beef.
- Flours of 16% or 23% tempered seeds treated to 115 °C had high lipoxygenase and peroxidase activities.

Therefore, flours of the following 5 treatments were selected for the model system experiments: (1) non-micronized lentil flour, (2) lentil flour from non-tempered seeds micronized to 115 ºC, (3) lentil flour from 16% tempered seeds micronized to 115 ºC, (4) lentil flour from 16% tempered seeds micronized to 150 ºC, and (5) lentil flour from 23% tempered seeds micronized to 115 ºC. Two replicated preparations of flour samples were used and all analyses were done in duplicate.

4.3.4.2 Preparation of oxymyoglobin

Oxymyoglobin solution was prepared fresh every day according to the procedures described by Yin & Faustman (1993) and modified by Wanasundara & Pegg (2007). A solution of myoglobin having a concentration of 5 mg/mL was prepared by using 25 mg of myoglobin from equine skeletal muscle (Sigma Aldrich) dissolved in 5 mL of citrate buffer (4 mM, pH 6.4). Sodium hydrosulfite (2.5 mg) was added to this myoglobin solution and mixed. The solution was oxygenated for 5 min using the bench top air supply while maintaining temperature at 4 ºC. Oxygenated myoglobin solution was passed through a mixed-bed ion exchange resin (AG 501-X8, Bio-Rad) column (1 cm × 10 cm) to remove residual sodium hydrosulfite and the collected oxymyoglobin solution was measured for spectra readings at 525, 545, 565, 572 and 730 nm. The oxymyoglobin concentration of the solution was calculated using the equations 4.3 and 4.4. (Krzywicki, 1982) and the volume of oxymyoglobin needed to get a concentration of 2.5 mg/mL was calculated. The value of 2.5 mg oxymyoglobin/mL was set as the required oxymyoglobin concentration for the experiments.

$$a = \frac{A_{572} - A_{730}}{A_{525} - A_{730}} \quad (4.3)$$

$$\text{Fraction of of oxymyoglobin} = 1 - (1.395 - a) \quad (4.4)$$

Where, A is the absorbance value at the particular wave length

4.3.4.3 Preparation of liposomes

Multi-vesicle liposomes containing phospholipids with unsaturated fatty acids were prepared to study the ability of lentil extract components in counteracting progression of oxymyoglobin oxidation due to lipid oxidation products. Liposomes were prepared by the method described in literature (Yin & Faustman, 1993; Wanasundara & Pegg, 2007). In the preparation of liposome lipid layer, 1,2-dilinoleolyl phospholipid, cholesterol and dicetylphosphate (Avanti Polar Lipids Inc.) were used. Stock solutions of 50 mg/mL phospholipid, 12 mg/mL cholesterol and 3 mg/mL dicetylphosphate were prepared in chloroform/methanol (2:1, v/v). To 50 mL round bottomed flasks, phospholipids (0.6 mL), cholesterol (1.0 mL) and dicetyl phosphate (1.0 mL) were transferred and the total volume was made up to 10 mL with chloroform/methanol (2:1, v/v). The flask was attached to a rotary evaporator at room temperature and rotated under vacuum until a thin lipid layer was formed on the flask wall. The flasks were flushed with nitrogen, covered with parafilm and stored in the freezer (at -20 °C) until ready for analysis.

4.3.4.4 Evaluation of effect of lentil flour extract on oxymyoglobin oxidation

The effect of lentil flour on oxidation of oxymyoglobin in the absence of lipids was determined by mixing oxymyoglobin (volume calculated to get 2.5 mg/mL in the reaction mixture) with NaCl (1 mL of 10 mg/mL solution), and lentil flour extract (2 mL). The total volume was made to 10 mL by adding 4 mM phosphate buffer (pH 6.8). Mixing was done at 4 °C. Control sample was prepared by mixing the same volumes of oxymyoglobin and NaCl and distilled water instead of the sample solution. Aliquots of 1 mL of the reaction mixture were drawn at 5, 40, 90 and 150 min intervals while temperature was maintained at 4 °C. Spectra readings were taken at 525, 545, 565, 572 and 730 nm and the oxymyoglobin concentration of the solution was calculated at each time as described in section 4.3.4.2.

Oxidation of oxymyoglobin in the presence of lipids was determined as follows. To the round bottom flask containing previously prepared lipid layer (section 4.3.4.3), 5 glass beads (1 mm diameter), oxymyoglobin (volume calculated to get 2.5 mg/mL in the reaction mixture), NaCl (1 mL of 10 mg/mL solution) and lentil flour extract (2 mL) were added. The total volume was made to 10 mL with 4 mM phosphate buffer (pH 6.8). The solutions were mixed using a mechanical shaker while maintaining the temperature at 4° C. Figure 4.2 summarises the assessment scheme for lentil flours with and without unsaturated lipids. Spectral readings of the resulting multi-vesicle liposomes containing oxymyoglobin-unsaturated lipids and lentil extract were taken at 525, 545, 565, 572 and 730 nm by drawing sample portions from the reaction mixture at 5, 40, 90 and 150 min intervals and the oxymyoglobin concentration of the solution was calculated. Control sample was prepared by adding the same volume of oxymyoglobin to the round bottom flask containing previously prepared liposomes and glass beads, NaCl (1 mL of 10 mg/mL solution) and distilled water (2 mL) instead of lentil flour extract.

4.4 Statistical analysis

The mean and standard deviation of each measurement for the samples were calculated. Treatment means were compared using the mixed procedure of SAS Institute Inc., Cary, NC, USA (2008). Mean separation was done using the least significant difference (LSD) procedure. The level of significance was set at $p < 0.05$.

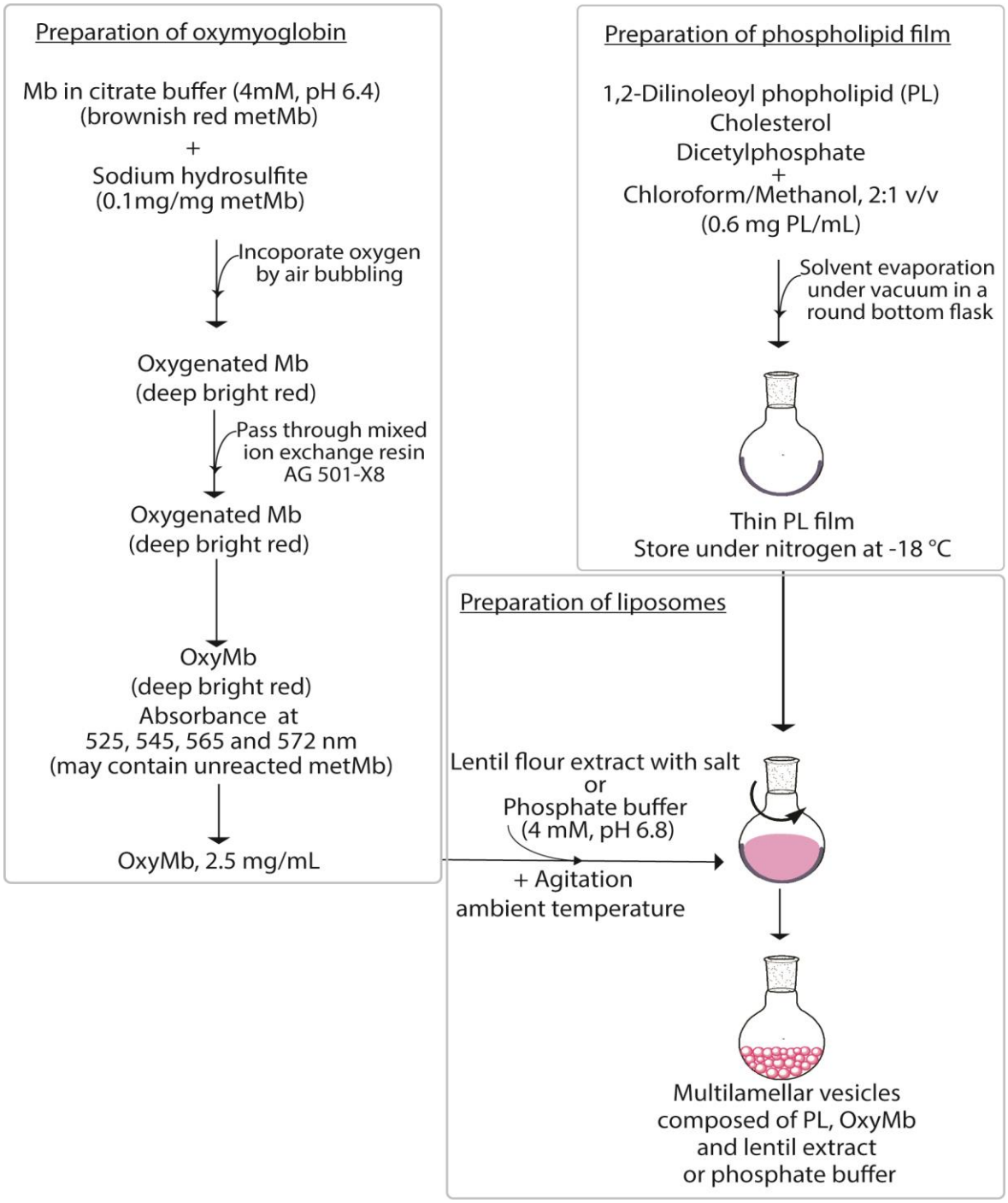

Figure 4.2 Summary of assessment of lentil flour aqueous extracts on oxidation of oxymyoglobin in the absence or presence of unsaturated lipids. Adapted from Yin & Faustman (1993); Wanasundara & Pegg (2007).

4.5 Results and discussion

4.5.1 Performance of lentil flours on stability of fresh colour and lipids of ground beef

In the first part of Study 2, lentil flour was incorporated into fresh ground beef to form burgers. The meat used in this study had an average pH value of 5.7; 68.3% moisture; 20.6% protein and 10.3% fat content as targeted after grinding. The 6% level of lentil flour addition was chosen according to the results of a previous study that showed no foreign flavour in the burgers was detected by a sensory panel (Der, 2010).

Colour of burgers was tracked for 5 days. The lightness (L*) of burgers of the 15 treatments (no binder, toasted wheat crumbs, flours from non-micronized lentil seeds and 12 micronization treatments) kept in the retail display case were similar from day 0 to 3 (Table 4.2). There was a slight decrease in lightness on day 4 for the burgers with flours from both micronized and non-micronized lentil seeds compared to the L* value of burgers with toasted wheat crumbs. No significant difference in the burger yellowness (b*) between the treatments was found during day 0 to 4 (Table 4.4). The visible changes in red colour in the fresh beef burger surface were reflected in the Hunter a* values (Table 4.3). All the burgers had high a* values on day 0 (between 17.6 and 20.3). On day 1, the control samples containing no binder, toasted wheat crumbs and lentil flour from non-micronized seeds showed a greater decrease in a* values compared to the burgers containing flours of micronized seeds. Deterioration of redness in burgers was visible in all samples over the days. By the day 4, the samples containing no binder, toasted wheat crumbs and lentil flour from non-micronized seeds showed the lowest a* values (Figure 4.3). Among the micronized treatments, the burgers containing flours from non-tempered and 115 and 130 °C showed the lowest redness (a*). These two treatments had higher lipoxygenase activity compared to the other non-tempered treatments. There was no statistically significant difference between the redness values of burgers containing micronized lentil flour from the other 11 treatments on day 4.

Table 4.2 Change of burger lightness (Hunter L* value) with storage time in retail display case at 4 °C[1,2].

Binder	Day 0	Day 1	Day 2	Day 3	Day 4
No binder	45.8 ± 8.0 [ab]	45.4 ± 8.8 [ab]	46.1 ± 9.3 [abc]	46.8 ± 9.1 [ab]	48.2 ± 8.8 [ab]
Toasted wheat crumbs	48.3 ± 3.8 [a]	47.7 ± 3.2 [a]	48.1 ± 3.3 [a]	48.8 ± 4.3 [a]	49.4 ± 4.0 [a]
Non-micronized lentil flour	47.0 ± 3.4 [ab]	46.3 ± 2.7 [ab]	46.4 ± 1.9 [abc]	46.1 ± 3.1 [ab]	45.3 ± 2.9 [bc]
Lentil flour from Non-tempered seeds					
Micronized to 115 °C	47.8 ± 5.6 [ab]	46.5 ± 3.0 [ab]	46.2 ± 3.6 [abc]	46.0 ± 4.3 [ab]	45.7 ± 3.5 [bc]
Micronized to 130 °C	46.8 ± 3.1 [ab]	45.8 ± 2.6 [ab]	45.6 ± 2.7 [abc]	45.0 ± 3.0 [b]	45.2 ± 2.7 [bc]
Micronized to 150 °C	47.9 ± 4.5 [ab]	46.9 ± 4.3 [ab]	47.1 ± 4.6 [ab]	46.9 ± 4.8 [ab]	45.9 ± 4.6 [bc]
Micronized to 165 °C	47.3 ± 4.6 [ab]	45.1 ± 3.2 [ab]	45.4 ± 2.6 [abc]	45.3 ± 3.6 [b]	45.7 ± 3.1 [bc]
16% tempered seeds					
Micronized to 115 °C	48.0 ± 5.5 [ab]	46.3 ± 3.2 [ab]	45.9 ± 3.0 [abc]	45.5 ± 2.9 [b]	46.0 ± 2.9 [bc]
Micronized to 130 °C	47.3 ± 6.3 [ab]	45.1 ± 4.3 [ab]	45.1 ± 4.1 [abc]	45.7 ± 4.6 [ab]	45.2 ± 4.3 [bc]
Micronized to 150 °C	46.6 ± 3.3 [ab]	45.2 ± 2.1 [ab]	44.8 ± 2.8 [abc]	45.3 ± 3.4 [b]	45.3 ± 3.0 [bc]
Micronized to 165 °C	45.5 ± 4.5 [ab]	43.6 ± 4.3 [b]	44.1 ± 4.6 [bc]	43.8 ± 4.3 [b]	43.9 ± 5.1 [c]
23% tempered seeds					
Micronized to 115 °C	47.0 ± 5.9 [ab]	44.9 ± 3.6 [ab]	45.1 ± 3.8 [abc]	44.8 ± 3.3 [b]	45.0 ± 4.9 [bc]
Micronized to 130 °C	45.4 ± 3.4 [ab]	44.5 ± 3.3 [ab]	44.6 ± 3.6 [abc]	44.0 ± 3.5 [b]	44.6 ± 3.5 [c]
Micronized to 150 °C	45.7 ± 3.6 [ab]	44.2 ± 2.8 [b]	44.5 ± 2.5 [abc]	43.7 ± 4.6 [b]	44.1 ± 2.4 [c]
Micronized to 165 °C	45.2 ± 4.9 [b]	43.8 ± 4.3 [b]	43.1 ± 5.3 [c]	44.8 ± 3.6 [b]	44.1 ± 4.0 [c]

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).
Note: for L* = 0 indicates black and L* = 100 indicates diffuse white.

Table 4.3 Change of burger redness (Hunter a* values) with storage time in retail display case at 4 °C[1,2].

Binder	Day 0	Day 1	Day 2	Day 3	Day 4
No binder	20.3 ± 0.8 [a]	18.5 ± 0.5 [abc]	15.3 ± 1.0 [ef]	11.4 ± 0.4 [f]	9.7 ± 0.2 [g]
Toasted wheat crumbs	19.6 ± 0.6 [ab]	17.6 ± 0.1 [cdef]	14.3 ± 1.6 [fg]	11.7 ± 1.8 [ef]	10.3 ± 0.7 [fg]
Non-micronized lentil flour	17.0 ± 0.9 [g]	15.5 ± 0.1 [g]	13.7 ± 0.4 [g]	12.6 ± 0.4 [def]	11.9 ± 0.3 [efg]
Lentil flour from					
Non-tempered seeds					
Micronized to 115 °C	18.1 ± 0.1 [def]	17.2 ± 0.2 [def]	15.7 ± 0.7 [def]	13.6 ± 1.1 [cde]	12.2 ± 0.7 [def]
Micronized to 130 °C	18.5 ± 0.9 [cdef]	17.6 ± 0.5 [bcdef]	16.4 ± 0.1 [bcde]	14.3 ± 0.4 [cd]	12.6 ± 1.0 [cde]
Micronized to 150 °C	19.0 ± 0.4 [bcd]	17.8 ± 0.5 [abcdef]	17.0 ± 0.6 [abcd]	16.6 ± 1.0 [ab]	15.2 ± 2.9 [ab]
Micronized to 165 °C	18.7 ± 0.4 [cde]	18.3 ± 0.7 [abc]	17.7 ± 0.2 [ab]	17.1 ± 0.1 [ab]	16.0 ± 1.4 [ab]
16% tempered seeds					
Micronized to 115 °C	18.4 ± 0.2 [cdef]	18.0 ± 0.0 [abcd]	16.9 ± 1.5 [abcd]	15.6 ± 3.0 [abc]	14.2 ± 3.5 [abcd]
Micronized to 130 °C	19.0 ± 0.2 [bcd]	18.7 ± 0.0 [a]	18.1 ± 0.3 [a]	17.6 ± 1.0 [a]	16.2 ± 2.4 [a]
Micronized to 150 °C	18.7 ± 1.0 [bcde]	18.0 ± 1.2 [abcde]	17.2 ± 0.9 [abc]	16.8 ± 0.5 [ab]	16.2 ± 0.0 [a]
Micronized to 165 °C	18.5 ± 0.0 [cde]	17.7 ± 0.2 [bcdef]	17.0 ± 0.4 [abcd]	16.4 ± 0.1 [ab]	15.4 ± 1.0 [ab]
23% tempered seeds					
Micronized to 115 °C	19.3 ± 0.3 [bc]	18.6 ± 0.2 [ab]	18.1 ± 0.4 [a]	17.3 ± 0.3 [a]	15.8 ± 1.7 [ab]
Micronized to 130 °C	19.0 ± 0.2 [bcd]	18.4 ± 0.4 [abc]	17.8 ± 0.8 [ab]	17.4 ± 1.2 [a]	16.4 ± 1.8 [a]
Micronized to 150 °C	17.6 ± 0.2 [fg]	16.8 ± 0.3 [f]	16.2 ± 0.0 [cde]	15.7 ± 0.0 [abc]	14.7 ± 0.3 [abc]
Micronized to 165 °C	18.0 ± 0.1 [ef]	17.0 ± 0.1 [ef]	16.1 ± 0.3 [cde]	15.2 ± 0.6 [bc]	13.9 ± 0.5 [bcde]

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).
Note: for a*, negative values indicate green while positive values indicate red.

Table 4.4 Change of burger yellowness (Hunter b* values) with storage time in retail display case at 4 °C[1,2].

Binder	Day 0	Day 1	Day 2	Day 3	Day 4
No binder	19.2 ± 1.5 ^{de}	18.3 ± 0.8 ^{ef}	17.2 ± 0.6 ^{efg}	16.7 ± 0.9 ^{cde}	17.3 ± 0.9 ^{abcd}
Toasted wheat crumbs	19.8 ± 0.6 ^{bcd}	19.0 ± 0.9 ^{abcde}	18.1 ± 0.6 ^{abcdef}	17.7 ± 1.1 ^{abc}	18.3 ± 1.2 ^{ab}
Non-micronized lentil flour	19.4 ± 1.3 ^{cd}	18.4 ± 1.4 ^{def}	17.5 ± 0.8 ^{defg}	17.4 ± 1.3 ^{abcde}	17.5 ± 1.4 ^{abc}
Lentil flour from Non-tempered seeds					
Micronized to 115 °C	19.9 ± 1.1 ^{bcd}	19.1 ± 2.0 ^{abcde}	18.1 ± 1.2 ^{abcde}	17.2 ± 1.5 ^{bcde}	17.2 ± 1.8 ^{abcd}
Micronized to 130 °C	20.4 ± 1.0 ^{ab}	19.5 ± 0.7 ^{abcd}	18.5 ± 0.0 ^{abc}	17.7 ± 0.5 ^{abcd}	17.7 ± 0.4 ^{abc}
Micronized to 150 °C	20.9 ± 0.4 ^{a}	19.0 ± 0.7 ^{abcde}	18.1 ± 0.4 ^{abcde}	17.9 ± 0.3 ^{abc}	17.6 ± 0.4 ^{abc}
Micronized to 165 °C	20.5 ± 0.6 ^{ab}	19.9 ± 1.5 ^{ab}	19.0 ± 0.9 ^{a}	18.4 ± 1.0 ^{a}	18.5 ± 0.0 ^{a}
16% tempered seeds					
Micronized to 115 °C	20.2 ± 0.2 ^{abc}	19.8 ± 0.9 ^{abc}	18.8 ± 0.0 ^{ab}	18.0 ± 0.4 ^{ab}	17.8 ± 0.1 ^{abc}
Micronized to 130 °C	20.2 ± 0.5 ^{abc}	20.0 ± 1.2 ^{a}	19.0 ± 0.5 ^{a}	18.4 ± 0.6 ^{a}	18.4 ± 0.5 ^{a}
Micronized to 150 °C	19.9 ± 1.5 ^{bcd}	18.7 ± 1.9 ^{cde}	17.7 ± 1.5 ^{cdef}	17.4 ± 1.4 ^{abcde}	17.5 ± 1.2 ^{abc}
Micronized to 165 °C	19.8 ± 0.3 ^{bcd}	18.8 ± 0.5 ^{cde}	17.9 ± 0.4 ^{bcdef}	17.4 ± 1.0 ^{abcde}	16.9 ± 0.4 ^{bcd}
23% tempered seeds					
Micronized to 115 °C	20.0 ± 0.7 ^{bcd}	19.4 ± 1.2 ^{abcd}	18.4 ± 0.9 ^{abcd}	17.8 ± 1.4 ^{abc}	17.3 ± 0.6 ^{abcd}
Micronized to 130 °C	19.8 ± 0.5 ^{bcd}	18.9 ± 0.3 ^{bcde}	18.0 ± 0.0 ^{bcdef}	17.6 ± 0.2 ^{abcd}	17.2 ± 0.4 ^{abcd}
Micronized to 150 °C	18.5 ± 0.4 ^{e}	17.5 ± 1.1 ^{f}	16.7 ± 0.6 ^{g}	16.3 ± 0.7 ^{e}	15.9 ± 0.7 ^{d}
Micronized to 165 °C	19.4 ± 0.8 ^{cd}	18.3 ± 0.4 ^{ef}	17.1 ± 0.2 ^{fg}	16.5 ± 1.3 ^{de}	16.7 ± 0.3 ^{cd}

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).
Note: for b*, negative values indicate blue and positive values indicate yellow.

However, the following treatments showed relatively high a* values (>16.0) on day 4; non-tempered 165 °C, 16% tempered 130 and 150 °C, and 23% tempered 130 °C. When the rate of decline of redness for the 4 days between the initial (day 0) and day 4 were compared, the highest rates were observed for burgers with no binder (2.7 a* value units/day) and toasted wheat crumbs (2.3 a* value units/day). Flours from non-micronized seeds and non-tempered 115 and 130 °C temperature treatments had higher rates of red colour decline (1.3, 1.5 and 1.5 a* value units/day respectively) compared to the other micronizing treatments which had rates between 0.6 and 1.1 suggesting that burgers containing flour from these treatments may have reduced consumer acceptability. Similarly, Der (2010) reported greater retention of redness in raw burgers containing 6% of lentil flour from 15% tempered seeds micronized to 135 °C than burgers containing flour from non-micronized seeds or toasted wheat crumbs, during stimulated retail display storage up to 5 days. These results show that when using lentil flour as a binder in burgers, micronized lentil flour will give better red colour retention than raw lentil flour and therefore, better consumer acceptability.

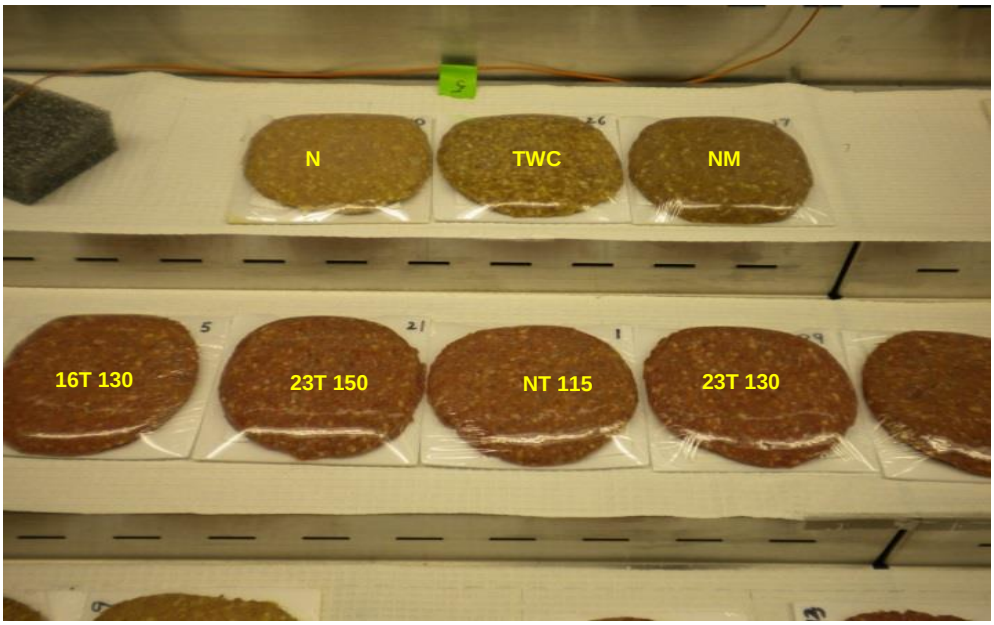

Figure 4.3 Burgers containing different binders in display case at 4 °C on day 4. N: no binder, TWC: toasted wheat crumbs, NM: lentil flour of non-micronized seeds, NT: non tempered, 16T: tempered to 16%, 23T: tempered to 23% and 115, 130, and 150 indicate micronizing temperatures. Pictures were captured using Canon PowerShot SX 700HS camera.

The TBARS value is used as an indicator of meat lipid oxidation (Fernandez et al., 1997). Malondialdehyde, a product of lipid oxidation is the main reactive substance with TBA and in addition to that, other oxidation products including α,β-unsaturated aldehydes and precursors of these substances are also found to be reactive (Fernandez et al., 1997). The results for TBARS on days 0, 2 and 4 for the burgers kept in the retail display case and the burgers for 4 months are shown in Table 4.5. There was no difference in the values of TBARS between the day 0 and 4 month frozen burgers containing lentil flours (from non-micronized and all the micronized seed treatments) but there was a slight increase in TBARS values for the burgers containing toasted wheat crumbs and for the burgers without a binder after 4 months indicating lipid oxidation had occurred during frozen storage.

The burgers without a binder and also with toasted wheat crumbs had higher values for TBARS on days 2 and 4 indicating higher level of lipid oxidation progression than others. All the burgers formulated with flours of micronized lentil seeds showed low TBARS and better oxidation stability of meat lipids than the control or the burger containing raw lentil flour. This could be due to the significant decrease in lipoxygenase activity in lentil flours from micronized seeds as observed in Study 1 (Table 3.7). Hunter a* values of burgers on day 4 showed a negative relationship ($r = -0.84$, $p<0.05$) with the TBARS indicating that oxidation of unsaturated lipids may have affected myoglobin oxidation because of the interrelationship of lipid and oxymyoglobin oxidation. Therefore at the level of lentil flour addition, most likely the compounds released from the flour protected oxidation of oxymyoglobin of meat particles and unsaturated lipids of the cell membrane. In the study by Modi et al. (2003), lower TBARS were observed for buffalo meat burgers incorporated with roasted legume flours (green/black/bengal gram at level of 8% w/w) at 4 °C storage compared to burgers with non-heated binders.

It is still not clear which factors of lentil contribute to the stabilization of meat lipids and oxymyglobin at refrigeration and frozen storage. One of the hypotheses formulated was that the presence of endogenous components of lentil flour provides antioxidant activity and delays the oxidation of unsaturated lipids and oxymyoglobin. Tempering and micronization treatments could have an impact on the compounds of the lentil seed that are responsible for providing antioxidant activity, especially on their availability and effectiveness. Therefore, tempering and micronization treatments could affect the antioxidant properties that lentil flour can exert on oxidizing substrates of the fresh ground beef.

Table 4.5 Thiobarbituric acid reactive substances (TBARS) of burgers in retail display case at 4 °C on days 0, 2 and 4 and frozen storage at -30 °C after 4 months[1,2].

Binder	TBARS (mg malondialdehyde per kg)			
	Day 0	**Day 2**	**Day 4**	**Month 4 (frozen)**
No binder	1.0 ± 0.1 ^{cd}	2.7 ± 0.2 ^{a}	4.1 ± 0.9 ^{a}	1.4 ± 0.3^{a}
Toasted wheat crumbs	1.1 ± 0.0 ^{bc}	2.1 ± 0.3 ^{b}	3.3 ± 0.2 ^{b}	1.4 ± 0.2^{a}
Non-micronized lentil flour	1.5 ± 0.0 ^{a}	1.6 ± 0.0 ^{c}	1.8 ± 0.3 ^{c}	1.5 ± 0.0 ^{a}
Lentil flour from Non-tempered seeds				
Micronized to 115 °C	1.2 ± 0.0 ^{b}	1.3 ± 0.1 ^{d}	1.5 ± 0.1 ^{cde}	1.2 ± 0.0 ^{ab}
Micronized to 130 °C	1.2 ± 0.0 ^{b}	1.3 ± 0.0 ^{d}	1.6 ± 0.1 ^{cd}	0.9 ± 0.0^{ab}
Micronized to 150 °C	0.9 ± 0.1 ^{de}	1.0 ± 0.1^{ef}	1.0 ± 0.1 ^{ef}	0.8 ± 0.2 ^{bc}
Micronized to 165 °C	0.9 ± 0.2 ^{de}	0.9 ± 0.1 ^{ef}	1.0 ± 0.2 ^{ef}	1.1 ± 0.2^{c}
16% tempered seeds				
Micronized to 115 °C	1.0 ± 0.1 ^{cd}	1.1 ± 0.1 ^{de}	1.4 ± 0.3 ^{cdef}	1.0 ± 0.1^{bc}
Micronized to 130 °C	0.9 ± 0.0 ^{e}	0.9 ± 0.0 ^{f}	1.0 ± 0.0 ^{ef}	0.9 ± 0.0^{c}
Micronized to 150 °C	0.8 ± 0.0 ^{e}	0.9 ± 0.0 ^{f}	0.9 ± 0.0 ^{ef}	0.9 ± 0.2 ^{bc}
Micronized to 165 °C	0.8 ± 0.0 ^{e}	1.0 ± 0.2 ^{ef}	1.1 ± 0.2 ^{def}	0.9 ± 0.0^{c}
23% tempered seeds				
Micronized to 115 °C	0.9 ± 0.0 ^{e}	0.9 ± 0.0 ^{f}	1.0 ± 0.0 ^{ef}	0.9 ± 0.0^{c}
Micronized to 130 °C	0.8 ± 0.0 ^{e}	0.9 ± 0.0 ^{f}	0.9 ± 0.1 ^{f}	0.8 ± 0.0^{c}
Micronized to 150 °C	0.8 ± 0.1 ^{e}	0.8 ± 0.1 ^{f}	0.9 ± 0.1 ^{f}	0.9 ± 0.0^{c}
Micronized to 165 °C	0.9 ± 0.0 ^{e}	0.9 ± 0.1 ^{f}	1.0 ± 0.1 ^{ef}	0.9 ± 0.0^{c}

[1] Values are means ± standard deviation.
[2]Means with different superscript within each column are significantly different ($p<0.05$).

4.5.2 Antioxidant activity and potential antioxidative compounds of lentil flour aqueous extracts

The phenolic compounds of lentil are reported as catechins, procyanidins, flavonols, flavones and hydroxycinnamic compounds (Aguilera et al., 2010). In the total phenolic content assay (Table 4.6), the soluble phenolics in aqueous salt solution was determined expecting that it represents the components that become available to meat particles in the burger product. The total phenolic content of untreated green lentil flour extract was low when compared with the values reported in literature mostly due to the aqueous nature of the extract used in the present investigation. Han and Baik (2008) reported 12 mg/g of total phenolics in 80% (v/v) ethanol extracts of lentil which is 3.5 times higher than the highest value obtained for non-micronized lentil flour in the present study (3.4 mg/g). Gharachorloo et al. (2012) reported that significantly different amounts of phenolic compounds from lentil were extracted into different solvents (hexane, methanol and acetone). The levels of phenolic compounds extracted from lentil into different organic solvents or aqueous alcohols are less relevant when lentil flour is used as a binder in fresh meat products.

Of the flour extracts investigated in this study, the phenolic compounds extracted into aqueous salt solutions decreased as the tempering moisture level and micronization temperature increased. Increasing tempering moisture level showed a linear significant relationship with the decreasing total phenolic content ($r = -0.70$, $p<0.05$). Similar reduction in phenolic compounds by thermal treatments was reported in the study by Aguilera et al. (2010) on the phenolics profile and antioxidant properties of lentil (var. *Pardina*). Soaking, cooking and industrial dehydration decreased the catechins, procyanidins, flavonols, flavones and hydroxycinnamic compounds which constituted about 85% of the total phenolic profile. It is thought that thermal treatment decreases the solubility and availability of phenolic compounds due to oxidative condensation or decomposition of thermo labile compounds (Aguilera et al., 2010). Some phenolic compounds are thought to be lost due to leaching or diffusion into the soaking or cooking water (Aguilera et al., 2010). However, the same study showed that hydroxybenzoic compounds, which represented 5% of the identified phenolics of lentil, increased with the thermal treatment implying that all phenolic compounds may not behave in the same way under thermal treatment.

Table 4.6 Total phenolic content and antioxidant activities of NaCl soluble components of lentil flours[1,2].

Treatment	Total phenolics (mg Gallic acid equivalents per 100 g)	Ferric ion reducing antioxidant power FRAP (Fe^{2+} mmol equivalents per 100 g)	Hydroxyl radical scavenging activity (%)	Superoxide radical scavenging activity (%)
Non-micronized	337.2 ± 13.6^{a}	1.5 ± 0.0^{abc}	76.4 ± 1.1^{cd}	10.8 ± 0.5^{a}
Non-tempered				
Micronized to 115 ºC	313.3 ± 27.7^{ab}	1.6 ± 0.1^{a}	75.6 ± 0.4^{d}	8.5 ± 0.2^{bcd}
Micronized to 130 ºC	294.1 ± 20.1^{bc}	1.5 ± 0.0^{abc}	76.8 ± 1.7^{bc}	6.2 ± 0.3^{e}
Micronized to 150 ºC	246.9 ± 11.4^{d}	1.4 ± 0.1^{bc}	73.6 ± 1.9^{f}	6.5 ± 0.3^{e}
Micronized to 165 ºC	217.1 ± 8.6^{ef}	1.4 ± 0.1^{bc}	73.2 ± 0.3^{f}	9.0 ± 0.0^{b}
16% tempered				
Micronized to 115 ºC	278.3 ± 23.6^{c}	1.6 ± 0.1^{ab}	77.0 ± 1.3^{b}	8.2 ± 0.1^{d}
Micronized to 130 ºC	233.4 ± 11.3^{de}	1.3 ± 0.1^{cd}	76.2 ± 0.3^{c}	8.3 ± 0.4^{cd}
Micronized to 150 ºC	200.6 ± 9.0^{fg}	1.3 ± 0.1^{cd}	74.9 ± 1.4^{e}	8.0 ± 0.0^{d}
Micronized to 165 ºC	201.3 ± 16.2^{fg}	1.6 ± 0.3^{a}	78.1 ± 0.3^{a}	8.0 ± 0.0^{d}
23% tempered				
Micronized to 115 ºC	217.6 ± 5.6^{ef}	1.1 ± 0.1^{de}	72.6 ± 0.9^{gh}	8.9 ± 0.4^{b}
Micronized to 130 ºC	167.8 ± 14.3^{h}	1.0 ± 0.0^{e}	71.8 ± 0.8^{h}	8.7 ± 0.4^{bc}
Micronized to 150 ºC	198.4 ± 14.6^{fg}	1.5 ± 0.0^{abc}	72.7 ± 1.8^{fg}	8.0 ± 0.0^{d}
Micronized to 165 ºC	188.4 ± 14.8^{gh}	1.6 ± 0.2^{ab}	67.4 ± 0.9^{i}	5.1 ± 0.2^{f}

[1]Values are means ± standard deviation.
[2]Means with different superscripts within each column are significantly different ($p<0.05$).

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Fernandez-Orozco et al. (2003) reported that antioxidant activity of lentil is mainly due to the phenolic compounds. Huang et al. (2005) categorized total phenolics as a measure of the sample's reducing capacity. Phenolic groups are excellent nucleophiles and as a result they can act as radical scavengers and also chelators of metal ions that induce oxidation (Han & Baik, 2008). Presence of phenolic compounds in micronized lentil flour, although at lower concentrations compared to raw flour, indicates potential antioxidant activity.

The stabilities of red colour and unsaturated lipids observed in beef burgers with lentil flours from micronized seeds as binders were hypothesised as the effects of antioxidant activities exerted by the NaCl soluble components of the flour. Loss of fresh, red meat colour is a result of oxidation of the central iron atom within the heme group of myoglobin, the heme protein responsible for red colour of meat, changing from red oxymyoglobin to brownish metmyoglobin. As reported by Faustman et al. (2010), during oxidation of oxymyoglobin, both superoxide anion and hydrogen peroxide molecules are produced and hydrogen peroxide can react with iron (II) to produce hydroxyl radical which has the ability to facilitate lipid oxidation. Hydrogen peroxide can also react with metmyoglobin to form prooxidative ferrylmyoglobin radical. Reactive oxygen species including superoxide, hydroperoxyl radical and hydrogen peroxide originated by the autooxidation of oxymyoglobin can cause damage to muscle lipids via oxidation reaction (Chaijan, 2008).

There is only a slight change in hydroxyl and superoxide radical scavenging abilities and ferric ion reducing antioxidant capacity of the flour extracts showing that micronization treatments had a minor effect on antioxidant potential of compounds in aqueous extracts (Table 4.6). Although the total phenolic assay showed a difference in the values (a reduction due to micronization), results of three antioxidant activity assays indicated that the antioxidant activity of lentil flours was still relevant and contributed to the stability of fresh meat colour and unsaturated lipids. According to Huang et al. (2005), Folin-Ciocalteu assay (used for the measurement of total phenolics) and FRAP assay are electron transfer reaction based assays for antioxidant activity. Electron transfer based assays measure the reducing capacity of the antioxidant. The H-atom transfer reaction is a key step in radical chain reactions. Therefore, superoxide anion and hydroxyl radical scavenging assays measure the ability of aqueous lentil extracts to mitigate radical chain reactions that accelerate oxidation of unsaturated lipids and its product-mediated oxymyoglobin oxidation. Ferric ion reducing capacity may indicate the ability

of maintaining oxidative stability of the central iron atom of the heme group in myoglobin. The ferric ion reducing capacity of the NaCl soluble components of lentil flour has not changed due to micronization, similar to hydroxyl and superoxide anion radical scavenging activities.

Han & Baik (2008) reported an increase in antioxidant activity by 10% and 36%, respectively for lentil var. *Pardina* and *Crimson* upon cooking. Contrary to these results, Aguilera et al. (2010) reported a significant decrease in the antioxidant activity in lentil (var. *Pardina*) due to treatments such as soaking, cooking and dehydration as observed by oxygen radical absorbance capacity assay (ORAC). According to these studies, the processing conditions of lentil seed seem to have an effect on antioxidant activity, but the results from different studies do not show a consistent increase or decrease.

Results of Study 1 showed lipoxygenase and peroxidase activities of lentil seeds were diminished due to micronization treatment and tempering moisture level of the seed also showed a significant decrease (Table 3.7). These two enzymes can be considered as pro-oxidants, because lipoxygenase induces oxidation of lipids containing 1,4-pentadiene systems containing fatty acids and peroxidases generate peroxide ions. According to the antioxidant activity assays, change in antioxidant potential of lentil flour extracts is minimal as shown by the results of electron transfer and hydrogen atom transfer assays (Table 4.6). However, pro-oxidants such as enzymes that support unsaturated lipid oxidation progression were diminished. These enzyme activities were determined in low ionic strength aqueous extracts; pH 6.9-7.0, 0.05-0.1M phosphate buffer. Therefore, the micronization treatment actually reduces the level of pro-oxidants in lentil that could be available for meat particles rather than elevating the level of compounds that pose antioxidant activity. Reduction of pro-oxidants may allow antioxidative compounds to provide better protection to unsaturated lipids and myoglobin and delay their oxidation.

4.5.3 Distribution of enzyme activities related to lipid oxidation in lentil seed components

Since lipoxygenase and peroxidase activities of lentil salt extracts show possible relationship with improved ability to delay unsaturated lipids and oxymyoglobin it is important to know the distribution of these enzymes in the physical components of lentil seed. Lipoxygenase and peroxidase activities of seed coats and cotyledons of heat treated and non-heat treated lentil seed components show a clear difference in the values as shown in the Table 4.7. The results show that these two enzymes are located mainly in the cotyledon and not in the seed coats and

therefore the removal of seed coat of lentil may not necessarily reduce lipoxygenase and/or peroxidase activities. These results also imply that any treatment to inactivate these enzymes should penetrate to the cotyledons to be effective.

Table 4.7 Enzyme activity of lentil seed components.

Treatment	Enzyme activity			
	Lipoxygenase[1] (10^5 units per gram of protein)		Peroxidase[2] (units per gram of protein)	
	Seed coat	Cotyledon	Seed coat	Cotyledon
Non heat treated	No activity	97.8 ± 5.7	1.4 ± 0.3	138.3 ± 6.1
Heat treated to 115 °C	No activity	23.7 ± 2.1	3.2 ± 0.7	102.4 ± 4.6
Heat treated to 150 °C	No activity	0.2 ± 0.1	2.1 ± 0.8	47.8 ± 5.3

[1]Activity of lipoxygenase was assessed in 0.05 M phosphate buffer (pH 6.9).
[2]Activity of peuroxidase was assessed in 0.1 M phosphate buffer (pH 7.0).

4.5.4 Oxymyoglobin-liposome model study

Lentil flour extracts from 4 seed micronization treatments and non-micronized seeds were used in the model system experiments. Results of this study showed that the addition of lentil flour aqueous extracts caused a decrease of oxymyoglobin content whether the assay system contained lipids or not (Tables 4.8 and 4.9). The calculated values for the rate of oxymyoglobin degradation in the first 5 min of the lentil aqueous extract containing assay mixtures were 2.7 to 4.2 times higher than the autoxidation of oxymyoglobin. Within the first 5 min, the rate of oxymyoglobin degradation was high for all lentil flour extracts and the rates differed according to the seed treatment but after that the degradation rate was fairly low. The largest drop in oxymyoglobin content in the system was observed for non-micronized lentil flour while the smallest drop was observed for the 16% tempered and 150 °C temperature treatment. According to these results, the soluble components that are released by lentil flour added to fresh meat systems can have a direct effect on oxymyoglobin oxidation. Micronization of lentil flour can reduce that effect depending on the conditions used for treatment.

Table 4.8 Rate of change of oxymyoglobin content with time in the absence of lipids[1].

Lentil seed treatment corresponding to the flour extract	Rate of reduction of oxymyoglobin concentration ($\times 10^{-3}$ mg/mL/min)			
	0-5 min.	5-40 min.	40-90 min.	90-150 min.
Control – no binder	58.9	0.4	0.5	0.1
Non-micronized	246.8	1.5	0.2	0.2
Non tempered micronized to 115 °C	205.9	0.2	0.3	3.6
16% tempered micronized to 115 °C	230.4	1.1	0.6	1.8
16% tempered micronized to 150 °C	163.4	0.5	0.1	0.4
23% tempered micronized to 115 °C	196.0	1.2	0.0	3.1

Initial oxymyoglobin concentration 2.5 mg/mL, [1]Values are means of two replicates.

Table 4.9 Rate of change of oxymyoglobin content with time in the presence of lipids[1].

Lentil seed treatment corresponding to the flour extract	Rate of reduction of oxymyoglobin concentration ($\times 10^{-3}$ mg/mL/min)			
	0-5 min.	5-40 min.	40-90 min.	90-150 min.
Control – no binder	320.1	2.6	1.1	0.7
Non-micronized	383.5	0.6	0.0	0.0
Non tempered micronized to 115 °C	368.2	2.1	0.4	0.6
16% tempered micronized to 115 °C	344.2	1.8	0.5	0.6
16% tempered micronized to 150 °C	341.0	1.5	0.7	0.8
23% tempered micronized to 115 °C	355.1	0.4	0.7	0.5

Initial oxymyoglobin concentration 2.5 mg/mL, [1]Values are means of two replicates.

When unsaturated lipids are present in the system, the oxidation process was accelerated compared to the assay without lipids. This was evident from the 5.4 fold rate of oxymyoglobin depletion in first 5 min of the control sample (Tables 4.8 and 4.9). The lentil extracts resulted in higher values for the calculated rate of oxymyoglobin depletion than the control and the non-micronized sample reported the largest value. This indicates that addition of lentil flour components can accelerate oxymyoglobin oxidation in the presence of lipids and the influence may be higher with untreated seed extracts. Presence of unsaturated polar lipids in the model system resulted in a considerable increase in the degree of oxymyoglobin oxidation confirming that lipid oxidation products enhance oxymyoglobin auto oxidation (Yin & Faustman, 1993).

A study by Wanasundara & Pegg (2007) using a similar liposome model system showed that plant binders such as wheat, soy and mustard have a marked effect on the autoxidation of both unsaturated polar lipids and oxymyoglobin. Since the flour extracts or the liposome model system does not contain all the components found in the actual meat system, the incorporation of flour from micronized lentil seed could have affected the oxidation reduction reactions in the meat which favour the slowing down of oxymyoglobin oxidation.

Among the aqueous extracts selected for the model system study, lentil flour of 16% tempered and 150 °C micronized seeds only showed statistically significant difference for the total phenolic content (Table 4.6). When these parameters are considered there are other treatment combinations that resulted in similar values, but their effect on the liposome study showed somewhat lower performance than the lentil flour from 16% tempered and 150 °C micronized treatment in terms of oxymyoglobin oxidation. Therefore, the prolongation of oxymyoglobin oxidation or depletion (related to red color stability of fresh beef) cannot solely be explained by the antioxidant potential of soluble components of lentil flour.

As indicated in Study 1, the activities of lipoxygenase and peroxidase were highly sensitive to moisture-IR heat combination. Lipoxygenase activity is directly related to linoleic acid oxidation and the peroxidases scavenge peroxy radical which facilitate lipid peroxidation. Therefore less activity of these two enzymes may have an effect on lipid oxidation product formation reducing the abundance of oxidation products that accelerate oxymyoglobin oxidation. Since these reactions occur simultaneously in the same system, the final outcome depends on both inhibitory and promoting reactions and responsible components in the same system when other factors such as temperature, pH and illumination are under control. The aqueous extracts of lentil flour from micronized seeds showed decreased activities of these lipid oxidation promoting enzymes (Table 3.7), and also the low protein dispersibility index (PDI) values (Table 3.5) at high temperature treatments indicate less soluble proteins. In legumes, these enzymes are part of the soluble fraction, even for the enzyme assays the preparations were performed in buffers (Section 3.3.7). Therefore less PDI is another indication that solubility of the enzymes are less as micronizing temperature and tempering moisture content goes up. Among all the seed treatments, high residual enzyme activities were observed for lentil seeds treated at 115 °C regardless of the tempering moisture level. In the samples assayed for the model system, only 16% tempered and 150 °C treated sample had residual enzyme activity much less (336 fold less for lipoxygenase and

23 fold less for peroxidase) than others. Although lower content of total phenolics (related to the phenolic compounds that provide antioxidant activity) in the aqueous extract was observed, the antioxidant activity assays showed unchanged capability. Therefore the ability of this lentil flour to retain fresh red colour of beef (Table 4.3 “a” values) may be related to the diminished activities of lipoxygenase and peroxidase compared to others that retained higher level of activity. From these results it is evident that the effect of micronization treatment for delaying of oxymyoglobin oxidation was a combined effect of inactivation of lipoxygenase and peroxidase and maintaining the same level of antioxidant activity of lentil flour. Since these enzymes are localized to the seed cotyledons (Table 4.7), the seed moisture and micronization temperature combinations that reduce their activities are needed for preparing lentil flours suitable for fresh meat product application. The model system can detect the effect of oxymyoglobin in isolation, but in actual meat system some other reactions can also influence the final outcome.

4.6 Conclusions

When the flours from micronized lentil seeds were used in beef burgers, burger redness was maintained and retardation of lipid oxidation were seen compared to the burgers without any binder or those containing toasted wheat crumbs or lentil flour from non-micronized seeds. Maintenance of the red colour of the beef burgers is important since the visual quality is highly valued by the consumers. The inactivation of lipoxygenase in lentil flour due to seed micronization may also have helped to reduce lipid oxidation leading to stabilization of myoglobin. The results showed that the stabilization of colour and lipid oxidation brought about by the addition of flour from micronized lentil seeds was not directly due to the phenolic compounds and antioxidant activities of flours. The model system results provided evidence that the colour stability of the meat systems was brought about by suppression of oxymyoglobin oxidation by the addition of lentil flour from micronized seed. Suppression of the formation of lipid oxidation products is also related to this event. Indirect relationship of lipid oxidation product formation due to oxidative enzyme inactivation seems to have an effect on oxymyoglobin oxidation. These oxidation promoting enzyme activities, particularly lipoxygenase and peroxidase, are localized to lentil cotyledons and micronization temperature and seed moisture level need to be considered for their inactivation.

5. GENERAL DISCUSSION

The overall objectives of studies 1 and 2 were to investigate how different moisture-temperature combinations during micronization of lentil seeds affect properties of lentil flours and the ability of flours from micronized lentil seeds to retain redness and provide stability to unsaturated lipids when used in beef burgers as a binder. It was hypothesised that the changes in lentil flour macromolecules would affect the physico-chemical properties and the minor components would affect lipid and oxymyoglobin oxidation.

This study showed that the tempering moisture levels of the seeds prior to micronization and the micronizing temperatures influenced the physico-chemical and functional properties of the resulting lentil flours. The effects of three seed moisture levels (8% natural moisture level and tempered to achieve 16 and 23% seed moisture levels) and four micronization temperatures (115, 130, 150 and 165 °C) were different on each of the above mentioned properties and also on the performance on fresh beef burger model. Since starch (44% on dry weight basis) and protein (26% on dry weight basis) comprise a large proportion of the lentil seed, the changes in functional properties taking place due to micronization are mainly related to these components. The results showed that high seed moisture level close to 23% prior to micronization was necessary for starch gelatinization. This was further confirmed from the DSC data which showed decreased enthalpy values for endothermic peaks corresponding to starch in flours from seeds tempered to 16 and 23%. Water holding capacity of flours also increased with higher seed moisture level and higher micronizing temperatures most likely due to changes that occurred in the carbohydrate and protein fractions. Decreased protein solubility (as PDI) with increasing moisture levels and micronization temperatures indicated soluble proteins were most likely heat denatured. DSC data supported that with diminished peak of denaturation assigned for proteins. Lipoxygenase and peroxidase activities were reduced or these enzymes were almost completely inactivated in lentil flours from micronized seeds indicating the possibility of long shelf life, but inactivation of lipoxygenase was not dependant on seed moisture level as was the peroxidase activity. Trypsin inhibitory activity, an antinutritional factor which limits the

utilization of lentil, was also reduced in flours. Changes that occurred in these enzyme activities showed they may have become insoluble due to denaturation and were not contributing to the soluble protein fraction. Therefore, lipoxygenase and peroxidase have less ability to cause adverse effects in flours following sufficient thermal treatment.

Some of the outcomes of the flour evaluation are summarized in Table 4.10 from the point of building functionalities into lentil flour, since without these treatments raw lentil flour has its own native functionalities only. By varying tempering moisture level and micronization temperature of seeds, different functionalities can be created in the resulting flour.

Subsequently, lentil flours (from micronized and non-micronized seeds) were incorporated into beef burgers at a level of 6% and the changes in colour parameters and lipid oxidation of the products were studied while keeping the burgers in a retail display case over 5 d. When the burgers without any binder, with lentil flour from non-micronized seeds and toasted wheat crumbs (commercially used binder) were compared, products with lentil flours from micronized seeds showed enhanced red colour retention and suppression of lipid oxidation, factors which are important for the consumer in making a decision on product purchasing. The results showed that all micronized treatments were effective in prolongation of redness and delaying lipid oxidation of burgers compared to the control samples used in the study. However, there were a few treatments which showed these effects more prominently than the others; non-tempered and treated to 165 °C, 16% tempered and treated to 130 and 150 °C, and 23% tempered and treated to 130 °C. Non-tempered and treated to 115 °C and 130 °C treatments showed the least colour and lipid oxidation stabilities. It was clear that most likely the inactivation of lipoxygenase and peroxidase during moisture-heat treatments of lentil seeds were responsible for the prolongation of oxymyoglobin oxidation and lipid oxidation in ground beef.

It was also observed that higher micronizing temperatures around 165 °C gave a roasted aroma to the flours which could change the sensory properties. The flours of those treatments, which showed comparatively high values for oil and water absorption, may retain more liquid in the product. Therefore the textural properties of the cooked product may be affected, if it is assumed that the retained liquids would not be released during cooking. Tempering to 16% and micronizing to 130 or 150 °C treatments could be recommended as the optimum treatments for enhancing burger redness and reducing lipid oxidation. Optimum micronization treatments for

maintaining sensory, textural and water holding parameters of cooked burgers need to be determined in a future study.

Table 4.10 Examples of properties that can be created in lentil flour and the treatment conditions (tempering moisture and micronization temperature) of the seeds that can provide the change.

Flour property	Tempering moisture %	Micronizing temperature °C
• High oil absorbing ability at ambient temperature	23	<130
• High water absorbing ability at ambient temperature	≥16	>150 for 16% tempering >115 for 23% tempering
• Less viscous paste	≥16	>150
• Less soluble protein at neutral pH	No tempering needed	>130
• Contains gelatinized starch	23	>115
• Small particles (over 90% of particles <350 µm)	No tempering needed 16 23	-
• Large particles (over 90% of particles >500 µm)	23	-
• Lipoxygenase inactivated (over 95% inactive)	No tempering needed	>115
• Peroxidase inactivated (over 95% inactive)	Natural moisture (8) 16 23	>165 >150 >115

Although it was anticipated that the retention of burger redness and prolongation of lipid oxidation could be due to enhanced antioxidant activities of flours as a result of micronization, a significant impact was not observed on the total phenolic contents and antioxidant activities of lentil flours that could be effective in similar conditions as in burgers. Total phenolics as

measured in this study represented possible active antioxidative compounds that could be extracted when water and sodium chloride are present around lentil flour particles in the burger system. The improvements observed in burgers could be related to the reduced enzyme activities, particularly lipoxygenase and peroxidase upon micronization. Both these enzymes catalyse oxidative reactions affecting oxymyoglobin and unsaturated lipids. These enzymes are primarily located in the lentil seed cotyledons and treatments should be effective to penetrate into seeds to inactivate them.

Although the results in this study did not show a significant increase in antioxidant activities of lentil flour soluble components which are endogenous, the effect of antioxidant compounds that were generated due to heat treatment or process-induced such as Maillard browning products on unsaturated lipids and oxymyglobin oxidation of lentil-fresh meat systems cannot be completely ignored. In addition to providing antioxidant activity, there may be a possibility of changing oxidation-reduction potential in meat system due to the endogenous and process-induced components in these lentil flours. Further in depth investigations at molecular interaction level on the moisture and infrared heat treatment on lentil may provide clarification to this macro-observation of prolongation of fresh-red meat colour and stability of unsaturated lipids. This would be helpful to the lentil ingredient processor in generating lentil flour suitable for fresh meat product application, because the consumer may prefer to have burgers containing health beneficial lentil which can also be suitable for gluten-sensitive individuals.

6. FINAL CONCLUSIONS

From the results of this study, it can be concluded that varying heat-moisture combinations of the lentil seeds during micronization bring about different functionalities in the resulting flours which could be utilized in a range of food applications. It was observed that the moisture-heat treatment of the lentil affected the fresh meat colour when lentil flour was used as a binder at 6% (w/w) level in beef burgers. Lentil flour from micronized seeds, especially non-tempered and treated to 165 °C, 16% tempered and treated to 130 and 150 °C, and 23% tempered and treated to 130 °C, could delay the red colour deterioration and lipid oxidation of burgers kept in a retail display set-up at 4 °C.

The antioxidant properties of soluble components of lentil flour from micronized seeds are not affected or reduced slightly due to moisture-heat treatments as shown by the results of total phenolics and antioxidant assays. However, reductions in oxidative enzyme activities, particularly lipoxygenase and peroxidase were shown for the soluble fraction of lentil flour from different moisture-heat treatments. Lipoxygenase and peroxidase activities were found to be concentrated in the lentil seed cotyledon and heat treatments which were effective in inactivation of these enzymes were able to provide protection to oxymyoglobin and unsaturated lipids resulting in prolonged redness and lipid stability of fresh meat systems.

7. FUTURE DIRECTIONS

Results of the present investigations provide the basis for developing future studies on lentil utilization in three different directions. One direction is to understand and fine-tune the moisture-heat treatment combination of lentil seed micronization to achieve variations of functional properties in resulting flours. Assessment of complete suite of functional properties of flours including more combinations of seed moisture and surface temperatures achieved in micronization is needed. Emulsifying and gelling properties of the flours which were not investigated in the present study due to less relevance to burger-type product applications should be included in future assessments. Finding out optimum treatment combinations for lentil seed that provide flours with required functionalities for different product applications (e.g. emulsified meat products such as salami and wieners, baked products such as crackers, unleavened breads, batters and coatings for deep frying) is necessary to advance lentil as an ingredient in food product formulation as well as micronization as a value-added processing treatment for lentil ingredient development. In the present study, the burger-lentil application was not assessed at the final consumable product stage. Therefore the effect of lentil flour obtained from different micronization treatments on the performance and sensory acceptability of the final cooked product need to be carried out.

The second direction is to understand the chemical basis of changes occurring in the lentil seed due to moisture-heat treatment. Considerations of macro- and micro components should be done. Even though the results from this study did not show any direct effect of micronization on phenolic compounds present in aqueous salt soluble fraction of lentil flour, the role of non-phenolic compounds and other components of lentil flour on prolongation of colour and lipid stabilities need to be further investigated. The role of oxidative enzymes in accelerating oxymyoglobin and unsaturated lipids and the exact mechanism and optimum level of micronization to achieve the product quality on the basis of chemical changes need to be investigated further.

A third direction would be to expand our understanding of the potential of Saskatchewan grown lentil for fresh meat product applications by examining different varieties, biotypes and

market classes. The present study was conducted on one variety, because the emphasis was on micronization treatment. Available lentil germplasm need to be investigated for the levels of oxidative enzymes (e.g. lipoxygenase, peroxidase), antioxidant potential and other macro- and micro components that are important in functionality variation. This can be done to understand not only the biological variation existing in lentil germplasm but also the effect of environmental factors on these components and functional properties.

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APPENDIX A.

Appendix A 1: Differential scanning calorimetry (DSC) data of lentil flours for the endothermic peak corresponding to starch[1,2].

Seed treatment	T_o (° C)*	T_p (° C)*	ΔH (J/g)*	T_c (° C)*
Non-micronized	61.7 ± 0.0^{c}	66.8 ± 0.1^{e}	2.15 ± 0.19^{bc}	74.1 ± 0.8^{f}
Non-tempered				
Micronized to 115° C	61.3 ± 0.3^{c}	66.6 ± 0.2^{e}	2.52 ± 0.03^{ab}	74.5 ± 0.2^{f}
Micronized to 130° C	61.2 ± 0.1^{c}	66.5 ± 0.2^{e}	2.44 ± 0.18^{ab}	74.7 ± 0.7^{f}
Micronized to 150° C	61.3 ± 0.0^{c}	66.9 ± 0.1^{de}	2.67 ± 0.14^{a}	75.4 ± 0.5^{def}
Micronized to 165° C	61.8 ± 0.1^{c}	68.9 ± 1.5^{d}	2.47 ± 0.02^{ab}	77.7 ± 1.1^{cde}
16% tempered				
Micronized to 115° C	61.8 ± 0.1^{c}	67.1 ± 0.1^{de}	1.98 ± 0.14^{c}	75.4 ± 0.5^{ef}
Micronized to 130° C	62.5 ± 1.4^{c}	68.1 ± 1.8^{de}	1.89 ± 0.38 ^{c}	76.0 ± 0.7^{def}
Micronized to 150° C	65.5 ± 1.2^{b}	71.5 ± 2.1^{c}	1.10 ± 0.30^{d}	78.4 ± 3.2^{bc}
Micronized to 165° C	66.8 ± 2.2^{ab}	73.6 ± 1.2^{ab}	1.05 ± 0.36^{de}	80.5 ± 0.7^{ab}
23% tempered				
Micronized to 115° C	66.1 ± 2.4^{ab}	71.9 ± 1.8^{bc}	0.82 ± 0.04^{de}	77.9 ± 1.3^{cd}
Micronized to 130° C	68.0 ± 1.0^{a}	74.0 ± 1.3^{a}	0.64 ± 0.0^{ef}	81.2 ± 2.2^{a}
Micronized to 150° C	55.9 ± 0.2^{e}	59.6 ± 0.5 ^{f}	0.13 ± 0.06^{g}	64.6 ± 0.1^{g}
Micronized to 165° C	53.3 ± 0.9^{e}	58.9 ± 0.8 ^{f}	0.34 ± 0.03^{fg}	65.7 ± 0.2^{g}

[1]Values are means ± standard deviation.

[2]Means with different superscripts within each column are significantly different ($p<0.05$).

*T_o – onset temperature, T_p – peak temperature, ΔH – change of enthalpy, T_c – concluding temperature.

Appendix A 2: Differential scanning calorimetry (DSC) data of lentil flours for the endothermic peak corresponding to protein[1,2].

Seed treatment	T_o (º C)	T_p (º C)	ΔH (J/g)	T_c (º C)
Non-micronized	80.6 ± 0.2^{d}	85.5 ± 0.4^{d}	0.46 ± 0.09^{a}	91.5 ± 1.6^{d}
Non-tempered				
Micronized to 115° C	80.9 ± 0.1^{d}	85.9 ± 0.5^{cd}	0.49 ± 0.07^{a}	92.1 ± 0.9^{cd}
Micronized to 130° C	80.6 ± 0.5^{d}	85.5 ± 0.2 ^{d}	0.44 ± 0.03^{a}	92.0 ± 0.1^{cd}
Micronized to 150° C	82.9 ± 0.6^{bc}	88.8 ± 1.4^{b}	0.36 ± 0.02^{ab}	95.6 ± 0.9^{ab}
Micronized to 165° C	86.4 ± 0.7 ^{a}	91.1 ± 0.5^{a}	0.15 ± 0.04^{c}	96.1 ± 0.4 ^{a}
16% tempered				
Micronized to 115° C	80.7 ± 0.5^{d}	86.3 ± 0.4^{cd}	0.51 ± 0.03^{a}	93.3 ± 1.1^{bcd}
Micronized to 130° C	81.8 ± 0.6^{cd}	86.7 ± 1.3 ^{c}	0.36 ± 0.07^{ab}	93.7 ± 1.2^{bcd}
Micronized to 150° C	NA	NA	NA	NA
Micronized to 165° C	NA	NA	NA	NA
23% tempered				
Micronized to 115° C	83.6 ± 0.8^{b}	88.6 ± 0.6^{b}	0.16 ± 0.02^{c}	94.0 ± 2.8^{abc}
Micronized to 130° C	NA	NA	NA	NA
Micronized to 150° C	70.6 ± 1.5^{e}	74.8 ± 1.2^{e}	0.21 ± 0.15^{bc}	80.5 ± 0.8 ^{e}
Micronized to 165° C	70.8 ± 0.6^{e}	75.1 ± 0.5^{e}	0.19 ± 0.13^{bc}	80.1 ± 2.3^{e}

[1]Values are means ± standard deviation.

[2]Means with different superscripts within each column are significantly different ($p<0.05$).

*T_o – onset temperature, T_p – peak temperature, ΔH – change of enthalpy, T_c – concluding temperature.

NA – Not applicable because no recognizable endothermic peaks were observed for these treatments.