

Addition of Micronized Black Bean (*Phaseolus Vulgaris*) Flour
Improves Sensory Qualities of Low Fat Beef Burgers

by

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ABSTRACT

Dehulled black beans were micronized at 90°C, 100°C, 110°C, 120°C, 130°C and 140°C; milled to flour and tested for lipoxygenase activity. Non micronized black bean flour was higher in lipoxygenase activity than flours at $\geq 120^\circ\text{C}$ ($p \leq 0.05$). Micronized (100°C, 110°C, 120°C) and non micronized black bean flour was added to low fat beef burgers (6%). C18:3 was significantly higher in the black bean flour samples (raw and cooked). Whole wheat flour had the highest amount of C18:2 in all samples ($p \leq 0.05$). The all beef control was significantly higher in Newton value, drip loss, cook loss and percent shrinkage compared to burgers with binders ($p \leq 0.05$). Ninety-three participants participated in a consumer sensory panel. Results showed higher acceptability of micronized burgers compared to all beef or whole wheat flour controls. This study demonstrated incorporation of black bean flour into low fat beef burgers can improve their physical, chemical and sensory properties.

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DEDICATION

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LIST OF ABBREVIATIONS

ALA- Alpha linolenic acid
ANOVA- Analysis of variance
CFIA- Canadian food inspection agency
CV- Coefficient of variance
CVD- Cardiovascular disease
FACT- Food action rating scale
GC- Gas chromatography
H₂O₂- Hydrogen peroxide
HDL- High density lipoprotein
IR- Infrared
LA- Linoleic acid
LDL- Low density lipoprotein
LOX- Lipoxygenase enzyme
MUFA- Monounsaturated fatty acid
n-3- Omega 3 fatty acid
n-6- Omega 6 fatty acid
O₂- Oxygen
PA- Proximate analysis
PLS- Partial least squares
PUFA- Polyunsaturated fatty acid
RPM- Rotations per minute
SAS- Statistical analysis system
SD- Standard deviation
SFA- Saturated fatty acid

SPSS- Statistical product and service solutions

T2DM- Type II diabetes mellitus

TG- Triglyceride

TPA- Texture protein analysis

WB- Warner Bratzler

USDA- United States department of agriculture

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Chapter 1 – Introduction

It is well known that there is an increase in the prevalence of obesity in Canada; the number of overweight and obese Canadians has more than doubled in the last 15 years (Colman, 2000). The obesity epidemic is now considered a public health crisis. The main chronic diseases directly related to obesity include: cardiovascular disease (CVD), type 2 diabetes mellitus, (T2DM), cancer, gallbladder disease and osteoarthritis (Luo, Morrison, De Groh, & Waters, 2007). There is a relationship between diet and health. Recent studies have shown a correlation between energy consumption, excessive fat intake and obesity (Lissner & Heitmann, 1995). Additionally, high fat intake, particularly saturated fat (SFA), has been associated with atherosclerosis and CVD in humans (Griel & Kris-Etherton, 2006; Lichtenstein et al., 1998). A reduction in SFA and cholesterol intake is now a world-wide recommendation (Health Canada, 2008). One of the main sources of SFA in the human diet is from red meats (Y. Wang & Beydoun, 2009).

Eating well with Canada's Food Guide 2007 recommends 2-3 servings per day of meat and alternatives for adults (Health Canada, 2007). While the majority of Canadians consume adequate amounts of meat, consumption of alternatives such as pulses, are low. Pulses are a good source of dietary proteins and are low in SFA. Health Canada recommends no more than 10% of total energy be from SFA and recommends substituting red meat for seafood or alternatives. The United States dietary guidelines 2010 states "choose a variety of protein foods, which include seafood, lean meat and poultry, eggs, beans and peas, soy products, and unsalted nuts and seeds". They also recommend replacing protein foods higher in solid fats with choices that are lower in solid fats and calories (US Department of Agriculture, 2010). Additionally, Eating Well with Canada's Food Guide 2007 recommends substituting meat for alternatives such as beans, lentils

and tofu more often (Health Canada, 2007). Recommendations for pulse consumption include 1.5 cups (375ml) beans, peas or lentils per week (Hermann & Male, 2011); however, most are not meeting these recommendations. A 2008 study reported that Canadians consumed 23.3 kg of red meat per capita, 11.2 kg of chicken and 6.6 kg of fish per person (Stats Canada, 2011). Data from 1999 showed that on a global scale average pulse consumption was 5.9 kg/capita per year; in the European Union it's averaged around 3.9 kg/capita per year (Akibode & Maredia, 2011). Furthermore, results from the Canadian Community Health Survey showed that only 13% of Canadians consume pulses (peas, lentils, beans, chickpeas) on a given day (Pulse Canada, 2012d).

One of the main reasons consumption may be low in Canada is due to the strong off flavour often associated with pulses and legumes. A food processing technique called micronization may be a solution to this problem. Micronization is a continuous heat treatment that involves using electromagnetic radiation in the infrared region of the spectrum (Arntfield et al., 2001b). This process has benefits in the food industry and has been shown to improve sensory and physiochemical properties of pulses, reduce cooking times and inactivate certain enzymes (Bellido, Arntfield, Scanlon, & Cenkowski, 2003). This process may create a product that can be used as a functional ingredient in foods such as meat products.

Out of the red meat consumed in Canada a large amount of it is in the form of hamburgers. A hamburger is usually made from ground beef, but may also be made from chicken, pork, turkey or bison. Hamburger consumption is on the rise; Canadians reported that 32% ate a hamburger at least once a week. This has increased from 28% in 2009 (MacKenzie, 2011). Beef is an important part of the North American diet and a big part of Canadian agriculture. We are seeing a large consumer demand for healthier, low fat products that of course

taste good. Therefore, if we can incorporate pulses in novel, convenient, and healthy food products we may benefit Canadians by helping to provide a healthier alternative to one of the country's most commonly consumed food.

The hypothesis of this study was:

Micronization of black bean flour will reduce lipoxygenase activity and addition of micronized black bean flour to low fat beef burgers will improve the nutritional composition, sensory and physiochemical properties of the product while retaining palatability for consumers

The objectives of this study were:

1. To determine the rate of lipoxygenase (LOX) activity at different micronization temperatures in black bean flour.
2. To create a beef burger with improved lipid profile through addition of black bean flour (micronized and non-micronized).
3. To assess consumer acceptability of the low fat beef burger with added black bean flour by conducting a sensory analysis study.

1.1 Literature Review

1.1.1 Pulse production: Canada

Pulses have been used as food for more than 10,000 years and are a part of the legume family which includes soy beans, peanuts, fresh peas and beans. A pulse however, only refers to the dried seeds of the plant, such as dried peas, lentils, beans and chickpeas (Pulse Canada, 2012f). Canada has seen a dramatic increase in pulse production since the 1990's and is one of the world's leading producers. Today Canada is the largest producer of peas, primarily green and yellow peas (Pulse Canada, 2012c). Canada also produces vast amounts of red lentils, laird (large green) lentils, beans and chickpeas (Pulse Canada, 2012b).

The Canadian Pulse industry saw its peak production in 2010 at 5.7 tons, with exports exceeding \$2.7 billion in 2011 (Pulse Canada, 2013). Provinces such as Manitoba, Ontario, Quebec, Saskatchewan and Alberta are the major pulse growing regions in Canada (Pulse Canada, 2013).

Manitoba's climate, soil content, agriculture, space, and growing season makes it conducive to pulse production (Government of Manitoba, n.db). It has seen a great increase in production of dry beans: (310,000 acres in 2002), and peas, (175,000 acres of peas per year). As a result, Manitoba now produces approximately 56.8 percent of Canada's total dry bean crop (63% of navy beans and 47% of coloured beans). While Saskatchewan is the greatest; Manitoba is now the third largest pea producing province in Canada (Government of Manitoba, n.da).

*1.1.2 Beans (*Phaseolus vulgaris*)*

Second to soybeans, the common bean (*Phaseolus vulgaris*) is the second most important legume worldwide and is a staple in the Middle East, India, South America and Asia (Xu &

Chang, 2009). On a global scale, The Food and Agricultural Organization (FAO) reported that dry beans are number one in production and consumption worldwide (Akibode & Maredia, 2011). Data showed that in the European Union, beans were the most frequently consumed pulse (46%) (Schneider, 2002). Furthermore a 2010 Ipsos Reid survey on 1100 Canadian households reported that beans are most commonly consumed of all the pulses (Ipsos Reid, 2010).

1.1.3 Black beans

There are many types of beans; however, black beans are eaten most frequently among a variety of cultures. They are a staple to North and South American cuisine, where they are incorporated into rice dishes, burritos, and tacos. Black beans are found in many Asian dishes being fermented, canned, dried, or as black bean sauce. Cuban and Caribbean meals also regularly include this food. USDA data showed that Americans consumed more than ½ lb black beans per person in 2010 and it is one of the fastest growing pulses eaten in the United States (Flipse, 2010).

1.2 Processing Dry Beans

1.2.1 Cleaning and dehulling

All pulse seeds are comprised of a seed coat, the cotyledons and the embryo which account for approximately 7-15%, 85% and 1-4% for the total seed mass respectively (Patil & Sokhansan, 2003). Although the majority of crude fibre is found in the coat, the bulk of nutrients are found in the cotyledons, and the removal of the seed coat, also referred to as dehulling is a common practice with pulses before preparation and cooking.

Certain standards are set before a legume or pulse can be prepared for commercial consumption; this includes some or all of the following: cleaning, separating, splitting, drying, polishing and sorting (USA dry bean council, 2010).

Prior to dehulling common treatments to whole beans include tempering, soaking and heating (Anton, Ross, Beta, Fulcher, & Arntfield, 2008). Dehulling can be performed manually for small amount of seed; however, for large scale commercial dehulling a mechanical device is used. This process has been shown to improve the protein quality, texture, and digestibility, reduce cooking times as well as anti-nutritive factors (Al-Obaidy & Siddiqi, 1981; Anton, Ross et al., 2008).

Dehulling was found to improve water and oil absorption capacity of ten different types of common beans (*Phaseolus vulgaris*) as well as improve gelling, foaming and emulsion properties (Deshpande, Sathe, Salunkhe, & Cornforth, 1982). Proximate analysis showed an increase in protein, ash and fat content of dehulled beans (Deshpande et al., 1982).

Akinjayeju, et al. (2011) studied the effects of manual and mechanical dehulling and compared it to unhulled black beans. Proximate composition, pasting, physical properties and anti-nutritive properties were examined. Both manual and mechanical dehulling results were similar in proximate analysis but were different from all the unhulled components of the analysis except for moisture. The unhulled beans were higher in protein, fat, ash and fibre but lower in total carbohydrate than the dehulled seeds. The functional properties such as water holding capacity and swelling were significantly different in dehulled black beans compared with unhulled beans. This was most likely due to the higher fibre in dehulled seeds. The hulled and dehulled seeds were milled and subsequently made into a black bean cake that was tested in a consumer panel. The scores showed that the black bean cakes made from dehulled seeds were more accepted by

consumers. These results demonstrate that dehulling black beans prior to milling may improve many physical, sensory and nutritive values of this food (Akinjayeju & Ajayi, 2011). These findings are consistent with other studies found in the literature. Moreover, the process of dehulling has also been shown to reduce LOX activity in the seed by up to 50% (Al-Obaidy & Siddiqi, 1981; Deshpande et al., 1982).

1.2.2 Milling

In order for the beans to be converted into flour for commercial use, they must be milled. This involves a number of steps such as grinding, particle size reduction, shifting, and purifying (Limsanquuan & Seiichiro, 2009), with the end result to reduce the particle size. There are generally two types of milling for pulses and legumes: *Impact milling*, in which the particle is fractured after being hit by a hard blunt force and then passed through a rotating assembly with a hammer-type blade. The second common milling process is: *Direct-pressure milling* where the pulse is pinched or compressed between two hard surface areas. This processing uses 1 or 2 rotating bars (USA dry bean council, 2010). The overall particle size and distribution depends on the type of mill used. A Burr mill is commonly used to crack the bean, which aids in the flow rate of the beans as they are processed. Other common mills include the plate mill (where the legumes are ground against two plates); the Wiley mill which uses rotating blades; or the pin mill in which flour is passed through rotor pins. Small scale kitchen mills are often used, for small batches of beans; ie: less than 15kg (USA dry bean council, 2010).

It has been observed that different milling process can affect the functional properties of legumes and grains (Glitso & Knudsen, 1999; Limsanquuan & Seiichiro, 2009; Liyana-Pathirana & Fereidoon, 2007). Limsanquuan et al, (2009) found that the use of a hammer mill for grinding

resulted in a product with higher antioxidant capacity, total phenolic compounds and resistant starch compared to other milling processes (Limsanquuan & Seiichiro, 2009). Furthermore, the anti-nutritive activity of tannins can be reduced in pulses and legumes by processing techniques such as milling (Reddy, Pierson, Sathe, & Salunkhe, 1985).

1.2.3 Micronization

Micronization is an infrared (IR) heat treatment ($\lambda = 1.8\text{-}3.4 \mu\text{m}$), that uses electromagnetic radiation (Arntfield et al., 2001b). It involves exposing an absorbent food source, for a short time on a vibrating bed to electromagnetic radiation in the infrared region of the spectrum. The process has been shown to improve food safety and shelf stability in the pulse industry by improving physiochemical, sensory properties, and reduce cooking times by affecting starch gelatinization (Bellido et al., 2003).

However, while micronization has been shown to improve cooking times; Arntfield et al. (2001) compared two different micronization temperatures of lentils and found that lentils which reached an internal temperature of 138°C had better functional properties than lentils micronized to 170°C . The higher temperature resulted in longer cooking times, a darker colour and a hardened lentil due to moisture loss (Arntfield et al., 2001a). Similarly, Bellido et al. (2006) observed that micronization has been shown to result in a darker colour (lower Hunter L^* values) of black beans (Bellido, Arntfield, Cenkowski, & Scanlon, 2006).

Micronization may improve the nutritional quality of pulses by increasing the protein quality and digestibility as well as reducing anti-nutritive factors (Davis, Arnold, & McCallum, 2002; Khattab, Arntfield, & Nyachoti, 2009; Melicion & Valdebouze, 1977; Metussin, Alli, & Kermasha, 1992). Furthermore, micronization can inactivate LOX enzymes (Der, 2010). LOX

enzymes are often thought to be responsible for causing the off flavour of pulses through the oxidation of certain PUFA. Processing pulse flours (green and red lentil), at micronization temperatures of 130-140°C has been shown to decrease or inactivate LOX activity (Der, 2010).

1.2.4 Lipoxygenase (LOX)

LOX enzymes are non-heme iron containing dioxygenases commonly found in plants, legumes, animals and fungi. Their activity in plants has been observed for almost 60 years (Siedow, 1991). LOX catalyzes the oxidation of PUFA to produce an unsaturated fatty acid and H₂O₂ in the presence of molecular O₂ (Siedow, 1991).

LOX has many physiological benefits to plants; it can aid in their growth, cell development, wound healing, and can act as a pesticide (Loiseau, Vu, Macherel, & Deunff, 2001). However, the hydroperoxide that produces certain alcohols and aldehydes derived from linoleic and linolenic acid can contribute to the off flavours that are often associated with legumes (Kermasha & Metche, 1986). These off flavours can occur through the many stages of storage and processing (Der, 2010).

Presence of LOX varies within different legume species, with soybean being one of the highest and different classes of beans including black beans containing medium levels of LOX (Loiseau et al., 2001). There are two main isoforms of LOX enzymes found in plants designated Type-1 and Type-2 LOX and are based on their pH and specificity for the H₂O₂ substrate. Type-1 is generally found only in soybeans and have an optimum pH 9-10 while Type-2 LOX is the common isoform found in other plant species and has also been shown to catalyse secondary reactions resulting in colour loss in pigments and production of oxydienoic acids (Loiseau et al., 2001). Type-2 LOX enzymes have an optimal pH of 6-7. The optimum pH for LOX activity in

beans ranges from 6.0-9.0 and it becomes inactive at a pH greater than 11 (Kermasha & Metche, 1986).

There are many different methods to determine LOX enzyme activity in legumes which include calorimetric method, manometric method, and spectrophotometric method (Al-Obaidy & Siddiqi, 1981). The presence of other phenolic compounds has been shown to interfere with absorbance reading using the spectrophotometric method; however this method is most commonly used for establishing LOX activity due to its high precision as it allows the reaction to be determined by measuring the product through a change in absorbance at certain wavelengths (Al-Obaidy & Siddiqi, 1981; Anthon & Barret, 2001; Busto et al., 1999; Chang & McCurdy, 1985; V. Kumar, Rani, Pandey, & Chauhan, 2006). It was discovered in 1964 that by solubilizing the fatty acid substrate (usually linoleic acid) with Tween 20 acting as an emulsifier, improved the enzyme activity over a wider range of pH. (Al-Obaidy & Siddiqi, 1981).

1.3 The Health Benefits of Pulses

The health benefits of pulses may be due to many different compounds found in the plants such as fibre, protein, complex carbohydrates, vitamins and minerals, fatty acids, as well as other functional substances (J. W. Anderson & Major, 2002). Pulses are also low in sodium and contain no cholesterol (Geil & Anderson, 1994). Black beans are an excellent source of protein, fibre, calcium, iron, potassium and folate. A complete nutrient profile of black bean flour is shown in Table 1.1.

Table 1.1: Nutrient profile of black bean flour

Nutrient	Unit	Amount per 100g
Energy	kcal	363
Total protein	g	22.99
Total carbohydrate	g	66.38
Fibre	g	14.7
Sugar	g	2.39
Total fat	g	1.51
Saturated fat	g	0.390
Monounsaturated fat	g	0.130
Polyunsaturated fat	g	0.650
Calcium	mg	131
Iron	mg	5.34
Magnesium	mg	182
Phosphorus	mg	375
Potassium	mg	1578
Sodium	mg	5
Folate	ug	473
Niacin	mg	2.080
Vitamin K	ug	6.4

(Adopted from Health Canada, 2012)

1.3.1 Carbohydrate and fibre

Pulses are an excellent source of fibre. Health Canada recommends that men consume 38 g/d fibre and women 25g/d (Health Canada, 2008). However, data shows that most North Americans are not consuming enough. Fibre is associated with cardio-protective benefits by lowering serum cholesterol levels (Duane, 1997). Fibre has been shown to have beneficial protective effects on certain types of cancers (colorectal, breast); furthermore, dietary fibre is inversely associated with T2DM (Verma & Banerjee, 2010). Although fibre content varies between pulses, they are all a high source of fibre averaging about 20g per 100g serving (Case & Fenster, 2011). Fibre also promotes satiety, and helps humans stay full longer. Thus high fibre

intake may also aid in weight management. The total dietary fiber content of black beans is approximately 26%, with approximately 85% being insoluble and 15 % soluble fibre (Hall, 2010).

1.3.2 Protein

For being of plant origin, pulses contain a great amount of protein; roughly twice the amount of other plant products such as wheat, oats, barley and rice (brown and white) (Case & Fenster, 2011). Pulses also contain many essential amino acids (methionine, valine, leucine, lysine, isoleucine, phenylalanine, threonine) (Wang & Daun, 2004), and can become complete proteins when consumed with other plant foods such as corn, cereals and grains. Compared with other bean varieties, black beans contain high amounts of essential amino acids lysine, isoleucine and threonine (Bressani, Marcucci, Robles, & Scrimshaw, 1954). Pulses are an excellent high protein alternative to meat products and may be especially important for people consuming vegetarian or vegan diets.

1.3.3 Fat

Pulses contain about 1-3% fat in total and are low in SFA (Sathe, Deshpande, & Salunkhe, 1984). They are however high in monounsaturated (MUFA) and PUFA such as C18:1 (oleic), C18:2 (LA) and C18:3 (ALA) (Wang & Daun, 2004). As previously discussed, there are health benefits to humans when SFA are replaced with MUFA and PUFA in the diet. ALA is the predominant PUFA in black bean flour (Health Canada, 2012).

1.3.4 Vitamin and minerals

Pulses are a great source of essential vitamins and minerals. They are especially high in iron, potassium, thiamin and folate. For example, pinto beans contain 59% of our daily value of iron, 53% of our daily potassium and 46% of our daily thiamin; per 100g dry (Pulse Canada, 2012a). One hundred g dry chickpeas contain 33% of our daily iron, 32% potassium, and 75% of our daily folate needs based on the % Daily Values (Pulse Canada, 2012b).

Specifically looking at black beans, as shown in Table 1.1, they are high in calcium, iron, folate, magnesium, phosphorus and potassium. One cup of cooked black beans contains 20% of one's recommended intake of iron, 64% of folate, 30% of magnesium and 172% of one's recommended intake of molybdenum (The George Mateljan Foundation, 2013).

1.3.5 Antioxidant properties

Pulses have many antioxidant containing phytochemicals. Antioxidants are substances that scavenge for free radicals in the body, protecting the cells from oxidative damage. This can help protect the body from certain types of cancers, and heart disease (U.S National Library of Medicine, 2013). Antioxidant activity in beans has been reported in the hull, seed coat and tannins of pulses. A study that looked at the effects of pre-hulling treatments on nutritional quality and changes in pinto and kidney beans found great variation in phenolic compounds depending on pre-hulling treatments. Heat treatments were associated with a significant increase in phenols related to antioxidant activity; the greatest amounts of phenols were found in the seed coat (Anton, Ross, Beta, Fulcher, & Arntfield, 2008). Other studies have shown that soaking beans can decrease phenol activity possible due to leeching of water soluble phenolic compounds into the liquid (Abd El-Hady & Habiba, 2003).

Important antioxidants in black beans include anthocyanins, flavonols and flavon-3-ols. Baojun et al. (2009) studied the effect of thermal processing on black and pinto beans. The study found that both steaming and boiling significantly reduced antioxidant activity in both types of beans; with boiling resulting in higher losses (Baojun et al. 2009).

Overall beans are an excellent source of antioxidants. However, since most pulses are processed and cooked before human consumption, the physical and nutritional changes from food processing must be taken into account.

1.3.6 Anti-nutritive aspects

Soaking and cooking pulses in water prior to consumption also affects some of the anti-nutrients in pulses such as phytates, tannins and trypsin inhibitors. These substances can lower nutrient availability by chelating certain compounds (calcium, iron, magnesium, zinc) resulting in decreased absorption, and can inhibit the action of trypsin and pepsin (Vidal-Valverde et al., 1994). Soaking and processing can also reduce flatulence-related substances such as raffinose and stachyose (Anton et al., 2008).

1.4 Pulses and Chronic Disease

Abundant evidence shows that consumption of pulses can have health benefits by helping to prevent or reduce chronic disease.

1.4.1 Diabetes

Diabetes is now considered a global epidemic and the rates of type 2 diabetes mellitus (T2DM) are on the rise. Today more than 9 million Canadians are living with this disease

(Canadian Diabetes Association, 2012). T2DM is associated with poor diet and lifestyle choices; smoking, having high cholesterol, and high blood pressure are also risk factors. The Canadian Diabetes Association recommends reducing the amount of high fat meats, (associated with SFA and cholesterol), and replacing it with lower fat healthier alternatives such as pulses and legumes (Canadian Diabetes Association, 2012). Clinical studies have shown pulses to be associated with improved diabetes control and improved insulin resistance (Jang, Lee, Kim, Park, & Lee, 2001; Rizkalla, Bellisle, & Slama, 2002). The glycemic index measures how fast carbohydrate food sources raise blood sugar; evidence has shown health benefits of consuming lower glycemic foods for improved glucose control (Jang et al., 2001; Rizkalla et al., 2002). Pulses are low on the GI, with black beans ranking about 44 on the scale (Hall, 2010).

A meta-analysis of 41 randomised control studies that examined the effects of pulses on people with or without T2DM was conducted. Results showed that out of 11 studies that looked at pulse consumption alone, fasting blood glucose and insulin were significantly reduced by incorporating pulses into the diet (Sievenpiper et al., 2009). Evidence shows that people living with T2DM may benefit health wise by increasing their pulse consumption.

1.4.2 Cardiovascular disease

Heart disease remains the leading cause of death in Canada and accounts for 29% of all deaths in the country (Heart and Stroke Foundation, 2012). Risk factors for heart disease include high fat diet, obesity, high cholesterol, high blood pressure, diabetes, decreased physical activity, and smoking (Heart and Stroke Foundation, 2012).

Pulse consumption has been shown to decrease the risk of CVD by reducing total and low density lipoprotein (LDL) cholesterol, reducing blood pressure, and assisting in weight

management (Stats Canada, 2011). A meta-analysis was conducted of 11 clinical trials on the effects of pulse consumption (excluding soybeans) on CVD in the past 20 years. The studies differed in type of pulse, preparation methods (cooked, canned, raw), length of time and amount ingested. The meta-analysis showed an overall significant 7% reduction in total cholesterol, 6.2% reduction in LDL cholesterol and a 17% reduction in triglycerides (TG). There was no significant reduction in high density lipoprotein (HDL) cholesterol (J. W. Anderson & Major, 2002).

A more recent meta-analysis evaluated the effect of non-soya legumes on cholesterol reduction. Ten clinical trials were analyzed, all studies were longer than 3 weeks in length, the majority of the subjects were men (70%) and subjects ranged from having, high to normal cholesterol levels. The average reduction in total cholesterol was -0.3 mmol/L, -0.2 mmol/L for LDL and for TG the averaged pooled net reduction was -0.02 mmol/L. This meta-analysis also reported an average increase in HDL cholesterol being 0.02 mmol/L (Bazzano, Thompson, Tees, Nguyen, & Winham, 2011). Furthermore after conducting a systematic literature review of the health benefits of pulse consumption, a report published in 2011 by the Food Regulatory Issues division, a Department of Agriculture and Agri-food Canada concluded that there was a highly consistent affect of pulse consumption on the reduction of total and LDL cholesterol (Agriculture and Agri-food Canada, 2011).

1.4.3 Other chronic diseases

Pulse consumption also may help reduce blood pressure and control weight. A recent study was conducted on the effect of legume intake and metabolic syndrome. The study used food frequency questionnaires to determine food intake. It found that subjects with the highest

legume intake had lower blood pressure, fasting blood glucose levels and highest HDL levels (Hosseinpour-Niazi et al., 2012). Pulses have been shown to play a role in healthy diets and reduce obesity by lowering body mass with increased consumption (Papanikolaou & Fulgoni, 2008). Several mechanisms may be responsible for weight maintenance; high levels of satiation and decreased caloric intake through high fibre, slowing of starch digestion and alterations of gut hormone secretions (Verma & Banerjee, 2010). Overall, there is strong evidence that pulse consumption can have positive cardiovascular effects by improving blood lipids, hypertension, fasting blood glucose, and obesity (Darmadi-Blackberry et al., 2004).

Chapter 2 - Functional Foods: Pulse Products

A functional food is defined as a food that has been modified (fortified or enriched with a specific component) usually with the intent to improve the health quality of the product and to help reduce the risk of chronic disease (Agriculture and Agri-food Canada, 2012). Many pulse flours (peas, beans, chickpeas, lentils) have been used to create functional foods such as baked goods, bread, tortilla, pasta, crackers, beverages, as well as meat products (Pulse Canada, 2012e).

2.1 Effects of Adding Binders to Various Meat Products

As previously discussed, red meat consumption remains higher than any other form of meat or alternative in Canada, and consumption of large amounts of red meat is associated with many chronic diseases. Red meat tends to be high in protein, fat, water, and many vitamins and minerals; however, lacks dietary fibre. Dietary fibre is inversely associated with CVD, cancer, T2DM, and may help with weight management (Verma & Banerjee, 2010). Dietary fibre has been shown to increase cooking yield because of its water and fat binding properties (Fernandez-Gines, Fernandez-Lopez, Sayas-Barberá, & Perez-Alveraz, 2006). One way to help improve the health of Canadians may be in incorporate high fibre foods such as pulses and its flours into red meat products.

Previous studies have looked into the addition of soluble and insoluble fibres into meat products. Oat bran fibre has been successfully added to ground beef and pork sausage (Keeton, 1994). Kumar et al. (2004) reported that addition of 4% barley flour to low fat pork patties had acceptable texture and flavour scores (Kumar & Sharma, 2004). Addition of wheat bran to meatballs improved lipid profiles of the meat by lowering trans-fats and increasing the ratio of unsaturated to saturated fatty acids (Yilmaz, 2005). Fruit fibres such as citrus fruits, alone or in

conjunction with cereal fibres have also been successfully incorporated into meat products which have improved the nitrite levels and nutritional properties of the product while still retaining acceptable sensory properties (Fernandez-Gines et al., 2006).

With regard to legumes and pulses, they are higher in both protein content and fibre than cereals and fruits. They also are lower on the glycemic index and contain more complex carbohydrates (resistant starch and oligosaccharides). Numerous trials have added soy products to meats (Chin, Keeton, Longnecker, & Lamkey, 1999; Muguera, Ansorena, & Astiasaran, 2003; Porcella et al., 2001). Soy has proven to be a good quality substitute due to its low cost and high protein content. However, soy is one of the most common food allergens, and therefore excludes a certain amount of the Canadian population (Canadian Food Inspection Agency, 2012).

Looking specifically at pulses, Muller et al. (2005) were able to effectively substitute beef mince with 0-15% navy beans, chickpeas, mung beans and kidney beans (Muller & Redden V., 1995). Modi and colleagues combined buffalo meat with soya bean, bengal gram, green gram and black gram. The study looked at the effect of including roasted (5 minutes at 150° C) or unroasted legumes in raw and cooked buffalo burgers as well as effects of storage on product quality (4 months storage). All types of flours were acceptable to the burgers with regard to sensory qualities. Soya flour yielded the highest protein content; however, the black gram flour burger had the overall highest yield, lowest shrinkage and lowest fat absorption compared to others (Modi, Mahendrakar, Narasimha Rao, & Sachindra, 2004).

Another study was conducted aiming to create a healthy and cost effective meat product that could be incorporated into national school lunch programs in the United States. Beef patties (20% fat) or pork sausage patties (18% fat) were combined with 23 different pulses at 35%,

42.5% or 50% substitutions. The researchers included a meat, fibre, and soy control product as well. The patties were then frozen at -29°C. Using the light red kidney bean to test nutrient profile, overall there was a reduction in kilocalories, fat, saturated fat and cholesterol compared to both beef and pork controls. As expected, the nutrient properties of each product improved with the higher pulse substitution (35% vs. 50%). Results showed that as fibre content increased, cooking loss decreased with the addition of pulse flour. Navy bean, light red kidney beans and small red beans were the generally most acceptable of all the 23 pulses (Holliday, Sandlin, Schott, Malekian, & Finley, 2011).

Inner pea flours were added to beef patties with 10% and 14% fat content. Sensory, shear force and cooking properties were studied compared to all beef patties with 10%, 14% and 18% fat content. Three different pea fibre formulations were used. All pea formulations had higher cooking yields than the all beef controls and were tenderer. There was no difference in fat retention among pea formulations. After cooking, the moisture content was similar between the 10% all beef and 14% pea flour patties. The patties with the pea formulation had a longer cooking time (18s) than the all beef controls. The authors postulate that this may be due to the pea flours ability to retain fat and moisture (Anderson & Berry, 2006).

Another study examined dehulled green and red lentil seed which was tempered and micronized to 135°C. LOX activity was measured as well as functional and physiochemical properties. Results showed LOX activity was decreased 100% at 135°C micronization temperature, as well water holding capacity was increased upon heat treatment. A decrease in colour was observed in the micronized seed. The flour was then incorporated into low fat beef burgers at 6% and 12% substitution. A toasted wheat crumb and all beef sample were used as controls. A trained and consumer sensory panel was conducted and properties of the burgers

were explored. Micronization improved cooking yield and resulted in significantly less shrinkage with increasing lentil flour. The sensory studies showed greater acceptance in the lentil burgers at 6% substitution compared to the controls and non micronized samples (Der, 2010).

Table 2.1: Addition of binders to meat products found in literature

Authors	Type of binder	Amount	Processing technique	Meat Product
Kumar & Sharma, 2004	Barley flour	4%	Milled	Low fat pork patties
Yilmaz, 2005	Wheat bran			Meatballs
Chin, Keeton, Longnecker, & Lamkey, 1999	Soy	0%-4.4%	Pre-hydrated	Low fat bologna
Muguerza, Ansorena, & Astiasaran, 2003	Soy	15-25%	Pre-emulsified	Pork sausage
Muller & Redden, 1995	Navy, chickpea, mung, kidney bean	0-15%	N/A	Beef mince
Modi, Mahendrakar, Narasimha Rao, & Sachindra, 2004	Soya, Bengal, green, black gram flour	8%	Dehulled, roasted	Buffalo burgers
Holliday, Sandlin, Schott, Malekian, & Finley, 2011	23 different pulses	35%, 42.5%, 50%	Cleaned, tempered, ground	Beef or pork sausage patties
Anderson & Berry, 2006	Inner pea flour	16%	Dehulled	Beef patties
Der, 2010	Lentil flour, wheat crumb	6% and 12%	Dehulled, tempered, micronized	Low fat beef burgers

2.1.1 Meat burgers

A burger is a type of sandwich which often consists of a ground beef patty, but may also include ground chicken, turkey, pork, lamb and bison. A patty is usually placed between two

buns. A burger can be fried, grilled or broiled and frequently comes with condiments such as cheese, ketchup, mustard, pickles and onion (WordIQ, 2010).

According to the Canadian Food Inspection Agency (CFIA), under the Canadian regulations act burgers are allowed to be formulated with fillers (Meat Inspection Regulations, 1990). However, CFIA requires that meat burgers contain a minimum of 13% and 15% total protein in the raw and cooked state, respectively. In addition, a minimum of 11.5% and 13.5% of the protein in the raw and cooked state, respectively, must come from a meat source (Meat Inspection Regulations, 1990). Therefore, these limits must be considered when adding non-meat constituents such as pulse flour to meat burgers. Based on these regulations, various studies have incorporated up to 20% binder in various meat products using flours from high-protein legumes (Hale, Carpenter, & Walsh, 2002; Prinyawiwatkul, McWatters, Beuchat, & Phillips, 1997; Yilmaz & Daglioglu, 2003).

2.1.2 Hamburger consumption

Meat is important for both biological and physiological aspects of appetite and satiety. (Fernandez-Gines et al., 2006). The red meat industry is Canada's single largest employer in the food industry with annual sales of more than \$20 billion. When it comes to meat and alternatives, Canadians consume more red meat per capita than chicken, pork, fish or alternatives (Stats Canada, 2011). Out of red meat purchased by Canadians approximately 35% is in the form of ground beef or hamburger patties. In 2011, 32% of Canadians reportedly ate a hamburger at least once a week, up from 28% in 2009 (MacKenzie, 2011).

Americans currently spend approximately \$134 billion each year on fast food. Out of the items purchased the hamburger is the number one top selling item. On average Americans

consume 13 billion hamburgers each year, totaling about 33.2 pounds of meat per person (American Meat Institute, 2009).

2.1.3 Health effects

It is evident from hamburgers popularity and from the increasing trends that consumers are not going to stop eating red meat. The meat industry is number one in Canada and in the world; however, new research into healthier products is imperative.

Although Canadians consume more hamburgers at home than Americans, the fast food industry is still the largest contributor of hamburgers to consumers. Burgers purchased from fast food outlets are associated with negative dietary images such as being energy dense, high in saturated fat, cholesterol and sodium and low in fibre. All of which are associated with chronic diseases such as CVD, T2DM, obesity, and cancers (Fernandez-Gines et al., 2006).

2.1.4 Texture of food

The texture of foods plays an important role in the overall acceptability of a food product. It includes aspects of food such as toughness, springiness and juiciness (de Huidobro, iguel, Blázquez, & Onega, 2005). When we consume foods, we expect them to feel a certain way in our mouths. Therefore the texture of the food product must be considered in the process of development. Texture analysis is an objective and reproducible way to measure meat quality. Sensory studies have shown meat texture to be one of the most important aspects in the acceptance of foods (Food technology corporation, n.d.).

Two common methods of analysis include Warner-Bratzler (WB) shear force analysis and texture profile analysis (TPA). There are large variations in the two methods. Shear force

analysis (WB) measures the amount of pressure in Newtons (N) that it takes to puncture or shear a food. It produces a force-time curve and peak force is measured (Der, 2010). It is the most commonly used method of measuring texture particularly in raw meat. TPA is another less common method used in the meat industry; however, it has its advantages by being able to assess more varieties of meat such as hardness, springiness, gumminess, adhesiveness and cohesiveness (de Huidobro et al., 2005).

Correlations can be made between texture analysis and sensory aspects of foods. Troutt et al. (1992) looked at the effect of texture and sensory aspects on different fat levels of beef burgers (5-30%). They found that the WB peak shear force was highest in the low fat burger (<5%) and that the cooking temperature also affected peak shear force. Patties cooked to an internal temp of 77°C also had higher WB shear force values than those cooked to 71°C (Troutt, Hunt, Johnson, Claus, Kastner, Kropf, & Stroda, 1992a).

Similarly, another study that examined the sensory quality, shear force and electromyography to determine variations in texture properties of beef also found that an increase in cooking temperature resulted in higher levels of shear force and elasticity. Overall they found a positive correlation between texture and sensory aspects (Mathevon, Mioche, Brown, & Culioli, 1995).

Equally to texture, appearance and flavour play an important role in the overall acceptability of a food product. Objective instrumental testing of foods such as colour analysis, pH, cooking and drip losses can be correlated with subjective organoleptic properties to determine overall meat quality.

2.1.5 Untrained sensory panel with low fat beef burgers

Sensory studies are imperative in the food industry when developing new products and the advantages of these studies for potential market success are well established (Lawless & Heymann, 1998). The purpose of conducting an untrained sensory study, or consumer study is to establish acceptability of a food according to a general audience and to learn what attributes of the food consumers prefer (The Food Technology Centre, 2010).

The atmosphere for sensory testing is important and certain variables must be taken into account such as lighting, temperature of the room, odours, randomized samples, appropriate temperature of the food product and using water to cleanse the palate between samples (Meilgaard, Civille, & Carr, 2007).

In a study of four European countries, it was found that appearance such as fat content and colour were the most important consumer attributes for meat acceptance (Grunert, 1997). Research also shows that demographics can affect consumer choice of meats (Resurreccion, 2004). For example, consumer segmentation by demographics was used in determining acceptability of boar meat. Subjects were asked to evaluate the flavour and odour of 5 different samples of cooked meat. Results showed variation depending on consumer demographics (Font i Furnols, Gispert, Diestre, & Oliver, 2003). Because of variations in age, gender, and ethnicity, that can affect choices, consumer studies can be further analyzed after being categorized into these different subgroups.

Chapter 3 - Materials and Methods

3.1 Experiment 1: Effect of Micronization Temperature on LOX Activity in Black Bean Flour

3.1.1 Black bean flour (processing, milling, micronization, shipping, storage)

One kg of black beans grown in Lethbridge Alberta, were sourced from an independent raw material supplier. The black beans were cleaned, colour sorted, polished and de-hulled at the supplier facility. The black beans were then shipped to Infaready Inc. in Saskatoon, Saskatchewan where they were subsequently micronized at six different temperatures 90°C, 100°C, 110°C, 120°C, 130°C, 140°C. A non-micronized sample of black bean flour was also included.

The micronization process was completed using a laboratory scale infrared heating system (Pilot Scale Micronizer Model # BF2A). The beans were fed onto a vibratory conveyor where they were passed through the infrared burner while being rotated through the conveyer. The time the seeds were processed under the burner was controlled electronically as to allow for different micronization temperatures to be reached while the beans were exiting the conveyer. To ensure targeted temperature ranges were being met, surface temperature was measured periodically (OakTon Infrared thermometers). The micronized beans were then cracked with a burr mill (CS Bell Co. Model # 60CM), and milled into flour with the use of a kitchen mill (K-TEC). The kitchen mill is used for small products, generally less than 15 kg. The reason that the beans were first cracked prior to milling was to aid in flow rate through the kitchen mill.

3.1.2 Control samples

A whole wheat flour control (Roger's 100% whole grain, whole wheat flour) was used in addition to an all beef control for substitution in the low fat beef burgers. The whole wheat flour

was purchased from a local grocery store (Safeway Inc.) The flour was stored at -20 °C for the duration of the project at the George Weston Sensory Research Laboratory (University of Manitoba, Winnipeg, MB)

3.1.3 Lipoxygenase activity in black bean flour

LOX activity was measured in the black bean flour according to the method of Sosulski and Gadan (1988) with some modifications. Crude protein was extracted by adding 0.5 grams pulse flour to 100ml of 0.2M sodium phosphate buffer (Na_3PO_4), pH 6.6. The solution was then slowly stirred for 60 minutes at 4°C. One ml from the solution was then transferred to a microcentrifuge tube and centrifuged (Sorvall/Heraeus Biofuge Pico D-37520, Germany) for 10 minutes at 10000 RPM (9503 x g)

The substrate was prepared by mixing 10 μl linoleic (99.5%) with 10 μl of the Tween-20 (polyethylene sorbitan monolaurate) in a 1 ml tube. Next 0.5 ml of 0.1 N potassium hydroxide (KOH) was added until the solution became clear. Six μl of the substrate mixture was subsequently aliquoted into a microcentrifuge tube along with 994 μl of sodium phosphate buffer (pH 6.6) to create a 0.37 mM linoleate solution.

To prepare the reaction, 2.7 ml of sodium phosphate (Na_3PO_4) buffer, 5 μl of the crude protein, and 0.3 ml of the 0.37 mM linoleate was mixed in a 5 ml centrifuge tube. 300 μl was then aliquoted into a UV plate micro well and read at an absorbance of 234nm using the Powerwave XS™ Microplate spectrophotometer (Biotek Inc). Analysis was completed using Gen 5 2.0 microplate data collection and analysis software (BioTek Inc.). Absorbance was read at 0 min, 1, 2, 4, 7, 12, 17, 27, 37, 47, 60, 70, 80, and 90 minute intervals. One unit of LOX activity was defined as an increase of 0.1 A_{234}/min (Sosulski & Gadan, 1988). Each sample was

tested in triplicate. The mean of three measurements was calculated and reported as the estimated LOX activity for each sample.

3.1.4 Statistical analysis

Statistical analysis was performed using one-way ANOVA. Post hoc tests included the Tukey test. A p-value of ≤ 0.05 was considered significant. SPSS version 17 was used for statistical analysis.

3.2 Experiment 2: Effect of Adding Micronized Black Bean Flour to Low fat Beef Burgers

3.2.1 Raw materials

The black bean flour and wheat flour used for the burger formulations were the same used for Experiment 1. Twenty kg of extra lean ground beef was purchased from Miller Meats, (Winnipeg MB). The 20 kg of beef was ground on February 24th, 2013 and was picked up from the supplier on February 25th, 2013. Sixteen burgers from each sample group were then prepared, frozen and stored at -20°C on that same day according to *Section 3.2.3*. The black bean flour obtained from Infaready Inc. Saskatoon, Saskatchewan arrived November 22, 2012 at the George Weston Sensory Research Laboratory (University of Manitoba) and was stored at 4°C in plastic ziploc bags, away from light and moisture. Six kg of extra lean ground beef was later purchased from the same local butcher (Miller Meats, Winnipeg, MB) on March 18th 2013; it had been ground the previous day. The meat was brought to the George Weston Sensory Research Laboratory was separated into 1 kg bags. Eight burger patties from each sample group were prepared that day; subsequently an additional eight burger patties were prepared according to *Section 3.2.3*. and frozen at -20°C on April 15, 2013

3.2.2 Burger formulations

The burger formulations are summarized in Table 3.1. Six burger formulations were prepared.

1. Control- All-beef
2. Control-Whole wheat flour
3. Non micronized black bean flour
4. 100°C micronized black bean flour
5. 110°C micronized black bean flour
6. 120°C micronized black bean flour

The low fat burgers were weighed to make 140 g patties. Black bean flour was used to replace 6% of the ground meat in extra lean ground beef. All beef and whole wheat flour at 6% substitution were used as controls. The preparation, cooking, and storage of the burgers were all completed at the George Weston Sensory Research Laboratory (University of Manitoba) Faculty of Human Ecology.

Table 3.1: Burger formulations

Sample	Beef %	Beef ¹ g	Flour %	Flour g	Water %	Water g	Salt %	Salt g
Control	88	123.2	0	0	11.1	15.54	0.9	1.26
Flour	82	114.8	6	8.4	11.1	15.54	0.9	1.26

¹Beef from extra lean ground beef

3.2.3 Preparation of the burgers

Equipment used:

Bowls
Containers for water, salt, and flour.
Mixer
Scale
Spoons
Patty burger maker
Spatula
Fork

Each ingredient was initially weighed separately (flour, beef, salt, water) using the *AND* (Model # FX-1000) scale. The meat that had been thawed the previous day at refrigeration temperature (4°C) was removed from the fridge and broken apart gently with a fork. Using a Kitchen Aid hand mixer (Model # K5SS, Made in the USA) the raw beef was pre-blended on low speed for 5 seconds. The water, salt and flour were combined and 1/3 of the liquid/flour slurry was added to the beef and mixed for another 5 seconds. The procedure was repeated twice more each time with 1/3 of the slurry and mixed for 5 seconds. The patties were then formed into a uniform shape approximately 7 cm in diameter and 4 cm high and placed in patty stacker (Starfit) where it was manually pressed down to form a consistent shape. The patties were subsequently labelled and placed in Tupperware containers between 2 pieces of wax paper, and frozen at -20 °C. The following day they were removed from the containers and stored Ziploc™ (Johnson) brand heavy duty freezer bags.

3.2.4 Cooking of the burgers

Before cooking the burgers, three conventional ovens (Frigidaire Electric Range, ES510 Control, Electrolux Canada Corp., Mississauga Ont.) were turned on to broil (550°F) and pre-heated for 15 minutes; oven racks were moved to ensure they were second from the top. Baking pans (45 x28 x 2cm) were lined with aluminium foil (Alcan™) so that no dripping escaped the edges. Pans were labelled with the 3 digit code for each burger sample.

The patties were randomly removed from the freezer and placed onto racks set on the foil covered baking pans. Two patties were placed on each baking pan. The pans were then placed in the oven one minute apart and a timer (Fisher Scientific) was set for 9 minutes. When the timer reached 9 minutes the patties were removed from the oven and checked to see if they had

reached an internal temperature of $55 \pm 2^\circ\text{C}$. The patties were then flipped and the baking pan was rotated before being placed back in the oven for another 5 minutes. When the time was up the temperature was again taken to ensure the patties had reached an internal temperature of $73 \pm 2^\circ\text{C}$. If they had not reached the desired internal temperature they were put back in the oven for an additional 2 minutes. The cooking times for the patties were approximately 14 ± 2 minutes and approximately 14 ± 4 minutes for those that contained binder and no binder respectively. The patties were then wrapped in heavy duty aluminum foil (Alcan™) and allowed to cool to room temperature.



Figure 3.1: Cooking of the low fat beef burgers (George Weston Sensory Research Laboratory)



Figure 3.2: Cooked product (low fat beef burger with added black bean flour)

3.2.5 Proximate composition of the black bean flour

Proximate analysis (PA) of the black bean flour was conducted at Central Testing Laboratory Ltd. (Winnipeg, MB). Two hundred and fifty g of micronized (120°C) and non-micronized flour was provided for testing. Percent moisture (AOAC method 930.15), ash (AOAC 923.03), crude fibre (AOCS Ba6a-05), crude protein (AOAC 990.03), and fat (AOCS Am 5-04) were measured. Total carbohydrate was calculated from the difference.

3.2.6 Proximate composition of the low fat beef burgers

PA of cooked black bean enriched burgers was conducted at Central Testing Laboratory Ltd. (Winnipeg, MB). Three patties from each sample group were cooked, cooled and stored as described in *Section 3.2.4*. A total of 200 g was provided for analysis. Percent moisture, ash,

crude fibre, crude protein and crude fat as well as the carbohydrate calculation was conducted according to the methods described in *Section 3.2.5*.

3.2.7 Statistical analysis

Statistical analysis was conducted using Microsoft Office Excel (2007). The mean, standard deviation and coefficient of variance (CV) were calculated for the different samples.

3.3 Instrumental Analysis

3.3.1 Shear force analysis

Shear force of the burger patties was conducted according to the Warner Bratzler Shear force testing method with some modifications (Chrystall et al., 1994). The burgers were cooked according *Section 3.2.4*, wrapped in heavy duty aluminum foil (Alcan™) and stored in Styrofoam holding containers for 15 minutes. The foiled wrapped patties were then transferred onto cooling racks and allowed to cool to room temperature for approximately one hour.

The Lloyd Texture measuring unit (Lloyd Texture Measuring Instrument, Model L1000R, Mississauga Ont.) was calibrated to 1-1000 N. The oval edges were cut from the burgers to produce a square shape. The patties were then cut into four pieces 1 cm in width and 5 cm in length. A piece of meat was then placed into the V shaped WB device attached to the Lloyd Texture Measuring Instrument with the top of the patty facing up. The maximum shear force was recorded in N using Test Loop Version 3 Software.

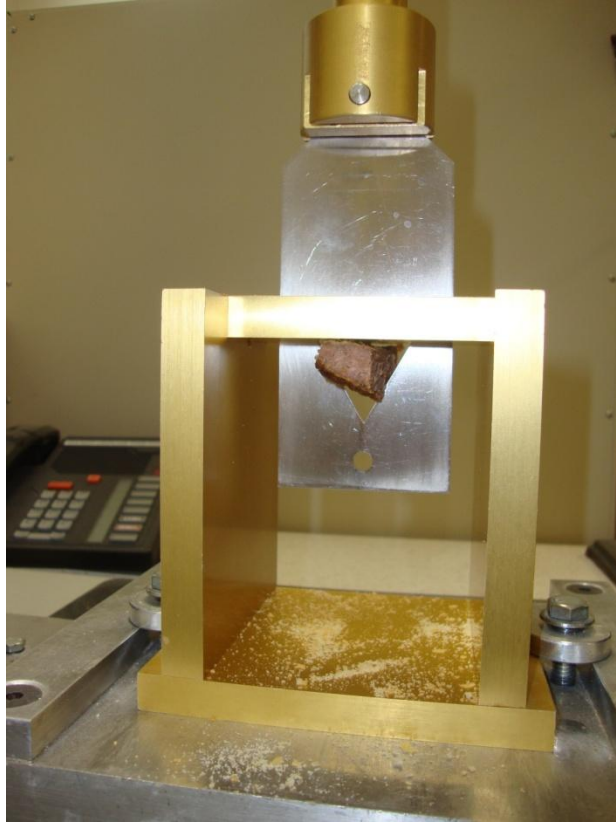


Figure 3.3: Lloyd Texture measuring unit with a Warner Bratzler shear device attachment

3.3.2 Lipid extraction of black bean flour

Lipid extraction was performed at the St. Boniface Research Centre (Winnipeg, MB) and was conducted according to the method of Folch et al. (1957) with some modifications (Folch, Lees, & Sloane-Stanley, 1957). Six samples (1 g) from the whole wheat and black bean flour groups (non-micronized black bean flour, 100°C, 110°C and 120°C micronized black bean flour) were weighed for analysis (Denver Instrument, Model # APX-200). Ten ml of 0.025% CaCl was added to the sample. The samples were then homogenized (Tissue Tearor Model 985370 Biospec Products Inc.) for 3 minutes. Thirty ml of chloroform methanol (2:1) was then added to each sample. They were vortexed (VWR mini-vortexer, Model M-3000, USA) for 1 minute and centrifuged at 2000 RPM (Eppendorf, Model # 5804R) for 10 minutes. The samples were

subsequently evaporated using a Pierce Reacti-Vap Evaporating Unit (Model 18780, Rockford IL) and stored overnight at -20°C . The following day samples were taken out of the freezer and allowed to thaw. One ml of toluene was added to the samples to allow lipids to dissolve, the mixture was then transferred to a 10 ml glass culture tubes and 1.2 ml of metholonic HCl was added. The samples were placed in a water bath at 80°C for one hour and allowed to cool for 10 minutes before addition of 1 ml reverse osmosis water and 1 ml hexane. Samples were then stored at -20°C until ready for gas chromatography.

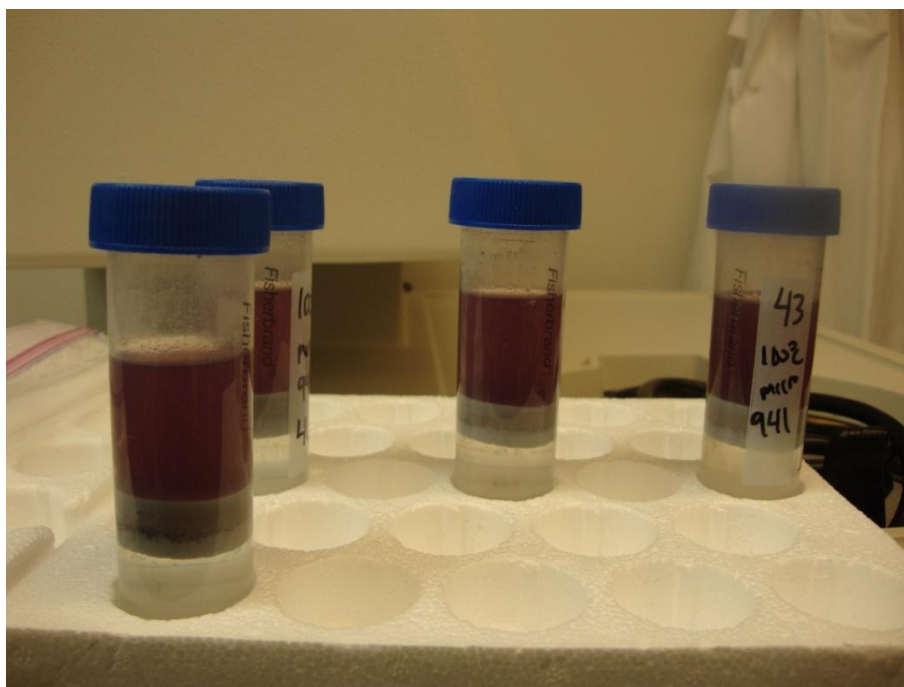


Figure 3.4: Lipid extraction of black bean flour

3.3.3 Lipid extraction of low fat beef burgers (raw and cooked)

Similarly to the black bean flour, lipid extraction of the low fat burgers was conducted according the standard Folch method (Folch et al., 1957) with modifications. Six samples (1 g) from each group (all beef, whole wheat flour, non-micronized black bean flour, 100°C , 110°C

and 120°C micronized black bean flour) were weighed (Denver Instrument, Model # APX-200) and placed in 50 ml centrifuge tubes. Lipid extraction was conducted in the same manner as described in *Section 3.3.2*. Percent total lipid was calculated as follows:

$$\% \text{ total lipid} = (\text{weight of lipid} + \text{container}) - (\text{container weight}) / \text{sample weight} * 100$$

3.3.4 Lipid analysis

Gas chromatography (GC) was conducted at the Richardson Centre for Functional Foods and Nutraceuticals (University of Manitoba, Winnipeg, MB). Methylated samples were separated on a Varian WCOT Fused Silica CP-SELECT FAME column (100m X 0.25 mm diameter and 0.25 µm film thickness; Varian Canada Inc., Mississauga, Ontario) using a Varian 450 GC with a flame ionization detector. The column was operated at 130°C for 2 minutes; the temperature was then raised to 175°C at 25°C/minute, held for 25 minutes, raised again to 240°C at 3°C/minute, and held for 10 minutes. Total run time was 60.47 minutes and the samples were run with a 20:1 split ratio and column flow of 0.8ml/min. (Injector temperature 270°C. Detector temperature 290°C). Hydrogen was used for the carrier gas.

3.3.5 Colour analysis of raw burgers

Colour analysis of the raw burgers was conducted on a Hunterlab miniscan instrument (Hunter Associates Laboratory Inc. Reston, VA). Illuminant D65/10° was used as the standard observer angle. Prior to measurement the machine was calibrated to a white tile measuring L*, a* and b* values. The raw patties were removed from the freezer (-20°C) and placed in re-sealable plastic bags at refrigeration temperature (4°C) for approximately 18 hours. The patties were removed from 4°C and allowed to "bloom" for 30 minutes. Three measurements from each

patty were taken on different surface areas that were covered by a clear glass plate. Two patties from each sample group were measured.



Figure 3.5: Colour analysis of raw beef burgers

3.3.6 Colour analysis of cooked burgers

Colour analysis of the cooked burgers was conducted using the same methods as *Section 3.3.5*. The patties were cooked according to the methods described in *Section 3.2.4* and allowed to cool to room temperature. Three measurements from each patty were taken on different surface areas that were covered by a clear glass plate. Two patties from each sample group were measured.

3.3.7 pH

The pH of low fat beef burgers was conducted using the method of Troutt et al. (1992)

with some modifications (Troutt, Hunt, Johnson, Claus, Kastner, Kropf, & Stroda, 1992b). The pH meter (Oakton Model 35624-35, Oakton Instruments, Vernon Hills, IL.) was initially calibrated to 5 and 10 pH. One hundred g of filtered water was placed into a mini food processor bowl (Cuisinart Canada). Ten g of raw, thawed, low fat ground beef was added. The samples were blenderized for 1 minute, strained through a strainer to remove fat fibres and stirred immediately. The pH electrode was then placed in 25g of the sample and read for two minutes. All samples were tested in duplicate.



Figure 3.6: pH analysis of low fat beef burgers

3.3.8 Cook loss

Cook loss of the patties was completed at the George Weston Sensory Research Laboratory (University of Manitoba). Aluminum foil (Alcan™) was measured to fit a baking pan (45 x28 x 2cm), labelled with the appropriate test code, and weighed using the AND (Model # FX-1000) electronic balance before being placed on the cooking pan. Heavy duty aluminum foil (Alcan™) was also labelled with the patty codes and weighed AND (Model # FX-1000). Weight of the raw patties was then recorded. The diameter and thickness of the patties was then measured in cm using a Fisher Scientific measuring apparatus. Both the diameter and thickness were randomly measured in three places. The patties were then cooked according to *Section 3.2.4*, with the exception of one patty being cooked per oven. The patties were then subsequently wrapped in labelled heavy duty foil (Alcan™) and placed in Styrofoam holding containers. After 15 minutes the patties were removed from the Styrofoam holding containers and allowed to cool to room temperature. Once the patties were cooled they were once again weighed. All cook loss samples were tested in quadruplicate. The percent cook loss was then calculated according to the formula:

$$\% \text{ Cook loss} = \frac{\text{weight of raw patty} - \text{weight of cooked patty}}{\text{weight of raw}} * 100.$$

3.3.9 Drip loss

Determination of drip loss was performed simultaneously with cook loss. Preparation was conducted as described in *Section 3.3.8*. Aluminum foil (Alcan™) was measured to fit a baking pan (45 x28 x 2cm), labelled with the appropriate 3 digit test code and weighed using the AND (Model # FX-1000) electronic balance; before being placed on the cooking pan. The patties were then cooked as described in *Section 3.2.4*, with the exception of one patty being cooked per oven.

The baking pans were then removed from the oven and allowed to cool to room temperature. At this time the racks were removed from the baking pans and the foil containing the drip loss was weighed. All drip loss samples were tested in quadruplicate. The percent drip loss was then calculated according to the formula:

$$\% \text{ Drip loss} = \text{weight of cooked patty} / \text{weight of drip loss} * 100.$$

3.3.10. Percent Shrinkage

The percent shrinkage of the low fat beef patties was conducted at the Weston Sensory Food Research Lab at the University of Manitoba and was performed simultaneously with cook loss and drip loss. The frozen raw patties were weighed and the diameter and thickness were measured according to *Section 3.3.8*. They were then cooked as described in *Section 3.2.4*, with the exception of one patty being cooked per oven. The patties were then subsequently wrapped in labelled heavy duty foil (Alcan™) and placed in Styrofoam holding containers. After 15 minutes the patties were removed from the Styrofoam holding containers and allowed to cool to room temperature for 1 hour. Once the patties were cooled to room temperature they were subsequently weighed *AND* (Model # FX-1000). The percent shrinkage was calculated according to the following formula:

$$\% \text{ Shrinkage} = [(RBT - CBT) + (RBD - CBD)] / (RBD + RBT) \times 100$$

Where:

RBT = Raw Burger Thickness

CBT = Cooked Burger Thickness

RBD = Raw Burger Diameter

CBD = Cooked Burger Diameter

3.3.11 Statistical analysis

Statistical analysis for all instrumental analysis (shear force, pH, lipid analysis, colour analysis, cook loss, drip loss and % shrinkage) was conducted using one-way ANOVA. Post hoc tests included the Tukey test. A p-value of ≤ 0.05 was considered significant. SPSS version 17 was used for statistical analysis.

3.4 Experiment 3: Consumer panel study of low fat beef burgers with added black bean flour

3.4.1 Consumer study

Ninety three volunteers were recruited to participate in a study on the acceptability of low fat beef burgers with added black bean flour. Participants were recruited from staff and students at the University of Manitoba, St. Boniface Research Centre and the general public. The only exclusion criterion was if the participants had a food allergy of any kind which was confirmed by a questionnaire (Appendix E). The study was performed between April 1st to April 9th, 2013. Subjects were required to judge the burgers on organoleptic properties such as appearance, flavour, aroma, texture and overall acceptability on a 9 point hedonic scale. The food action rating scale (FACT) (Schutz 1965) was another method used to determine how often consumers would eat the burgers. They selected from the following categories: 9 = I would eat this everyday; 8 = I would eat this very often, 7 = I would eat this frequently; 6 = I like this and would eat it now and then; 5 = I would eat this if available but wouldn't go out of my way; 4 = I do not like this but would eat it on occasion; 3 = I would hardly ever eat this; 2 = I would eat this only if there were no other food choices; 1 = I would eat this only if forced. The judges responded to how much they liked or disliked various sensory attributes (Appendix B). All who participated were given a small honorarium.

3.4.2 Burger treatment groups

The burger formulations used for the consumer study are outlined in *Section 3.2.2*. The meat samples were cooked according to *Section 3.2.4* depending on the number of volunteers scheduled for the session. After the burgers were cooked they were then cut into 6 uniform triangle pieces wrapped in heavy duty aluminum foil (Alcan™) and held in Styrofoam holding containers until the participants were ready. Approximately 12 g of each sample was placed into plastic serving cups consistent with each sample code and delivered to the consumers. Overhead fluorescent light was used and filtered water was available to cleanse the palate between samples. Participants were seated in individual stations equipped with a computer with Sensory software (Sensory Integrated Management System, Morristown NJ, 2000).



Figure 3.7: Preparation of low fat beef burgers for sensory study



Figure 3.8: Consumer panellist cubicle for sensory study

3.4.3 Ethics approval

Participants were recruited according to the Joint-Faculty Research Ethics Board and the University of Manitoba (Appendix A). An email was sent out to potential participants (Appendix C). All participants were required to complete a consent form and questionnaire (Appendix D & E).

In order to maintain confidentiality, all participants were assigned a number and the data was recorded anonymously. All the data related to personal information was stored in a locked filing cabinet for up to 5 years or until the data is published, after which all information will be destroyed.

3.4.4 Statistical analysis

Statistical analysis for the consumer testing was conducted using SAS Statistical system software (2003), Version 9.2 (Statistical Analysis System, Cary, NC). Four-way ANOVA was conducted using a mixed method model with judge (J) as the random effect and burger formulation (F), age, (A) and gender (G) and the fixed effects. Two-way interactions between gender by age, formulation by gender, and formulation by age was also analyzed. Post hoc tests included the Tukey test. A p-value of ≤ 0.05 was considered significant.

3.4.5 Partial least squares regression analysis

Partial least squares regression (PLS) was used to analyze associations between sensory attributes, instrumental results and the observations. Correlations between the X variables, (sensory attributes of the consumer panel) and Y variables (physical and chemical properties) along with the six sample formulations (observations.) were analyzed using XLSTAT 2012 statistical software. A biplot figure using PLS was generated to show a relationship among all attributes with significant results ($p \leq 0.05$).

Chapter 4: Results and Discussion

4.1 Experiment 1: Effect of micronization temperature on LOX activity in black bean flour

4.1.1 Lipoxygenase activity of black bean flour

Estimated LOX activity for the black bean flour is presented in Figure 4.1. LOX is responsible for catalyzing the oxidation of molecular oxygen. The hyperperoxides it produces result in the formation of many aldehydes and alcohols thought to be responsible for the off-flavours in pulses and legumes. These off flavours are a by-product after the plant has been harvested and processed (Sessa, 1979). The rate of enzyme activity may have a significant impact on storage times and stability of black bean flour. To examine the effect of micronization on LOX activity the black bean flour was measured before and after micronization process.

Overall a trend was observed, as micronization temperature increased, LOX activity decreased. LOX activity ranged from 1.3×10^5 units/ml to 35×10^5 units/ml. As shown in Figure 4.1., non micronized black bean flour had the highest estimated LOX activity (35×10^5 units/ml). It was significantly higher than that in the 120°C, 130°C and 140°C micronized flour (18×10^5 units/ml, 6×10^5 units/ml and 1.3×10^5 units/ml, respectively). The micronization process has a significant effect on LOX activity, and it can become almost completely inactivated in black bean flour at temperatures of 140°C. Since pulses contain high amounts of PUFA, the potential for oxidative rancidity is great and decreasing or inactivating LOX enzymes may result in potential consumer and market benefits.

Variations in LOX reported in legumes and pulses in the literature is possibly the result of different analytical methods, substrate preparation, pH values, and protein extraction conditions (Chang & McCurdy, 1985; Kermasha & Metche, 1986). Although the temperature that LOX enzymes become inactivated vary depending on the type of legume, our findings are consistent with the literature. It has been reported that high heat temperatures of $\geq 80^{\circ}\text{C}$ can denature LOX in various pulses (Walker & Kochhar, 1982). Der (2010), found that micronization temperatures of 135°C decreased LOX activity by 100 fold in both dehulled green and red lentil. LOX eactivity was also significantly decreased at a micronization temperature of 100°C in soya beans (Žilić, Šobajić, S. S.' Mladenović-Drinić, S. D.' Kresović, B. J., & Vasić, 2010).

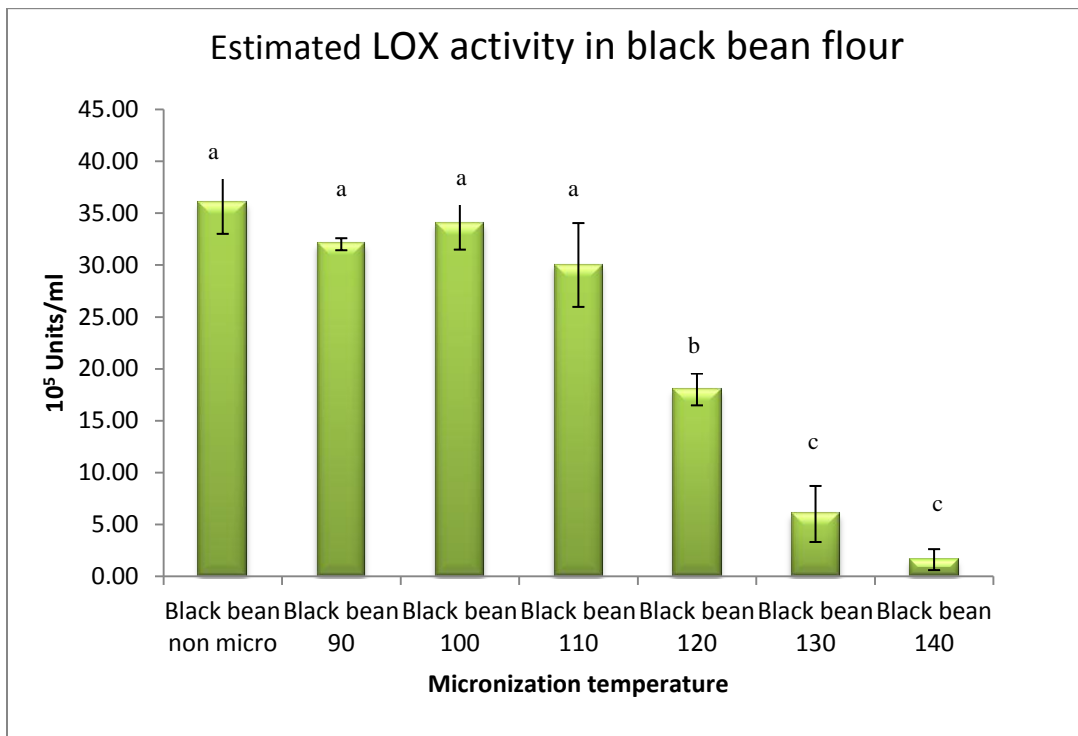


Figure 4.1 Estimated LOX activity of micronized and non micronized black bean flour Data are means and standard deviation, $n=3$, Values with the same letter are not significantly different ($p \leq 0.05$)

4.2 Experiment 2: Effect of adding micronized black bean flour to low fat beef burgers

4.2.1 Proximate composition of black bean flour

Proximate composition of the black bean flour is presented in Table 4.1. The means with standard deviation (SD) and coefficient of variance (CV) is presented in Table 4.2. For the purpose of this study 200g of non-micronized black bean flour and 200g of 120°C micronized flour was analyzed and compared. Moisture content was lower in the micronized flour (5.93%) compared to non-micronized flour (6.79%). The results are similar to what has been published in the literature with the exception of moisture and carbohydrate which were both lower in the black bean samples (Berrios, Swanson, & Adeline Cheong, 1999). These results may be due to the effect of heat treatment, length of storage time or that the beans for this study were not tempered prior to the micronization process

The micronization process has been shown to improve the water holding capacity of other pulse flours (green and red lentil). Similarly with other pulses, moisture content in large green lentil was shown to decrease in micronized (6.8% -5.7%) and non-micronized (9.6%-8.6%) flour over a one year period (Der, 2010). A study on the differences in PA in untreated and heat treated black beans observed no change in moisture content after heat treatment up to 121°C. Additionally no change in moisture content was observed after nine months' storage (Molina, Baten, Gomez - Brenes, King, & Bressani, 1976). Conversely, after two years of storage, it was found that moisture content of black beans decreased from 9.25% to 8.32% (Berrios et al., 1999). Protein content was slightly higher in the micronized flour (23.64%), than non-micronized flour (23.07%), which is consistent with the literature (Berrios et al., 1999). No major difference was observed in carbohydrate, (61.63% vs. 61.75%), fat (1.59% vs. 1.5%). or

ash content (3.84% vs. 3.90%) respectively between non-micronized and micronized black bean flour. In order to compare the variability of two or more data sets, the CV is often used. CV is the percentage variation of a mean and the higher the percentage the greater the variability (UCLA Statistical Consulting Group, 2013). The bean flours had the highest CV for moisture (9.56%), indicating the highest degree of variation. CV for carbohydrate was lowest 0.14%.

4.2.2 Proximate composition of low fat beef burgers

Three different patties were used for PA equaling 250 g. For the purpose of the study, only cooked burger samples were analyzed. The PA is displayed in Table 4.1. The means with SD and CV is presented in Table 4.3. The all beef control had the lowest moisture content (53.99%) compared with the whole wheat and black bean flour. The whole wheat flour had the highest moisture content (58.98%) and the black bean flours ranged from 56.06% -58.82%, with the non micronized sample having higher moisture compared to the micronized samples. Protein content was higher in the all beef control (30.70%) compared to the whole wheat flour (24.17%), non-micronized black bean flour (23.05%) 100°C (24.62%), 110°C (23.41%) and 120°C (24.58%). This study used 6% substitution of black bean flour into low fat beef burgers. It has been observed that increasing the amount of other pulses (red and green lentil) from 6% to 12% substitution in low fat beef burgers decreased the moisture and protein content of the burgers (Der, 2010).

Another study on the addition of 20% soy legume or vegetables (peas and carrots) to lean beef burgers ($\leq 20\%$ fat), found that the textured soy sample had significantly higher protein (19.20%) than the all beef control or vegetable sample (Kassem & Emara, 2010). It was thought that the whole wheat flour binder would result in lowest protein content than the black bean flour; however, this was not the case in these samples.

Total fat content increased in the cooked burgers, which is consistent with the literature (Der, 2010; Kassem & Emara, 2010). This is most likely due to moisture and drip loss. The all beef control has the highest total fat content (15.49%) and the whole wheat flour control had the lowest (10.46%). Among the micronized burger samples, the 120°C micronized burgers had the lowest lipid content (11.18%) and the 100°C burger had the highest (12.20%). Ash content was lower in the all beef control (1.69%). There was no major difference in ash content among all other samples with added binders. The greatest CV in the burger formulations was fibre (93.41%) and carbohydrate, (52.11%). PA results showed the range of fibre to be 0.12 (all beef) and 1.35 (100°C micronization). The range of carbohydrate in the burgers was 0.00 (all beef) to 5.17 (120°C micronized flour).

Table 4.1: Proximate composition of black bean flour and burgers with various binder formulations^a

Component	Black Bean Flour		Burgers (cooked)					
	Non-micronized	120°C micronized	All beef	Whole wheat flour	Non-micronized	100°C micronized	110°C micronized	120°C micronized
Moisture (%)	6.79	5.93	53.99	58.98	58.82	56.06	57.87	56.48
Protein (%)	23.07	23.64	30.70	24.17	23.05	24.62	23.41	24.58
Carbohydrate (%) ^b	61.63	61.75	0.00	3.62	3.83	3.54	4.4	5.17
Fiber (%)	3.10	3.31	0.12	0.69	0.11	1.35	0.26	0.5
Fat (%)	1.59	1.50	15.49	10.46	12.12	12.20	11.83	11.18
Ash (%)	3.84	3.90	1.69	2.10	2.10	2.25	2.25	2.10

^a Expressed as dry weight basis (dwb)

^b Calculated from the difference

Table 4.2: Coefficient of variance of non micronized and micronized black bean flour.

Component	Mean \pm SD¹	Coefficient of Variance (%)
Moisture (%)	6.36 \pm 0.61	9.56
Protein (%)	23.36 \pm 0.40	1.73
Carbohydrate (%)	61.69 \pm 0.08	0.14
Fibre (%)	3.21 \pm 0.15	4.63
Fat (%)	1.55 \pm 0.06	4.12
Ash (%)	3.87 \pm 0.04	1.10

¹Values are mean \pm SD of non-micronized and 120°C micronized black bean flour

Table 4.3: Coefficient of variance among 6 burger formulations

Component	Mean \pm SD¹	Coefficient of Variance (%)
Moisture (%)	57.03 \pm 1.91	3.34
Protein (%)	25.09 \pm 2.82	11.24
Carbohydrate (%)	3.43 \pm 1.79	52.11
Fiber (%)	0.51 \pm 0.47	93.41
Fat (%)	12.21 \pm 1.73	14.20
Ash (%)	2.08 \pm 0.21	9.87

¹Values are mean \pm SD of 6 burger formulations

4.3 Properties of burgers

4.3.1 Lipid composition of black bean flour

4.3.1.1 Total lipid content of black bean flour

The total lipid content of the black bean and whole wheat flour is presented in Figure 4.2. The whole wheat flour control had the highest fat content (2.52%) and was significantly higher than all of the black bean flours which ranged from 1.91% to 2.03% regardless of micronization temperature. On average most pulses contain approximately 1-3% fat in total. These results demonstrate that micronization temperature up to 120°C do not affect total lipid content.

4.3.1.2 Fatty acid content of black bean flour

The individual fatty acid content of the whole wheat and black bean flour is presented in Table 4.4. Palmitic acid (C16:0) is a common SFA and it was significantly higher in the whole wheat flour compared to the black bean flours (19.80% versus 16.29-17.60%). The 120°C micronized black bean flour had the significantly lowest amount of C16:0 (16.29%). Stearic acid (C18:0) was significantly higher in the non micronized black bean sample (3.34%) compared to the micronized samples and whole wheat flour; while C18:0 in whole wheat flour was significantly lower compared to all other samples (1.65%). C18:1 (oleic acid) is a common fatty acid in vegetables and meats, and is referred to as an omega-9 fatty acid. The whole wheat flour and non micronized black bean flour had the highest content of C18:1 (15.55% and 15.37% respectively) and was significantly higher than all other samples. The 120°C micronized sample had the lowest content of oleic acid (13.34%).

C18:2(n-6) (LA) is one of two essential PUFA, meaning we cannot synthesize it on our own and we must obtain it from exogenous sources. The whole wheat flour control had significantly higher amounts of linoleic acid than all the black bean samples (54.55% vs 24.54-26.90%) respectively. Although the n-6 content of the black bean flours was almost half of the amount of whole wheat flour, the 120°C micronized flour had the second highest quantity of n-6 FA (26.90%), and it was significant. Furthermore the non micronized black bean flour had the least amount of n-6 (24.54%) and was significantly lower than all the other black bean flours. C18:3 (ALA) is another essential PUFA that is found abundantly in plants. The health benefits of ALA have been previously discussed. Pulses, including black beans are a rich source of ALA. Similar to n-6 fatty acid, the 120°C micronized sample had the highest amount of ALA (34.73%) and was significantly higher than all other samples. Out of the black bean flours, the non

micronized sample had the lowest amount of ALA (31.51%), and the differences were significant. Comparable to the n-6 fatty acid content of black bean flour, a trend was observed; as micronization temperature increased the amount of LA and ALA increased. It may be postulated that this has to do with the inactivation of LOX enzyme at high micronization temperatures. PUFA constitute the majority of fatty acids in black bean flour, therefore the potential for oxidative rancidity is great. Although micronized chickpea flours were not explored, Sosulski et al. (1988), observed the same relationship among different chickpea cultivators and LOX activity. The researchers proposed that the inactivation of LOX enzymes by heat treatment may improve shelf life and stability of chickpea flours, and therefore may increase the potential to be used in food products (Sosulski & Gadan, 1988). Whole wheat flour is not a major source of n-3 PUFA (Health Canada, 2012), and contained significantly less compared with all black bean samples (3.55% vs. 31.51%-34.73%).

Studies have demonstrated cardioprotective effects when SFA was replaced with MUFA or PUFA in the diet (Keys, 1970). PUFA have significant cardioprotective benefits on humans (Kris-Etherton & Yu, 1997). It is recommended that a healthy diet contain 1-4:1 n-6:n-3 PUFA; however, the typical western diet associated with high red meat consumption contains a ratio closer to 10-20:1 n-6:n-3 PUFA. Thus, reducing the n-6:n-3 PUFA along with improving the PUFA:SFA ratio in red meat could also improve quality of life from a population prospective. The 120°C micronized black bean flour had a significantly higher PUFA:SFA (2.9:1), which makes sense as it had the highest content of total PUFA (61.78%) and lowest amount of SFA (21.59%). Non-micronized black bean flour had the lowest PUFA:SFA (2.3:1). There was no significant difference among the 100°C, 110°C micronized black bean flour and the whole wheat flour. Due to the large quantity of n-6 in the whole wheat flour, and small amount of n-3, it was

observed that it had a significantly higher n-6:n-3 compared to the bean flours (14:1). There was no significant difference among the black bean flours; the n-6:n-3 ranged from 0.77:1 - 0.79:1.

Wheat flour or wheat crumbs is a common binder used when making ground beef patties. However, results show that black bean flour has a superior fatty acid profile; it's higher in ALA, and has a greater PUFA:SFA and n-6:n-3. Furthermore, by inactivating LOX activity by the micronization heat treatment it reduces the availability of the enzyme to oxidize the vast amount of PUFA in the flour. Therefore it has the potential to benefit consumers if it was used in place of wheat products in low fat beef burgers.

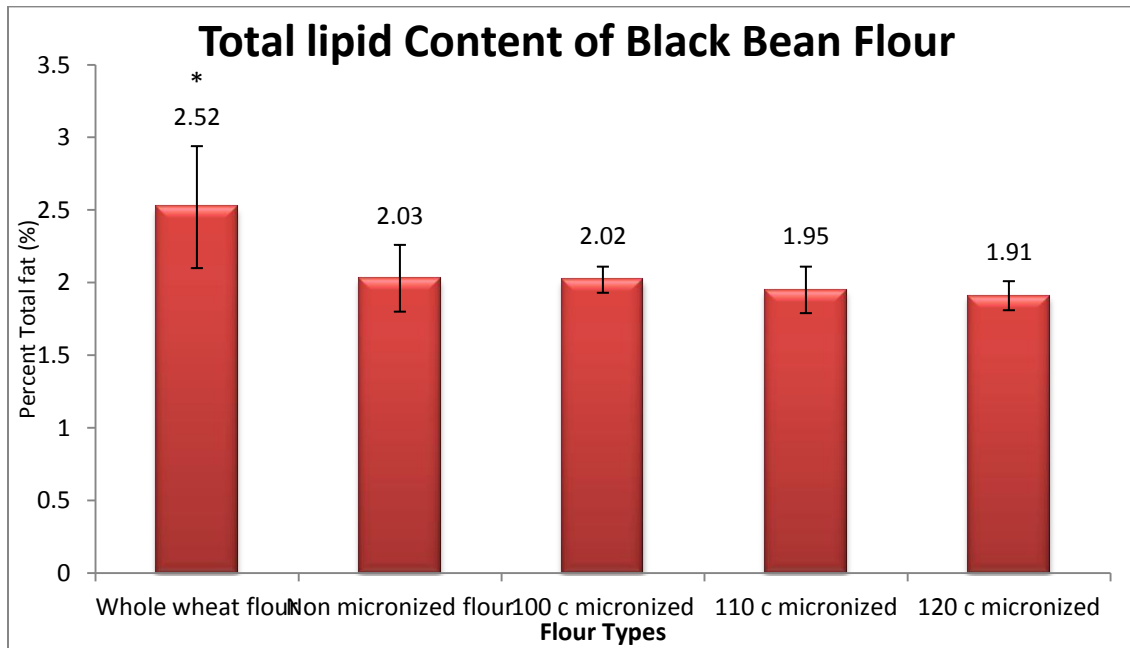


Figure 4.2: Total lipid content of black bean flour
* = Significantly different from other flour types

Table 4.4: Fatty acid profile of whole wheat and black bean flour¹

Fatty Acid Type	Flour Type				
	Whole wheat flour	Non-micronized	100°C micronized	110°C micronized	120°C micronized
C16:0	19.80 ^{a2} ± 0.13	17.60 ^b ± 0.45	17.05 ^b ± 0.40	17.49 ^b ± 0.25	16.29 ^c ± 0.15
C18:0	1.65 ^c ± 0.05	3.34 ^a ± 0.36	2.76 ^b ± 0.06	2.75 ^b ± 0.07	2.57 ^b ± 0.06
C18:1	15.55 ^a ± 0.17	15.37 ^a ± 0.60	13.83 ^{bc} ± 0.25	14.00 ^b ± 0.11	13.34 ^c ± 0.05
C18:2 (n-6)	54.55 ^a ± 0.13	24.54 ^d ± 0.60	26.03 ^c ± 0.16	26.22 ^c ± 0.30	26.90 ^b ± 0.09
C18:3 (n-3)	3.55 ^d ± 0.04	31.51 ^c ± 0.95	32.80 ^b ± 0.51	32.77 ^b ± 0.43	34.73 ^a ± 0.13
PUFA:SFA	2.54 ^b ± 0.03	2.30 ^c ± 0.14	2.51 ^b ± 0.08	2.53 ^b ± 0.11	2.86 ^a ± 0.03
n-6:n-3 ³	14.18 ^a ± 0.24	0.78 ^b ± 0.14	0.79 ^b ± 0.01	0.79 ^b ± 0.01	0.77 ^b ± 0.00
Total SFA	23.04 ^c ± 0.24	24.46 ^a ± 0.91	23.42 ^{ab} ± 0.46	23.47 ^{ab} ± 0.78	21.59 ^c ± 0.30
Total MUFA	15.55 ^a ± 0.17	15.37 ^a ± 0.60	13.82 ^{bc} ± 0.25	14.00 ^b ± 0.24	13.34 ^c ± 0.05
Total PUFA	58.40 ^b ± 0.14	56.07 ^c ± 1.49	58.83 ^b ± 0.64	59.41 ^b ± 0.70	61.78 ^a ± 0.28

¹Values are means ± standard deviation

²Means with the same letter were not significantly different

³Values are for total amount of n-6 and n-3 in samples (not all data shown)

4.3.2 Lipid composition of raw low fat beef burgers

4.3.2.1 Total lipid content of raw low fat beef burgers

The total lipid content of the raw black bean burgers is presented in Figure 4.3. There was no significant difference in total lipid content among any of the formulations. The average lipid content was 7.8% and thus all burgers were <10% total fat meeting the requirements to be considered a low fat burger. The highest lipid content was in the 100°C micronized patty at 8.9%. In this study 6 samples (1g each) were taken from each formulation to account for the variations in lipid content throughout ground beef. However these results show that substituting low fat beef burgers with 6% black bean flour does not affect total lipid content in raw patties.

4.3.2.2 Fatty acid content of raw low fat beef burgers

Individual fatty acids of the raw low fat beef burgers are described in Table 4.5. C16:0 was significantly lower in the 110°C micronized flour (26.31%) compared to all other formulation. Stearic acid (C18:0) is one of the most common SFA found in animals (Encyclopedia Britannica, 2013). The all beef control had the highest amount of C18:0 (15.07%) and was significantly higher than the 110°C micronized sample (13.45%). All of the black bean flour formulations had lower C18:0 compared to the controls. C18:1 was highest in the 110°C micronized sample (38.80%) and was significantly higher than the whole wheat flour control (36.72%). The whole wheat flour control had the highest amount of C18:2(n-6) (3.45%) and was significantly higher than the non-micronized and 100°C micronized black bean flour formulations (1.94% and 2.60%) respectively. In general, whole wheat flour contains more n-6 than black bean flour (Health Canada, 2012). The difference in n-6 content among the micronized and non-micronized black bean formulations may be due to the fact that as LOX enzyme activity decreased with increasing temperature; there are fewer enzymes to act on the substrate to oxidize the PUFA. There was significant difference in C18:3 (n-3) between all of the black bean formulations, regardless of micronization processing, and the all beef and whole wheat control. There was also significant difference between the 120°C micronized samples which had the highest amount (1.05%) and the other black bean formulations. Furthermore there was a trend among the black bean formulations, as micronized temperature increased, n-3 (ALA) content increased. Similarly to n-6 PUFA, it maybe postulated that inactivating the LOX enzyme through the micronization process may improve the n-3 content due to fewer enzymes to oxidize this PUFA. Arachidonic acid (C20:4) is another n-6 fatty acid. It was significantly higher in the all beef control compared with all other samples (0.73% vs. 0.28%-0.48%). C20:4 is

predominantly found in red meat. These results show that substituting 6% black bean or whole wheat flour may significantly affect the amount of this fatty acid.

The PUFA:SFA ratio in the patty formulations ranged from 0.01-0.12:1 and are presented in Table 4.5. The only significant difference was in the non-micronized sample which had a ratio of 0.01:1. The non-micronized samples also had the lowest amount of total PUFA which again may be explained by the fact this sample had the highest LOX activity. The whole wheat flour control had the highest n-6:n-3 ratio (4:1) and was significantly higher than the non-micronized and 120°C micronized samples (2.5:1 and 2.6:1) respectively. All samples including the whole wheat flour however, fell into the recommended range of 1-4:1 n-6:n-3. Total SFA was significantly higher in the all beef and whole wheat flour controls (47.75% and 47.01%) respectively, compared with the 110°C micronized flour sample (44.82%). The 110°C micronized flour had the highest MUFA (38.80%) and the all beef control had the highest total PUFA content (5.35%) and was significantly different than the non-micronized sample (4.04%).

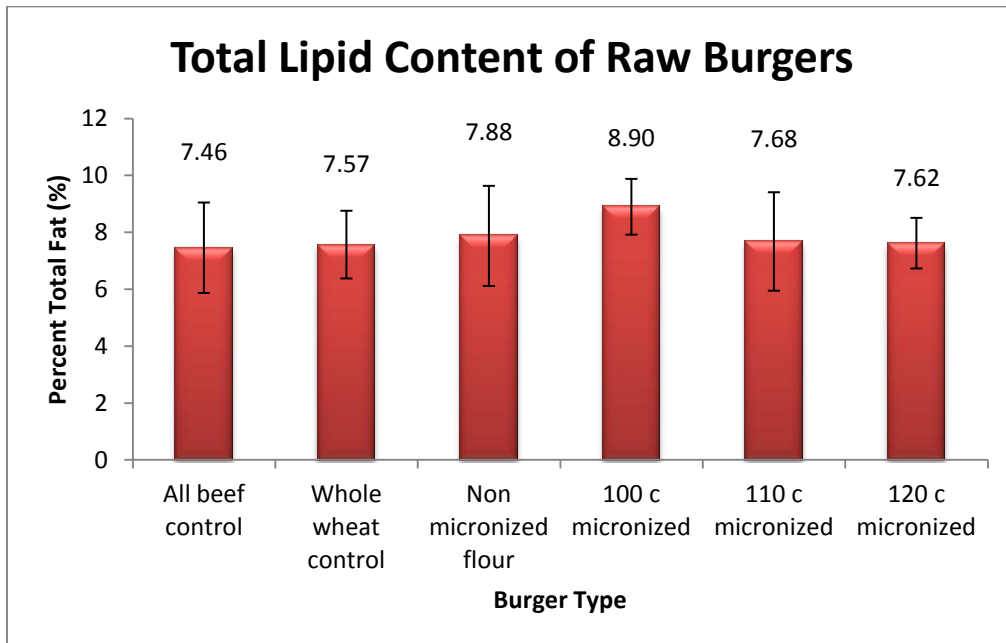


Figure 4.3: Total lipid content of raw low fat beef burgers

Table 4.5: Fatty acid profile of raw, low-fat beef burgers with added pulse flour¹

Fatty Acid Type	Burger Type					
	All Beef	Whole Wheat Flour	Non-micronized	100°C micronized	110°C micronized	120°C micronized
C16:0	27.58 ^{a2} ± 0.44	27.34 ^a ± 0.25	27.20 ^a ± 0.29	27.28 ^a ± 0.86	26.31 ^b ± 0.53	27.05 ^{ab} ± 0.26
C18:0	15.07 ^a ± 0.98	14.48 ^{ab} ± 0.84	14.40 ^{ab} ± 0.70	14.05 ^{ab} ± 0.24	13.45 ^b ± 1.09	13.75 ^{ab} ± 0.60
C18:1	37.08 ^{ab} ± 1.29	36.72 ^b ± 0.69	38.32 ^{ab} ± 0.99	37.63 ^b ± 1.09	38.80 ^a ± 1.25	37.22 ^{ab} ± 0.51
C18:2 (n-6)	2.74 ^{abc} ± 0.16	3.45 ^a ± 0.33	1.94 ^c ± 0.91	2.60 ^{bc} ± 0.24	2.68 ^{abc} ± 0.22	3.07 ^{ab} ± 0.52
C18:3 (n-3)	0.51 ^c ± 0.03	0.51 ^c ± 0.03	0.73 ^b ± 0.07	0.81 ^b ± 0.08	0.84 ^b ± 0.07	1.05 ^a ± 0.17
C20:4	0.73 ^a ± 0.13	0.34 ^b ± 0.28	0.42 ^b ± 0.05	0.48 ^b ± 0.09	0.46 ^b ± 0.07	0.28 ^b ± 0.23
PUFA:SFA	0.11 ^a ± 0.01	0.12 ^a ± 0.01	0.01 ^b ± 0.02	0.11 ^{ab} ± 0.01	0.11 ^a ± 0.01	0.12 ^a ± 0.02
n-6:n-3 ³	3.77 ^a ± 0.26	4.0 ^a ± 1.25	2.51 ^b ± 0.87	2.93 ^{ab} ± 0.12	2.93 ^{ab} ± 0.1	2.61 ^b ± 0.31
Total SFA	47.74 ^a ± 1.25	47.01 ^a ± 0.94	46.74 ^{ab} ± 0.71	46.54 ^{ab} ± 1.22	44.82 ^b ± 1.67	45.88 ^{ab} ± 0.57
Total MUFA	37.08 ^{ab} ± 1.29	36.72 ^b ± 0.69	38.32 ^{ab} ± 0.99	37.63 ^{ab} ± 1.09	38.80 ^a ± 1.25	37.22 ^{ab} ± 0.51
Total PUFA	5.35 ^a ± 0.38	5.70 ^a ± 0.62	4.04 ^b ± 0.98	4.89 ^{ab} ± 0.46	4.98 ^{ab} ± 0.35	5.51 ^a ± 0.63

¹Values are means ± standard deviation²Means with the same letter were not significantly different³Values are for total amount of n-6 and n-3 in samples (not all data shown)

4.3.3 Lipid composition of cooked low fat beef burgers

4.3.3.1 Total lipid content of cooked low fat beef burgers

Total lipid content of the cooked burger formulations is presented in Figure 4.4. All formulations had an increase in total lipid after cooking ranging from 8.6%-13.97%. The all beef control had the highest amount of total lipid (13.97%) and was significantly higher than the other formulations. The larger amount of lipid in the all beef control is most likely due to the fact that this sample had significantly higher drip loss, lower pH and less moisture compared to all the other formulations. Since drip loss is primarily composed of water and some fat, particularly SFA (Campbell & Turkki, 1967). These findings are consistent with another study that found an increase in total lipid content 16.3% vs. 19.1% after cooking ground beef (Campbell & Turkki,

1967). Conversely, a different study observed a decrease in crude fat after broiling beef patties. The lipid content was reduced from 18.1% raw, to 10.0% cooked (Janicki & Appledorf, 1974).

4.3.3.2 Fatty acid content of cooked low fat beef burgers

Individual fatty acids of the cooked burgers are shown in Table 4.6. There was no significant difference among C16:0 among the samples which ranged from 26.1% -28.2%. There was no significant difference in the levels of C18:0 among the formulations. The values ranged from 12.84% (non-micronized sample) to 13.98% (120°C micronized sample). Similarly, there was no significant difference in C18:1 in any of the formulations; however, the 120°C micronized samples had the lowest amount (34.29%). The whole wheat flour control had the highest amount of C18:2 n-6 after cooking (5.46%) and was significantly higher than the other samples which ranged from 3.68%-4.34%. C18:3 (n-3) was greatest in the 120°C micronized sample at 1.45%, and was significantly higher than that in the all beef, whole wheat and non-micronized black bean flour (0.48%, 0.65% and 1.10% respectively). Similar to the raw patties, a trend in n-3 fatty acid content was observed; as micronization activity increased, n-3 content increased, most likely due to the lesser amount of LOX. There was no significant difference among the levels of C20:4 in any of the samples.

The amount of PUFA is related to the amount of lipid oxidation in cooked meats. The PUFA:SFA ratio was highest in the whole wheat flour control (0.2:1) and was significantly higher than all other samples with the exception of the 120°C micronized flour sample. The amount of n-6 increased after cooking in all of the samples. The n-6:n-3 FA ratio was highest in the whole wheat flour control and was significantly greater than that in all other samples. These results were expected as we observed in the raw samples that whole wheat flour contained significantly larger amounts of n-6 PUFA. There was no significant difference among total SFA

or MUFA among the samples, however, total PUFA was highest in the whole wheat flour (8.68%), but, was only significantly different from the all beef control.

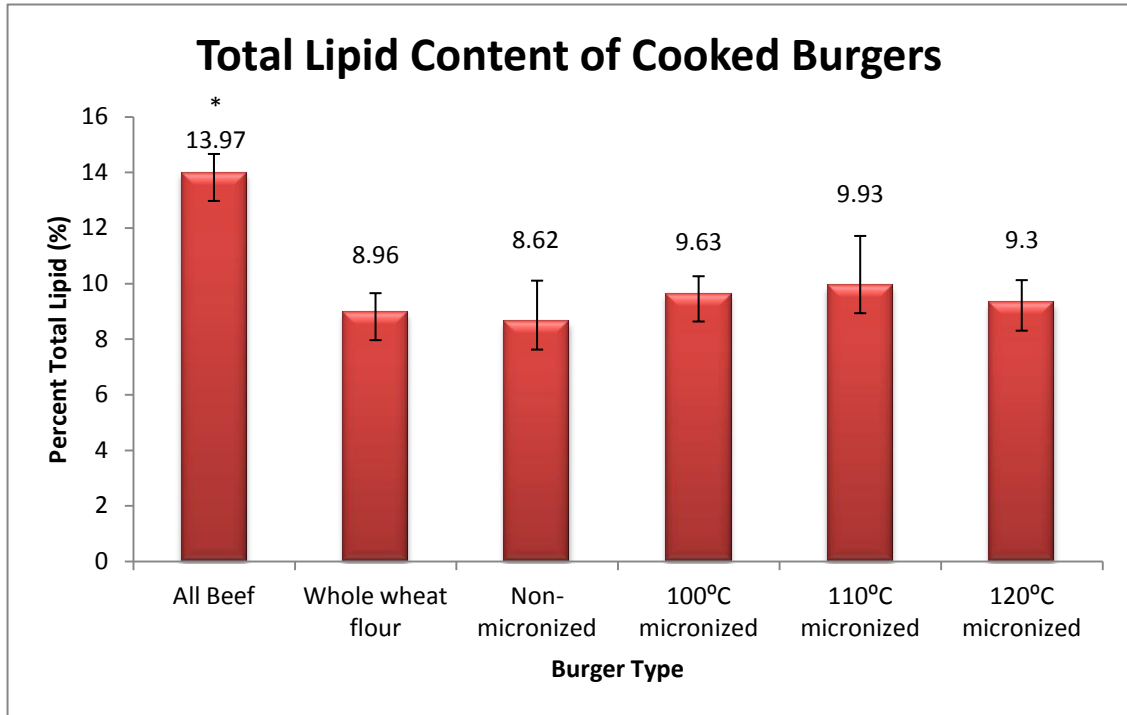


Figure 4.4: Total lipid content of cooked low fat beef burgers
* = Significantly different from other flour types

Table 4.6: Fatty acid profile of cooked low fat beef burgers with added pulse flour¹

Fatty Acid Type (%)	Burger Type					
	All Beef	Whole Wheat Flour	Non-micronized	100°C micronized	110°C micronized	120°C micronized
C16:0	27.71 ^a ± 0.23	26.12 ^a ± 0.34	27.25 ^a ± 0.29	27.47 ^a ± 0.27	26.10 ^a ± 0.38	28.20 ^a ± 5.29
C18:0	13.18 ^{a2} ± 0.24	13.21 ^a ± 0.34	12.84 ^a ± 0.21	13.23 ^a ± 0.31	13.39 ^a ± 2.68	13.98 ^a ± 1.11
C18:1	37.77 ^a ± 0.79	38.04 ^a ± 0.42	38.39 ^a ± 0.54	37.59 ^a ± 0.45	38.93 ^a ± 0.53	34.29 ^a ± 4.90
C18:2 (n-6)	3.68 ^b ± 0.40	5.46 ^a ± 0.36	3.99 ^b ± 0.30	4.29 ^b ± 0.51	3.69 ^b ± 0.31	4.34 ^b ± 1.07
C18:3 (n-3)	0.48 ^c ± 0.02	0.65 ^c ± 0.06	1.10 ^b ± 0.11	1.17 ^{ab} ± 0.11	1.16 ^{ab} ± 0.13	1.45 ^a ± 0.35
C20:4	0.04 ^a ± 0.00	0.01 ^a ± 0.03	0.00 ^a ± 0.00	0.01 ^a ± 0.00	0.02 ^a ± 0.00	0.00 ^a ± 0.00
PUFA:SFA	0.14 ^b ± 0.01	0.20 ^a ± 0.02	0.15 ^b ± 0.01	0.16 ^b ± 0.02	0.16 ^b ± 0.02	0.18 ^{ab} ± 0.03
n-6:n-3 ³	2.0 ^{bcd} ± 0.05	2.50 ^a ± 0.24	2.04 ^{bc} ± 0.05	2.13 ^b ± 0.36	1.71 ^{cd} ± 0.13	1.66 ^d ± 0.15
Total SFA	45.59 ^a ± 0.37	43.90 ^a ± 0.63	44.81 ^a ± 0.59	45.52 ^a ± 0.71	44.18 ^a ± 0.60	46.92 ^a ± 8.84
Total MUFA	37.76 ^a ± 0.79	38.04 ^a ± 0.42	38.39 ^a ± 0.54	37.59 ^a ± 0.45	38.93 ^a ± 0.53	34.29 ^a ± 12.07
Total PUFA	6.51 ^b ± 0.65	8.68 ^a ± 0.75	6.79 ^{ab} ± 0.55	7.12 ^{ab} ± 0.75	6.86 ^{ab} ± 0.73	8.23 ^{ab} ± 2.07

¹Values are means ± standard deviation

²Means with the same letter were not significantly different

³Values are for total amount of n-6 and n-3 in samples (not all data shown)

4.3.3.3 Comparison of fatty acids among raw and cooked burgers

Comparisons between raw and cooked patties are presented in Table 4.7. The whole wheat flour control had the greatest change in C16:0, (27.04% vs. 26.12%) raw vs. cooked and was the only sample that was significant. The other formulations, with the exception of 110°C micronized flour all had slight increases in C16:0 after cooking. All samples had a decrease in C18:0 after cooking with the exception of 120°C micronized sample that increased from 13.75%-13.98%. This is consistent with the literature that found a decrease in C18:0 after broiling ground beef (Campbell & Turkki, 1967; Janicki & Appledorf, 1974). However, there was no significant difference in the levels of C18:0 among raw and cooked patties. All formulations had an increase in C18:1 after cooking with the exception of the 110°C and 120°C micronized sample which both

had slight decreases. These findings are similar to Campbell et al. (1967), who observed an increase in C18:1 in ground beef patties from 39.5% to 42.0% in the raw and cooked state respectively (Campbell & Turkki, 1967). However, Janicki et al. (1974), found no difference among C18:1 in raw and cooked beef (Janicki & Appledorf, 1974).

There was an increase in PUFA after cooking of the patties. The amount of C18:2 (n-6) increased in all samples and was significantly higher in the whole wheat flour (3.45% vs. 5.46%), raw versus cooked respectively; non-micronized black bean flour (1.94% vs. 3.99%); 100°C micronized flour (2.60% vs. 4.29%); and the 120°C black bean flour (3.07% vs. 4.34%).

These results are similar to other studies in the literature that found a significantly higher amount of n-6 after broiling beef burgers (Janicki & Appledorf, 1974; Scheeder, Casutt, Roulin, M., Escher, F., Dufey, & Kreuzer, 2001). With the exception of the all beef control, C18:3 (n-3) increased in all the formulations after cooking. It was significantly higher in the black bean flours regardless of micronization temperature. However, the 120°C micronized sample had the highest amount (1.45%). This is important as it shows that addition of black bean flour to low fat beef burgers can improve the nutritional quality of the product by improving the fatty acid profile; and that the results are still significant after cooking. However, these findings are different from other studies that looked at the changes in fatty acids in ground beef after cooking. One study observed a higher amount of n-3 after cooking (0.57% vs. 0.62%), although the results were significant (Scheeder et al., 2001). In contrast, Janicki et al. (1974) found no significant difference in n-3 FA after cooking (both levels were 1.2%) (Janicki & Appledorf, 1974). C20:4 decreased significantly after cooking in all of the samples. It may be hypothesized that the majority of C20:4 is lost in the drip upon broiling. However, no change in C20:4 was observed in another study after broiling ground beef burgers (Janicki & Appledorf, 1974).

Overall, cooking of the burgers resulted in a decreased amount of SFA (C18:0) and an increased in most of the MUFA and PUFA. Phospholipids contain a large amount of PUFA and little is lost in the drip upon cooking (Campbell & Turkki, 1967). Therefore, the retention of MUFA and PUFA on our samples may be due to that fact that the majority of unsaturated fatty acids are part of the structural components of phospholipids and are less likely to be lost in the drip (Janicki & Appledorf, 1974)

Table 4.7: Comparison of fatty acid composition of raw and cooked burgers¹

Formulations	Fatty Acids					
	C16:0	C18:0	C18:1	C18:2 (n-6)	C18:3 (n-3)	C20:4
All beef						
Raw	27.58 ^{ab} ± 0.44	15.07 ^{a2} ± 0.98	37.08 ^{de} ± 1.29	2.74 ^{def} ± 0.16	0.51 ^f ± 0.03	0.73 ^a ± 0.13
Cooked	27.71 ^a ± 0.23	13.18 ^{ab} ± 0.23	37.77 ^{abcde} ± 0.79	3.68 ^{bcd} ± 0.40	0.48 ^f ± 0.02	0.04 ^c ± 0.05
Whole wheat flour						
Raw	27.34 ^{abc} ± 0.25	14.48 ^{ab} ± 0.79	36.72 ^e ± 0.69	3.45 ^{bcde} ± 0.33	0.51 ^f ± 0.04	0.34 ^b ± 0.28
Cooked	26.12 ^d ± 0.34	13.21 ^{ab} ± 0.34	38.04 ^{abcde} ± 0.42	5.46 ^a ± 0.36	0.65 ^{ef} ± 0.06	0.01 ^c ± 0.03
Non-micronized flour						
Raw	27.20 ^{abc} ± 0.29	14.05 ^{ab} ± 0.70	38.32 ^{abcde} ± 1.00	1.94 ^f ± 0.92	0.73 ^{ef} ± 0.07	0.42 ^b ± 0.05
Cooked	27.25 ^{abc} ± 0.29	12.84 ^b ± 0.22	38.39 ^{abcd} ± 0.54	3.99 ^{bc} ± 0.30	1.10 ^{bc} ± 0.11	0.00 ^c ± 0.00
100°C micronized						
Raw	27.28 ^{abc} ± 0.86	14.05 ^{ab} ± 0.24	37.63 ^{bcde} ± 1.09	2.60 ^{ef} ± 0.24	0.81 ^{de} ± 0.08	0.48 ^b ± 0.09
Cooked	27.47 ^{ab} ± 0.27	13.23 ^{ab} ± 0.32	37.59 ^{bcde} ± 0.45	4.29 ^b ± 0.51	1.17 ^b ± 0.10	0.01 ^c ± 0.02
110°C micronized						
Raw	26.31 ^{cd} ± 0.53	13.45 ^{ab} ± 1.09	38.80 ^{abc} ± 1.25	2.68 ^{def} ± 0.22	0.84 ^{cde} ± 0.07	0.46 ^b ± 0.07
Cooked	26.10 ^d ± 0.38	13.39 ^{ab} ± 0.27	38.93 ^{ab} ± 0.53	3.69 ^{bcd} ± 0.31	1.16 ^b ± 0.12	0.02 ^c ± 0.01
120°C micronized						
Raw	27.05 ^{abcd} ± 0.26	13.75 ^{ab} ± 0.60	37.22 ^{cde} ± 0.51	3.07 ^{cde} ± 0.52	1.05 ^{bcd} ± 0.17	0.28 ^b ± 0.23
Cooked	28.20 ^a ± 5.29	13.98 ^{ab} ± 2.68	34.29 ^a ± 0.43	4.34 ^b ± 1.07	1.45 ^a ± 0.35	0.00 ^c ± 0.00

¹Values are means ± standard deviation

²Means with the same letter were not significantly different

4.4 Colour analysis of raw burgers

Hunter lab colours have been in use since 1966 and are more visually uniform compared to the previous XYZ scale (Hunter lab, 2008). Hunter lab values for raw burger formulations are presented in Table 4.8. The different burger formulations had a significant effect on L* values which indicates degree of lightness. The whole wheat flour had the highest value (40.05) and was significantly different from samples with black bean flour and the all beef control. This result was expected as whole wheat flour is very light in colour, while black bean flour is darker. It has been shown that the micronization process can influence the colour of both red and green lentils resulting in a darker colour (Bellido et al., 2006; Der, 2010).

In our samples the non-micronized black bean flour had the lowest L* value indicating the darkest colour. There was significant difference observed between the non-micronized and 110°C micronized burger samples. These findings are contradictory to what has been observed in another study that examined the colour of whole black beans after micronization in which a darker colour (lower L* value) was observed after heat processing mostly due to the Maillard browning effect (Bellido et al., 2003). However, Bellido and colleagues used whole black beans and it has been shown that milling pulses and other plant materials into flour results in an overall decrease in colour. Additionally, Arntfield et al. (2001) observed an inverse relationship with micronization temperature and L* values in Laird No. 1 lentils (Arntfield et al., 2001a).

Positive Hunter a* lab values indicate degree of redness, where as negative a* value would indicate more green colour (Hunter lab, 2008). The whole wheat flour control had significantly higher a* value (10.72) than all the other burger formulations. The 120°C micronized black bean flour burger formulation had the second highest value (9.28) and was

significantly different from the other formulations as well. These findings are consistent with the literature; comparison of micronized and non-micronized black beans showed more positive a^* values after heat treatment (Bellido et al., 2003). It was shown that a higher micronization temperature resulted in a redder colour of green lentil seed (Arntfield et al., 2001a; Der, 2010). However micronizing at 135°C resulted in a lower a^* value (less red) in red lentil seed (Der, 2010).

Positive Hunter b^* values indicates the degree of yellowness; whereas negative b^* values would represent greater blue colour. The whole wheat flour control had the highest b^* value, (most yellow), (17.15), and it was significantly different compared to all other formulations. The all beef control had the second highest b^* value (14.72) and was significantly different than all other formulations. The non micronized sample had the lowest b^* value (11.23), indicating the least yellow of all samples and was significant.

Out of the black bean formulations, the 120°C micronized sample had the highest b^* value (12.12) and was significantly different from all other formulations. Der et al. (2010) found that addition of 6% micronized green and red lentil flour to low fat beef burgers resulted in higher b^* values compared to addition of non-micronized lentil flour (Der, 2010). Conversely, another study found that micronization of black beans resulted in more negative b^* values (more blue) (Bellido et al., 2003).

Table 4.8: HunterLab L, a and b colour values¹ for burgers formulated with micronized and non-micronized black bean flour

Raw Burgers (n=6)	L*¹	a*	b*
Burger Types			
<i>All Beef</i>	36.30 ^{b2} ± 1.4	8.40 ^{bc} ± 0.6	14.72 ^b ± 0.4
<i>Whole wheat flour</i>	40.05 ^a ± 1.0	10.72 ^a ± 0.8	17.15 ^a ± 0.3
<i>Non- micronized</i>	32.97 ^d ± 1.0	8.12 ^c ± 0.4	11.23 ^d ± 0.6
<i>100°C micronized</i>	33.58 ^{cd} ± 1.2	8.17 ^c ± 0.4	11.75 ^{cd} ± 0.7
<i>110°C micronized</i>	34.85 ^{bc} ± 0.6	7.68 ^c ± 0.5	11.73 ^{cd} ± 0.6
<i>120°C micronized</i>	33.87 ^{cd} ± 0.6	9.28 ^b ± 0.6	12.12 ^c ± 0.3

¹Values are means ± standard deviation

L* = lightness; a* = redness; b* = yellowness

²Means with the same letter were not significantly different

4.5 Colour analysis of cooked burgers

Various binders and additives can affect colour of meat products. The exterior of the cooked samples containing the different burger formulations were analyzed and the results are presented in Table 4.9. The all beef control had the highest L* values (40.15), indicating the lightest colour and was significantly different from the whole wheat flour control and the 110°C micronized burger. This could be due to the fact that the all beef control had the highest fat content. The whole wheat flour control had the lowest L* value (33.88). In another study it was observed that there was no significant difference among L* values of cooked low fat burgers with added green and red lentil with the exception of green lentil which was lighter (Der, 2010).

Hunter a* values were significantly higher in the all beef and whole wheat control (4.53 and 4.83) respectively, indicating a redder colour than all the black bean formulations. There was no significant difference between the micronized and non micronized black bean formulations. Turhan et al. (2005) added hazelnut pellicle to low fat beef burgers at 0-5% substitutions and

found that addition of the binder resulted in lower a^* values compared to the controls (Turhan, Sagir, & Sule Ustun, 2005)

The all beef control had the highest b^* values (yellowness) at 13.92 and was significantly different compared to the other formulations. This again may be due to the higher fat content of this sample. The whole wheat flour control had the second highest value (12.03) and was significantly different than all other formulations. The non-micronized sample had the lowest b^* value (9.80), (ie: least yellow) however, there was no significant difference between any of the black bean formulations. Overall there was no significant difference in colour between the non-micronized and micronized black bean burgers. There was significant difference between the controls and the black bean formulations with regard to a^* and b^* values. The evidence shows that addition of black bean flour (non-micronized and micronized) to low fat beef burgers results in a decrease in both red and yellow colour. Addition of hazelnut pellicle to low fat beef burgers had a similar effect (Turhan et al., 2005).

The colour of food products has an impact of sensory attributes. Consumers expect a certain appearance of ground beef when purchased at a store, generally a redder pigment associated with oxymyoglobin is generally preferred as it is associated with meat quality and freshness. A study that investigated the effect of low fat beef burgers containing 6% and 12% micronized and non micronized green and red lentil colour found an inverse relationship with length of storage time and redness in raw burgers. However, after measuring colour at 0, 3, 5, and 7 days, the burgers containing the micronized flour had significantly higher a^* (red values) than the non micronized and control samples in both green and red lentils (Der, 2010).

Table 4.9: HunterLab L, a and b colour values¹ for burgers formulated with micronized and non-micronized black bean flour

Cooked Burgers (n=6)	L*	a*	b*
Burger Types			
<i>All Beef</i>	40.15 ^{a2} ± 1.9	4.53 ^a ± 0.3	13.92 ^a ± 0.6
<i>Whole wheat flour</i>	33.88 ^b ± 3.2	4.83 ^a ± 0.5	12.03 ^b ± 1.1
<i>Non- micronized</i>	37.17 ^{ab} ± 1.3	3.25 ^b ± 0.2	9.80 ^c ± 0.5
<i>100°C micronized</i>	37.53 ^{ab} ± 3.3	2.92 ^b ± 0.3	10.13 ^c ± 1.2
<i>110°C micronized</i>	35.32 ^b ± 2.6	2.97 ^b ± 0.6	10.22 ^c ± 0.3
<i>120°C micronized</i>	36.93 ^{ab} ± 2.5	3.33 ^b ± 0.5	10.47 ^c ± 0.8

¹Values are means ± standard deviation

L* = lightness; a* = redness; b* = yellowness

²Means with the same letter were not significantly different

4.6 pH

Average pH of animal muscle before slaughter is approximately 7.1. Due to the increase in lactic acid post slaughter the pH usually decreases, with beef normally reaching its lowest pH values ranging from 5.4-5.9 after about 18-24 hours (Eutech Instruments Pte Ltd, 1997). The pH of the low fat beef burgers are shown in Table 4.10. The values ranged from 5.94-6.06. There was significant difference between the pH values of the all beef (5.94) control and the 120°C micronized patty (6.06). There was no significant difference between pH among the other formulations.

Der et al. (2010), found that the pH of low fat beef burgers with green and red lentil flours ranged from 5.5-5.7, which is lower than all burgers presented in our study (Der, 2010). Similarly, the all beef control had the lowest pH (5.5); however, the wheat flour control and the lentil burgers had the same pH (5.5). Conversely, our whole wheat flour control measured at pH 6.04. Another study that investigated the effect of substituting 2.5-5% chickpea flour into low fat

bologna reported pH ranges from 6.2-6.3 with no significant difference between the samples (Thushan, Sanjeewa, Wanasundara, Pietrasik, & Shand, 2010).

It has been reported that ultimate pH can have significant effect on water holding capacity and shear force of bovine meat and mutton (Bouton, Harris, & Shorthose, 1971; Bouton, Carrol, Harris, & Shorthose, 1973; Eutech Instruments Pte Ltd, 1997). One study postulated that this may be due to the effect of fibre contraction (Bouton et al., 1973). We observed a significant difference in the all beef control with the lowest pH value and shear force, drip loss, cook loss and % shrinkage.

4.7 Shear force

The WB shear force method is the most common way of measuring the tenderness of meat. The results of shear force are presented in Table 4.10. The all beef control was significantly higher (20.03 N) compared to all other formulations which ranged from 9.56 N-12.63 N. No significant difference was observed between any of the black bean or wheat flour formulations.

These findings are consistent with other studies in the literature. Der et al (2010) observed a significant difference solely in the all beef control compared to wheat flour, wheat crumb and green and red lentil at 6% and 12% substitutions in low fat beef burgers (Der, 2010). Likewise, another study observed significantly higher peak shear force values in the all beef controls compared with 16% inner pea flour substitution into beef patties. These findings were significant regardless of fat content of the patties (10%, 14% and 18%) (Anderson & Berry, 2006). Additionally a study that used 23 different pulses at 35%, 42.5% and 50% substitutions into beef and sausage patties observed significant differences between the all beef, as well as a

rice fibre and soy protein control and shear force compared to the pulse patties (Holliday et al., 2011).

In our study, the all beef control had significantly higher cook and drip loss, as well as % shrinkage. Therefore, the ability for high fibre pulse flours to hold and retain fat and moisture may play an important role in the overall tenderness and juiciness of the patties.

4.8 Cook loss/drip loss

The percent cook loss and drip loss is presented in Table 4.10. There was significant difference between the all beef control and all other flour formulations, regardless of micronization temperature. The all beef control had almost 50% cook loss indicating poor retention of fat and moisture which is consistent with the PA findings which showed the all beef patty to have significantly less moisture after cooking. The whole wheat flour control had the lowest cook loss (28.45%) and drip loss (5.03%) compared with the black bean flour formulations although the results were not statistically significant. There was no significant difference in cook loss or drip loss between the micronized and non-micronized black bean flours, even though micronization has been shown to improve water holding capacity (Der, 2010). This may be due to the limited amount of black bean flour (6%) added to the patties.

Addition of 6% and 12% lentil flour to low fat beef burgers resulted in significantly higher cooking yields than the all beef control and was comparable to whole wheat flour or wheat crumb (Der, 2010). Additionally a similar study observed significant cook loss between the three control samples (all beef, rice fibre and soy protein) compared to 23 different pulse flour substitutions into beef burgers (Holliday et al., 2011). The average cook loss for beans in the study was 10.6%, whereas our present study observed 29.77% - 31.88% loss. This is most

likely due to the greater amount of pulse flour substitution compared to ours and the ability of pulses to retain moisture and fat during cooking. Holliday et al. (2011), observed less cook loss (37.9%) in the all beef patty compared to our study (47.97%). This may be due to the higher fat content of the patties (10%, 14% and 18%) compared with our low fat burger patties (<10%).

4.9 Shrinkage

The percent shrinkage of the patties is shown in Table 4.10. The all beef control had significantly higher shrinkage (25.28%), than all other formulations. There was no significant difference between micronized and non micronized burgers with black bean flour and the whole wheat flour control; however, the whole wheat control had the lowest % shrinkage (18.47%).

Increasing fibre has been shown to result in less shrinkage of low fat beef patties (Desmond, Troy, & Buckley, 1998). Our results illustrate that addition of high fibre black bean flour, even at 6% may result on more acceptable product.

Addition of 12% lentil flour (green and red) resulted in the least amount of shrinkage compared to the 6% flour substitutions and all beef controls in another study using low fat beef burgers (Der, 2010). Anderson et al. (2006) observed less shrinkage with addition of 16% pea flour to beef patties compared to all beef controls with 18% fat content (Anderson & Berry, 2006). Another study that examined cooking and physiochemical properties of adding soya bean, bengal, green and black gram flour into buffalo burgers found that the black gram flour had the least amount of shrinkage (5%) and was significantly different than the soya bean formulation. The researchers did not use an all beef control, however, they did observe the effects of roasted and unroasted pulse flour and found significantly less shrinkage with the roasted flours with the

exception of the green gram flour (Modi et al., 2004). This is interesting to note that other heat treatments, besides micronization may result in improved moisture and fat retention.

The diameter of the all beef patties had the greatest loss (-34.2 cm) compared to all the other formulations. This is similar to another study that found less shrinkage in low fat patties containing oat flour compared to no binder (Desmond et al., 1998). However, the results are contradictory to other studies in the literature that found no difference in diameter loss by addition of plant flours to meat products (Berry, 1997; Troutt, Hunt, Johnson, Claus, Kastner, & Kropf, 1992).

Table 4.10: Instrumental analysis of low fat beef burgers with added black bean flour

Burger Type	Shear Force¹ (N)	Cook Loss (%)	Shrinkage (%)	pH	Drip Loss (%)
All Beef	20.03 ^{a2} ± 3.4	47.97 ^a ± 1.90	25.28 ^a ± 1.5	5.94 ^b ± 0.1	16.16 ^a ± 3.60
Whole wheat flour	9.56 ^b ± 2.4	28.45 ^b ± 1.98	18.47 ^b ± 0.7	6.04 ^{ab} ± 0.0	5.03 ^b ± 2.08
Non- micronized	10.91 ^b ± 2.8	31.35 ^b ± 1.42	19.43 ^b ± 2.1	6.01 ^{ab} ± 0.0	8.45 ^b ± 1.39
100°C micronized	11.32 ^b ± 4.2	31.44 ^b ± 2.61	19.40 ^b ± 1.4	6.01 ^{ab} ± 0.0	8.86 ^b ± 3.06
110°C micronized	9.79 ^b ± 2.5	29.77 ^b ± 4.06	17.05 ^b ± 2.0	6.03 ^{ab} ± 0.0	7.16 ^b ± 3.53
120°C micronized	12.63 ^b ± 3.2	31.88 ^b ± 1.91	19.13 ^b ± 1.3	6.06 ^a ± 0.0	9.99 ^b ± 2.70

¹Values are means ± standard deviation

²Means with the same letter were not significantly different

4.10 Experiment 3: Consumer panel study of low fat beef burgers with added black bean flour

4.10.1 Consumer panel

To determine the acceptability of the low fat beef burgers with added pulse flour a consumer sensory study was carried out at the George Weston Sensory Research Laboratory (University of Manitoba). Ninety three participants were recruited and judged 6 burger

formulations on aroma, appearance, flavour, texture overall acceptability and FACT. The goal of the sensory evaluations was to draw conclusions from a general population. Therefore the judges weren't selected for any specific qualities or sensitivities and were thus considered random.

4.10.2 Demographics

The age distribution of the participants in this study compared with the Canadian population is illustrated in Figure 4.5. Out of the 93 participants in the study 32% were between the ages of 18-24 (n=30), 22% between the ages of 25-34 (n=20), 15% were between the ages of 35-54 (n=14); 12% between the ages of 45-54 (n=11) and 19% of the participants were 55+ (n=18). This is somewhat representative of the Canadian population with the exception of the 18-24 and 25-34 age groups. These results are expected as the majority of the participants were students recruited from the University of Manitoba and therefore the demographics consisted of a younger population; this is similar to other consumer studies conducted at Universities (Der, 2010). Regarding gender, our goal was for a 1:1 ratio of males to females however, 74% of the study participants were females (n=69) and 26% were males (n=24) (Table 4.11).

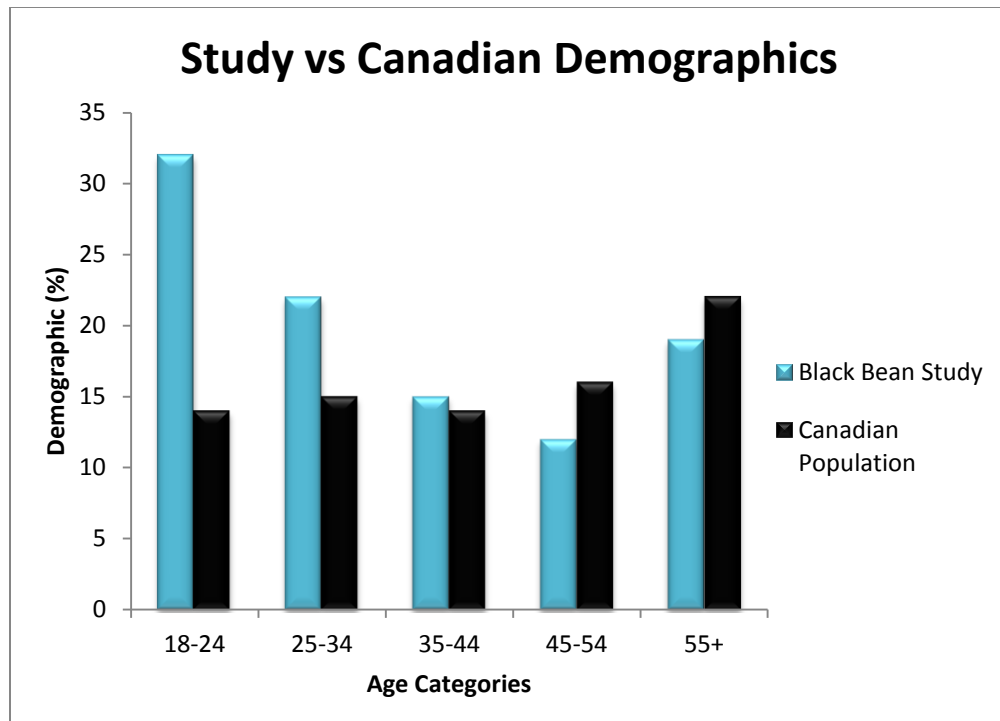


Figure 4.5: Age distribution of consumer sensory study (n=93) and the Canadian population (Stats Canada, 2012)

4.10.3 Acceptability scores for low fat burgers with added black bean flour

The consumer acceptability results are shown in Table 4.11. There was very highly significant difference observed for judges (J), in our study. This is to be expected as each individual has their own subjective criteria for acceptance of food products and is consistent with other consumer studies (Aliani, Ryland, Williamson, & Rempel, 2013). Gender differences showed significant difference for FACT only, (ie: how often they would eat the burger). Significant difference was observed between age and appearance in the study. There was no significant difference with the sample formulations and aroma or appearance, however very highly significant difference was observed between the formulations and flavour, texture, overall acceptability and FACT. Interactions between gender and age, gender and sample formulation,

and sample formulation and age were analyzed; the only significant difference was observed between sample formulation and age regarding aroma.

Mean values for gender and acceptability scores for aroma acceptability was 6.7 meaning that it was liked slightly according to the 9 point hedonic scale. Similarly the mean value for appearance acceptability was 6.6. For both flavour and texture, the values ranged from 6.5 for females to 6.8 for males, showing that males overall preference for flavour and texture was slightly higher, however both fell into the “like slightly” point on the scale. Overall acceptability of the burgers was somewhat higher in the males compared to females (6.8 vs. 6.4); as was FACT, males (6.1) and females (5.5), indicating that females neither liked nor disliked the samples. Overall females preferred the aroma of the burgers more than males; conversely, males preferred the appearance, flavour, texture, overall acceptability and FACT.

Similarly to gender, there was no significant difference between age and aroma acceptability scores; however, there was a trend, as age increased acceptability scores increased. For aroma acceptability, age groups ranged from 6.5-6.9 (like slightly), except for 55+ where the mean value was 7.0 (like moderately). There was significant difference among appearance acceptability and age groups. The mean value for the 18-24 group was 5.9 (neither like nor dislike), 25-54 ranged from 6.6-6.9 (like slightly) to 7.3 (like moderately) in the 55+ group. There was no significant difference among flavour, texture, overall acceptability and FACT and age groups, again there was a trend that as age increased, acceptance increased in all categories.

Regarding the different burger formulations; there was no significant difference between aroma and any of the samples. The 100°C and 110°C micronized samples had the highest scores (6.9) and the whole wheat flour had the lowest (6.6). All of the acceptability scores in the

present study were higher compared to another study that conducted a consumer analysis of low fat beef burgers with added micronized red and green lentil flour. An average score of 4.3 on a 6 point hedonic scale for aroma was observed for all samples (all beef control, 6% non-micronized lentil flour, 6% micronized lentil flour and a 6% toasted wheat crumb) (Der, 2010). Likewise, there was no significant difference among appearance and any of the burger formulations. All of the micronized samples had higher scores; the 110° micronized flour sample had the highest score (6.8), indicating that it had the highest acceptability while the all beef, whole wheat flour control and non-micronized samples all had scores of 6.5.

There was significant difference among all of the black bean flour samples (non-micronized and micronized) and the all beef and whole wheat flour control regarding flavour acceptability. Flavour acceptability was highest in the 100°C formulation (7.1) indicating “like moderately” and lowest in the all beef control (5.8) “neither like nor dislike”. These results demonstrate that addition of black bean flour to low fat beef burgers improves the flavour compared to using wheat flour or no binder. A similar study that measured flavour of low fat beef burgers with added lentil flour found that the non-micronized sample and the all beef control had the lowest acceptability (4.2) and that addition of micronized flour was most favoured (4.7) (Der, 2010).

Texture acceptability was significantly higher in all of the black bean flour (micronized and non-micronized) compared to the controls. The non-micronized and 110°C sample had the highest scores (7.1). The lowest texture acceptability score was the beef control (4.9). It was significantly lower than the whole wheat flour (6.3). As previously discussed, the all beef control had significantly higher cook loss (50%) and drip loss as well as % shrinkage than all of the other samples. Proximate analysis showed that the all beef control had the least amount of

moisture. Due to the increased moisture and fat loss the all beef control would have been drier and tougher than all other samples. Although the addition of any binder to low fat beef burgers improved texture acceptability, addition of black bean flour was significantly greater than using whole wheat flour. This is most likely due to the increased water holding capacity of pulse flour as texture aspects and sensory attributes are highly correlated. Similarly, Der et al. (2010) found that the all beef control had the lowest texture acceptability scores (4.0) compared to addition of binder of any kind and the non-micronized lentil flour sample was the most acceptable (Der, 2010).

Overall acceptability was significantly higher in all the black bean flour samples with the 100°C micronized sampled being the highest (7.1). The all beef control had the lowest acceptability (5.3) and was significantly lower than the whole wheat flour. The frequency of how often one would consume the burgers was investigated. The consumer panellist showed that all of the black bean burger samples would be eaten more frequently than the whole wheat or all beef control samples. The 100°C micronized flour formulation had the highest sensory score (6.3), however all the black bean formulations had scores that feel within the “I like this and would eat this now and then” category. The whole wheat flour control had a FACT score of 5.0, “I would eat this if available but would not go out of my way” and the all beef control was significantly lowest (4.9) “I do not like this but would eat this on occasion”.

Part of this study was to investigate whether LOX activity affected acceptance of low fat beef burgers with added pulse flour. Although this study showed that micronization significantly reduced estimated LOX activity, there was no significant difference among the non-micronized and micronized samples regarding acceptability. However, in general the 100°C and 110°C samples were most accepted. This is contradictory to other studies who found that non-

micronized lentil flour was less accepted than toasted wheat crumb (Der, 2010), or that addition of legume flours (soy and chickpea) decreased overall sensory scores in meatloaves due to off flavours (Shaner & Baldwin, 1979).

Furthermore, by separating consumers according to certain demographics (gender and age) we were able to distinguish significant difference in acceptance regarding the different burger formulations. These findings play an important role at a consumer level when determining a target group for marketing purposes.

Table 4.11: F-value associated probabilities and mean value (standard deviation of the mean) for consumer acceptance of black bean burgers from four- way ANOVA

(J= Judge [n=93]; G= Gender [n=2]; A=Age [n=5]; S= sample formulation [n=6])

Attribute	Source of variation (F-value)							Mean value for gender		Mean value for age				
	J	G	A	S	G x A	S & G	S & A	Female n=69	Male n=24	8-24 yrs n=30	25-34 yrs n=20	35-44 yrs n=14	45-54 yrs n=11	55 + yrs n=18
Aroma ¹	3.0 ***	0.1 NS	1.1 NS	1.7 NS	1.3 NS	1.78 NS	2.0 **	6.8 ^a (1.3)	6.6 ^a (1.4)	6.5 ^a (1.4)	6.8 ^a (1.2)	6.7 ^a (1.3)	6.8 ^a (1.5)	7.0 ^a (1.3)
Appearance ¹	3.8 ***	0.2 NS	4.9 **	0.9 NS	0.7 NS	2.0 NS	1.34 NS	6.5 ^a (1.5)	6.6 ^a (1.5)	5.9 ^c (1.6)	6.6 ^b (1.3)	6.6 ^b (1.6)	6.9 ^b (1.6)	7.3 ^a (1.1)
Flavour ¹	3.2 ***	1.6 NS	0.5 NS	11.5 ***	1.3 NS	0.4 NS	0.5 NS	6.5 ^a (1.8)	6.8 ^a (1.4)	6.5 ^a (1.6)	6.5 ^a (1.8)	6.5 ^a (1.8)	6.4 ^a (1.8)	7.0 ^a (1.5)
Texture ¹	3.0 ***	1.9 NS	1.0 NS	21.6 ***	0.8 NS	1.1 NS	1.0 NS	6.5 ^a (1.8)	6.8 ^a (1.6)	6.3 ^a (1.9)	6.6 ^a (1.6)	6.5 ^a (1.8)	6.6 ^a (1.8)	7.0 ^a (1.4)
Overall acceptability ¹	2.9 ***	2.2 NS	0.5 NS	16.2 ***	1.8 NS	0.8 NS	0.6 NS	6.4 ^a (1.7)	6.8 ^a (1.4)	6.4 ^a (1.7)	6.5 ^a (1.6)	6.4 ^a (1.8)	6.4 ^a (1.7)	6.9 ^a (1.5)
FACT ²	3.2 ***	4.6 *	1.4 NS	15.25 ***	1.9 NS	1.1 NS	0.7 NS	5.5 ^a (1.7)	6.1 ^a (1.4)	5.4 ^a (1.6)	5.5 ^a (1.6)	5.6 ^a (2.0)	5.6 ^a (1.8)	6.3 ^a (1.3)

Table 4.11 Cont.d

Mean value for formulation						
Attribute	AB	WW	Non micronized	100°C	110°C	120°C
Aroma ¹	6.7 ^a (1.3)	6.6 ^a (1.5)	6.7 ^a (1.3)	6.9 ^a (1.4)	6.9 ^a (1.4)	6.7 ^a (1.4)
Appearance ¹	6.5 ^a (1.7)	6.5 ^a (1.6)	6.5 ^a (1.4)	6.7 ^a (1.5)	6.8 ^a (1.4)	6.4 ^a (1.7)
Flavour ¹	5.8 ^b (1.8)	5.9 ^b (2.0)	6.8 ^a (1.4)	7.1 ^a (1.4)	6.9 ^a (1.5)	6.9 ^a (1.5)
Texture ¹	4.9 ^c (2.0)	6.3 ^b (1.7)	7.1 ^a (1.2)	7.0 ^a (1.5)	7.1 ^a (1.3)	6.8 ^a (1.5)
Overall acceptability ¹	5.3 ^c (1.9)	5.9 ^b (1.8)	6.9 ^a (1.3)	7.1 ^a (1.4)	7.0 ^a (1.4)	6.8 ^a (1.4)
FACT ²	4.6 ^c (1.8)	5.0 ^b (1.9)	6.0 ^a (1.5)	6.3 ^a (1.4)	6.1 ^a (1.3)	6.0 ^a (1.4)

ANOVA (analysis of variance); AB= All-beef control; WW= whole wheat flour control; 100°C= 100°C micronization temperature; 110°C= 110°C micronization temperature; 120°C= 120°C micronization temperature. Mean values within the same variable “gender” “age group” “formulation” with the same letter in the same row (attribute) are not significantly different with a p-value <0.05.

¹9= Like extremely; 8=like very much; 7=like moderately; 6= like slightly; 5= neither like or dislike; 4= dislike slightly; 3= dislike moderately; 2= dislike very much; 1= dislike extremely.

²9= I would eat this every opportunity I had; 8=I would eat this often; 7=I would frequently eat this; 6=I like this and would eat it now and then; 5= I would eat this if available but would not go out of my way; 4= I do not like this but would eat this on occasion; 3= I would hardly ever eat this; 2= I would eat this if there were no other food choices; 1= I would eat this only if forced.

NS= P ≥ 0.05, *= P < 0.05, **= P < 0.01, ***= P < 0.001

4.11 Partial least squares analysis

PLS analysis is a common method for constructing models that have many factors and are highly collinear. It is to some extent an advanced model compared to multiple regression analysis, which is often used when correlating variables with few, non-collinear factors. The principle behind PLS is that the *X* and *Y* variables are chosen in a way so that the relationship between factors is as strong as possible (Tobias, 2011). PLS has been used in to correlate various sensory attributes, physical and chemical properties of food products, including meat, from trained and consumer panels (Aliani et al., 2013; Williamson, 2011).

The biplot in Figure 4.6 illustrates the correlation between *X* variables (sensory attributes of consumer panel) and *Y* variables (physical and chemical characteristics). The *Observations* (6 burger formulations) are also shown to explain which components are related to them. The 6 low fat beef burger formulations are as follows: All beef control, whole wheat flour control, non-micronized black bean flour, 100°C micronized black bean flour, 110°C micronized black bean flour, and 120°C micronized black bean flour at 6% substitution.

The biplot divides the formulations into four sections throughout the figure; the non-micronized and 110°C micronized formulations are in the upper right quadrant and the 100°C and 120°C micronized black bean flour formulations reside in the lower right quadrant. Whole wheat flour is in the upper left quadrant, the all beef control in the lower left. The biplot shows which characteristics are related to each other. The sensory aspects all fall into the right two quadrants. Because an untrained sensory panel was used to determine acceptability of the patties at a consumer level all of the sensory attributes (texture, flavour, aroma, taste, FACT) were only measuring acceptability of the product. Specific aspects such as hardness, juiciness, saltiness,

bitterness etc. are what would be analyzed using a trained sensory panel. Therefore it is also important to note that from the results of this study, we cannot determine which aspects were more favoured in each acceptability category.

The biplot shows associations between texture acceptability and non-micronized and 110°C micronized, which are also related to LOX activity and pH. Ultimate pH has a significant effect of shear force and water holding capacity of meat. Therefore we would expect to see some correlation between pH and texture. It makes sense that the LOX activity was closely associated to non-micronized black bean flour. As previously discussed, when LOX activity was measured at the different micronization temperatures, we observed the non-micronized sample to have significantly more LOX activity than the 120°C, 130°C and 140°C micronized flours.

The remaining sensory attributes from the consumer study (flavour acceptability, overall acceptability and FACT), are all related to the 100°C and 120°C micronized black bean flour formulations as well as C18:1 and C18:3 fatty acids. The flavour qualities of the micronized black bean flour burgers (100°C and 120°C) were more accepted. This indicates that the micronization process, possibly through the inactivation of LOX enzymes, may improve the organoleptic properties of these burgers.

No sensory attributes were associated with the all beef or whole wheat control. This demonstrates that all black bean formulations, regardless of micronization temperature, were in general more accepted. The all beef control in the lower left quadrant is associated with many of the physical characteristics (shear force, cook loss, drip loss, % shrinkage). This was expected as the all beef control was significantly harder, and had higher cook loss, drip loss and shrinkage than the other formulations. It is also associated with C18:0 and C20:4 fatty acids. Correlations

with colour show that the all beef control was associated with Hunter L* and b* values of cooked patties. This shows that these burgers are lighter and more yellow in colour. As previously examined these patties had significantly higher L* and b* values than the other formulations and it was postulated that this may be due to the higher fat content of this sample.

The whole wheat flour formulations as well had no relationship with any of the sensory attributes but was however correlated to Hunter L*, a* and b* colour values of raw burgers. This formulation had significantly higher L*, b* and a* values compared to all other formulations meaning that it was lighter, more red and more yellow. This may be explained as whole wheat flour is very pale and yellowish in colour and would affect the colour attributes especially in the raw state. The formulation is also associated with cooked fatty acids (C18:3, and C18:2). To a lesser extent it is associated with Hunter colour a* cooked and C18:2 fatty acid in the raw burger.

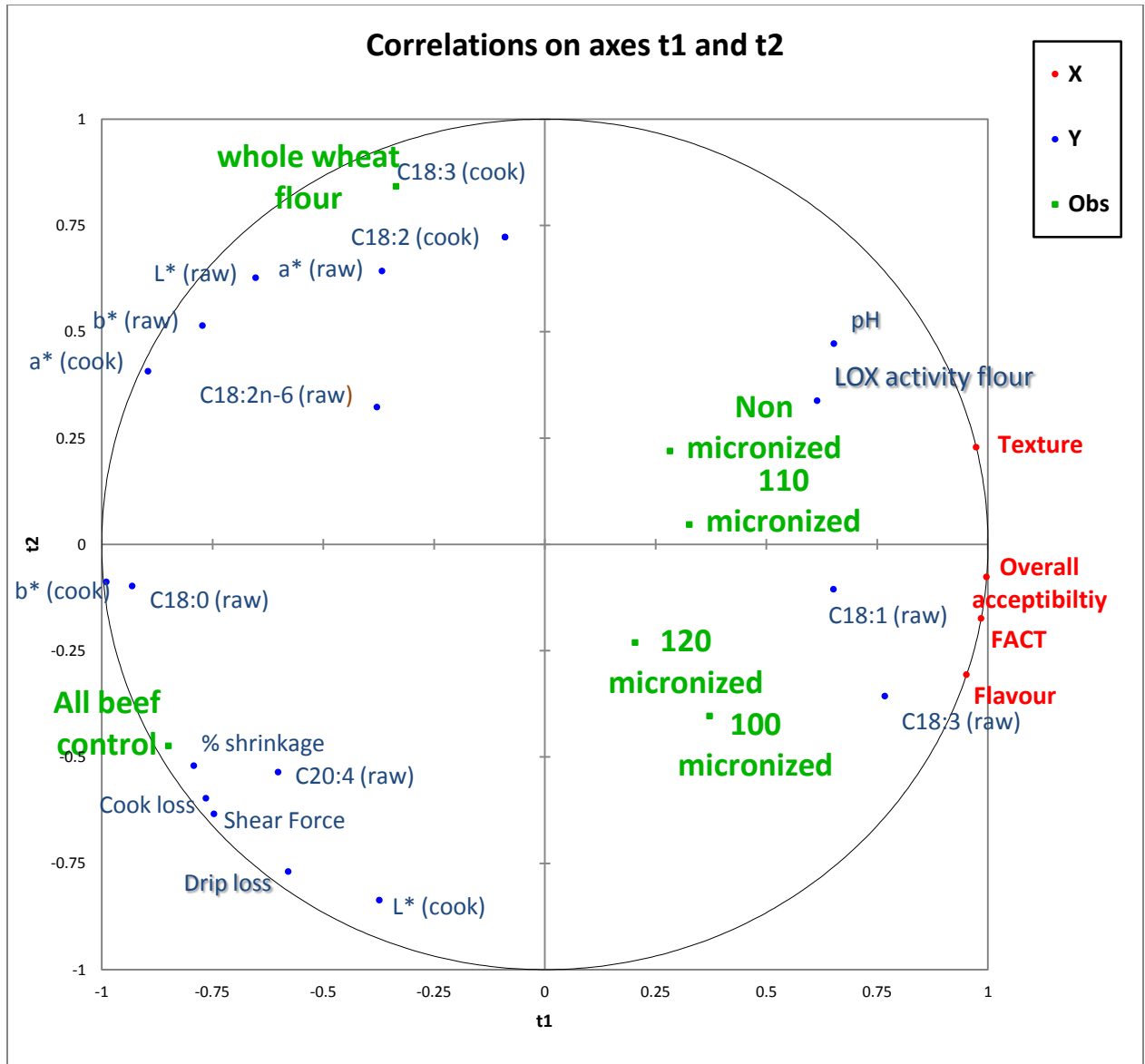


Figure 4.6: Partial least squares (PLS) analysis correlations. X = sensory components (Texture, Flavour, Overall acceptability, FACT). Y= physical and chemical components for raw and cooked samples (Hunter L*, a* b*, C18:0. C18:2, C18:3. C20:4, shear force, drip loss, cook loss, pH, % shrinkage, Obs= 6 burger formulations-all beef, whole wheat flour, non-micronized, 100°C microneurized, 110°C microneurized, 120° microneurized.

Chapter 5: General Discussion

5.1 Overall summary

The objective of this research project was to determine the possible application of using micronized black bean flour as a binder in low fat beef burgers; additionally, to see if it was acceptable at a consumer level. We used six different burger formulations, an all beef control, a whole wheat flour control, non micronized black bean flour; and black bean flour micronized at 100°C, 110°C and 120°C. Six percent substitution was used for all of the flours. There is evidence that using pulse flours as a substitute in meat products may improve the nutritional quality of the products. However, it may also negatively affect some of the sensory properties of the food. Pulses and legumes contain a great amount of PUFA which have the potential to become oxidized; therefore, the inhibition of LOX enzymes through micronization was explored to determine if it affected organoleptic properties.

The effect of adding black bean flour to low fat beef burgers was evaluated by analyzing LOX enzyme activity, conducting proximate analysis, fatty acid analysis, and instrumental analysis (shear force, pH, drip loss, cook loss, and % shrinkage). Additionally an untrained sensory study was performed to determine acceptability at a consumer level. Finally, partial least squares regression was conducted to establish correlations between sensory and instrumental data compared to the different burger formulations.

In the first part of the study, crude protein was extracted from micronized and non micronized black bean flour and LOX activity was tested. It was observed that LOX enzyme activity was significantly decreased at micronization temperatures of 120°C 130°C and 140°C

compared to the non micronized sample. This has the potential to improve market value of the low fat beef burgers through improved shelf life and flavour quality.

The next aspect of the study was to determine the effect of black bean flour in low fat beef burgers on physiochemical and functional properties of the food. PA of the black bean flour showed lower moisture content after micronization, possibly due to the high heat treatment. However, the micronized sample had slightly higher protein content which may be due to the fact that micronization or other heat processes can affect protein denaturation. PA of the cooked burgers showed a higher amount of protein and lower moisture content in the all beef control; it also had higher total lipid content. This is most likely due to the greater amount of drip loss and the fact that addition pulse flour has been shown to improve water holding capacity in beef burgers. Fibre content wasn't affected by 6% pulse flour substitutions; however, incorporating greater amount of pulses may increase the content.

Total lipid content of the black bean flour wasn't affected by micronization temperature. However, compared to whole wheat flour, the black bean flour had a much more desirable lipid profile. The 120°C micronized black bean flour had the highest amount to LA and ALA compared to the other black bean samples. A trend was observed in both LA and ALA, as micronization temperature increased, so did the amount of LA and ALA. This has to do with the inactivation of LOX enzyme at high micronization temperatures, resulting in fewer enzymes to oxidize the fatty acids; which is important since PUFA make up the majority of black bean flour. There was no difference in total lipid in the raw burgers; however, total lipid increased in all samples after cooking mostly due to the amount of moisture lost in the drip. In general, substituting black bean flour into low fat beef burgers resulted in a higher amount of ALA, it

improved PUFA:SFA and n-6:n-3 and it is important to note that these results were still significant after cooking.

Colour analysis of the raw burgers showed that the whole wheat flour control was lightest, and had the most red and yellow attributes. The non micronized sample was darkest, compared to the other black bean samples, this is expected as high heat treatments have been shown to result in lower L* values of pulses. Upon cooking, the all beef control had the highest L* and a* values, which is possibly a result of the greater fat content in this sample. The appearance of a meat product plays an important role in the acceptability of the food. Overall there was no significant difference in colour between the non micronized and micronized black bean samples after cooking. Addition of black bean flour (both micronized and non-micronized) resulted in a less red and yellow burger. This may improve potential market value by producing a more neutral colour binder that could be added to food products.

Furthermore, the process of micronization decreased or inhibited LOX activity in pulses. LOX activity can influence lipid oxidation which can then result in iron oxidation (Yin, Faustman, Riesen, & Williams, 1993). Myoglobin, is responsible for the red colour in meat after slaughter and is associated with meat quality. When the iron compound in myoglobin is oxidized it is converted into metmyoglobin which results in a brown colour that is not as consumer acceptable. Therefore, the use of micronization as a food processing technique to pulse flour may have many benefits to consumers and improve the potential market value of these food products.

Instrumental analysis showed significant differences in the all beef control regarding shear force, cook and drip loss and % shrinkage, it also had the lowest pH value. This means the

all beef control would be harder, tougher, smaller and drier than the samples containing a binder. Since none of these properties are typically desired in a beef burger, it may be postulated that addition of a binder may improve the functional attributes of this food. In general, the 110°C micronized black bean flour beef patty had the most acceptable sensory properties of all pulse flour formulations, and is comparable to the whole wheat flour formulation. Whole wheat flour or wheat crumb is a common binder used in beef burgers; however, pulse flour is much higher in protein, fibre and contains more n-3 PUFA than wheat flour and is therefore a superior alternative.

A consumer sensory study was carried out, which consisted of 93 participants to evaluate the aroma, appearance, flavour, texture overall acceptability and FACT of the 6 burger formulations. The majority of participants were between the ages of 18-24 and were female. In general, males had higher acceptability of the burgers, with the exception of aroma. The 55+ age group preferred the aroma, appearance, flavour, texture, overall acceptability and FACT than any other age group.

Regarding the burger formulations, overall the 100°C micronized black bean flour was most accepted; however, it was not significantly different from the non-micronized flour as has been shown in other studies (Der, 2010). It was found addition of black bean flour to low fat beef burgers improved the flavour, texture, overall acceptability and FACT of low fat burgers compared to burgers with wheat flour or no binder. Black bean flour is higher in protein and fibre; it is more nutrient dense and was more accepted in the sensory study compared to the all beef or whole wheat flour. These results show that addition of black bean flour to low fat beef burgers makes them more acceptable at a consumer level.

5.2 Significance of research and further studies

There is strong evidence that creating a healthier meat product by the addition of pulse flours may improve the nutritional quality, fatty acid profiles, sensory and physiochemical properties. The meat industry is a one of the largest industries in the world. Meats are high in consumer demand and producers are in constant competition with one another to develop new products. Therefore, the development of a burger with the addition of pulse flours may benefit the meat sector from a marketing point of view. Additionally, health benefits such as increased vegetable consumption, higher fibre intake and lower fat intake may be observed among consumers who eat these novel meat products.

Future investigation is warranted to determine the effects of adding different pulse flours to meat products, increasing the percentage of pulse flours added, the interactions of these pulses with meats, and analyzing how different compound such as vitamins and minerals, bioactives, phyto-nutrients and antioxidants are affected by high heat treatments. As well a more in-depth sensory panel to determine overall consumer acceptance towards the new manufactured goods should be conducted. Beef is an essential part of the North American diet and a big part of Canadian agriculture. We are seeing a large consumer demand for healthier products that taste good. Therefore, if we can incorporate pulses in novel, convenient, and healthy food products we may benefit Canadians by helping to provide a healthier alternative to one of the country's most commonly consumed food.

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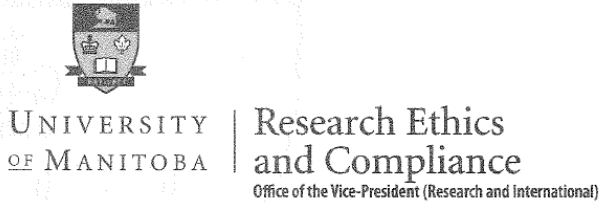
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Appendix A-Ethics Approval




Human Ethics
208-194 Dafoe Road
Winnipeg, MB
Canada R3T 2N2
Phone +204-474-8880
Fax +204-269-7173

APPROVAL CERTIFICATE

November 9, 2012

TO: Michel Aliani
Principal Investigator

FROM: Wayne Taylor, Chair 
Joint-Faculty Research Ethics Board (JFREB)

Re: Protocol #J2012:184
"Consumer Acceptability of Beef Burgers with Pulse Flours - Study 2"

Please be advised that your above-referenced protocol has received human ethics approval by the **Joint-Faculty Research Ethics Board**, which is organized and operates according to the Tri-Council Policy Statement (2). **This approval is valid for one year only.**

Any significant changes of the protocol and/or informed consent form should be reported to the Human Ethics Secretariat in advance of implementation of such changes.

Please note:

- If you have funds pending human ethics approval, the auditor requires that you submit a copy of this Approval Certificate to the Office of Research Services, fax 261-0325 - please include the name of the funding agency and your UM Project number. This must be faxed before your account can be accessed.
- if you have received multi-year funding for this research, responsibility lies with you to apply for and obtain Renewal Approval at the expiry of the initial one-year approval; otherwise the account will be locked.

The Research Quality Management Office may request to review research documentation from this project to demonstrate compliance with this approved protocol and the University of Manitoba *Ethics of Research Involving Humans*.

The Research Ethics Board requests a final report for your study (available at: http://umanitoba.ca/research/orec/ethics/human_ethics_REB_forms_guidelines.html) in order to be in compliance with Tri-Council Guidelines.

Appendix B- Questionnaire presented to consumers for Sensory Study

PARTICIPANT INSTRUCTIONS

TASK

Rinse your mouth with the water provided to cleanse your palate as required.

PART 1 - SAMPLE EVALUATION

The task is to evaluate your degree of liking of the aroma, appearance, flavor, texture and the overall acceptability of the meat samples in addition to indicating how often you would eat them.

PART 2 - GROUP CHARACTERISTICS

Respond to the final 4 questions which will enable us to describe our group of tasters.

PROGRAM STEPS

1. CLICK ON START TEST.
2. CONFIRM 6 different sample codes on tray.
3. REVIEW instructions.
4. Select the RIGHT hand direction at the top of the screen to MOVE AHEAD and the LEFT direction to GO BACK to previous screens.
5. FIRST SAMPLE CODE is at the top of the screen.
6. CLICK ON the appropriate descriptor to record your response. Responses can be changed by selecting the desired descriptor.
7. COMMENTS can be made at any time. CLICK ON the notebook icon on the top of the screen to enter.
8. Complete ALL questions for the first sample.
9. Once completed the NEXT SAMPLE box will appear and the next sample number to evaluate will be displayed at the top of the screen.
10. CONTINUE the same procedure for the remaining six samples.
11. COMPLETE PART 2 – Group Characteristics.
12. CLICK ON the RED END BOX to complete your data entry.
13. Please see the researcher to sign for and receive your honorarium.

1. AROMA

Sample No. _____XXX_____

Remove the cover from the meat sample, smell it and determine how much you like/dislike the AROMA.

- Like Extremely
- Like Very Much
- Like Moderately

- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

2. APPEARANCE

Look at the meat sample and determine how much you like/dislike the APPEARANCE.

- Like Extremely
- Like Very Much
- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

3. FLAVOR

Taste the sample and determine how much you like/dislike the FLAVOR of the meat.

- Like Extremely
- Like Very Much
- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

4. TEXTURE

Sample No. _____

Taste the sample and determine how much you like/dislike the TEXTURE of the meat.

- Like Extremely
- Like Very Much

- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

5. OVERALL ACCEPTABILITY

How much do you like/dislike the meat overall?

- Like Extremely
- Like Very Much
- Like Moderately
- Like Slightly
- Neither Like nor Dislike
- Dislike Slightly
- Dislike Moderately
- Dislike Very Much
- Dislike Extremely

6. FREQUENCY OF EATING

Please indicate how often you would eat this meat.

- I Would Eat This Every Opportunity I Had
- I Would Eat This Very Often
- I Would Frequently Eat This
- I Like This and Would Eat It Now and Then
- I Would Eat this If Available But Would Not Go Out of My Way
- I Don't Like this but Would Eat It on an Occasion
- I Would Hardly Ever Eat This
- I Would Eat This If There Were No Other Food Choices
- I Would Eat This Only If Forced



Appendix C-Recruitment Email for Sensory Evaluation

Weston Sensory Centre
Rm 400 Human Ecology
Bldg.

Faculty of Human Ecology Department of Human Nutritional Sciences

March 14, 2013

Dear Colleague,

We are recruiting volunteers to participate in a research study on the acceptability of beef burgers with added pulse flours. Pulses are dried seeds that can be eaten from a group of plants that belong to the legume family. The most common pulses are lentils, beans, chickpeas, yellow and green peas. In this study flour from black beans will be added to the beef burgers. You would have the opportunity to learn a research method for collecting data regarding consumer acceptability of the sensory attributes of a food product. **The only criterion is that participants have no allergies to food products.** This letter explains what your commitment would be. If you have any questions please call me, [REDACTED]

Approximately 50 to 100 volunteers will take part in the study. The 20-30 minute session can be scheduled for one of the following dates and times:

April 1 and 8 (Mondays) - 11:30, 12:30, 1:30, 2:30

April 2 and 9 (Tuesdays) - 11:30, 1:00, 2:30

April 4 (Thursday) - 11:30, 1:00, 2:30

April 5 (Friday) - 11:30, 12:30, 1:30

Participants will be required to smell and taste burger samples and respond regarding how much they like/dislike the color, aroma, flavor, texture, overall acceptability as well as how often they would be eaten. Other details regarding the commitment are provided in the attached consent form. An honorarium of a \$10.00 gift card from the University of Manitoba Bookstore will be offered to those completing the required session. The study will take place on the Fourth Floor in the Human Ecology Building.

Completion of the enclosed questionnaire will confirm that no food allergies exist. If you are interested in helping us with this research [REDACTED] e. Please complete the attached consent form and questionnaire required by the Joint Faculty Research Ethics Board and send them back by e-

mail, fax or mail to confirm attendance.

We hope that you will be able to take part in this study and look forward to hearing from you. Alternatively, if you know of anyone else that might be interested in participating we would appreciate it if you could forward this information to them. Thank you.

Sincerely,

[Redacted]

[Redacted]

[Redacted]



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Appendix D- Consent Form for sensory evaluation

Faculty of Human Ecology Department of Human Nutritional Sciences

Weston Sensory Centre
Rm 400 Human Ecology
Bldg.

CONSENT FORM

Research Project Title: Consumer Acceptability of Beef Burgers with Added Pulse Flours – Study 2. Sponsored by: Tiffany Nicholson has a University of Manitoba Graduate Fellowship

Researchers: Dr. Michel Aliani, Dr. Mohammed Moghadasian, Tiffany Nicholson and Donna Ryland, Department of Human Nutritional Sciences

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The study is being done to determine the acceptability of beef burgers with added pulse flours. **A potential risk would be allergic reactions to food products. Due to this risk people with food allergies will not be allowed to participate. Completion by participants of the accompanying questionnaire will confirm that no food allergies exist.**

Participants will be requested to observe, smell and taste as much as they want of not more than seven samples each containing about 12 grams of minced beef (maximum approx 100 g). They will be asked how much they like/dislike the color, aroma, flavor, texture, acceptability overall, and how often they would eat the product. Responses will be indicated by checking the appropriate descriptor on a category scale. Questions regarding gender, age and frequency of eating beef burgers and pulses will also be asked. There will be one 30 minute session held at a scheduled time during the weeks of April 1 and April 8 between the hours of 11:30 and 3:00. An honorarium of a \$10 gift card from the University of Manitoba Bookstore will be offered to those completing the session. Participants may request a copy of the publication when available by contacting the researchers noted above. The study will take place on the Fourth Floor in the Human Ecology Building.

All data will be recorded anonymously and therefore all participants will remain anonymous. Data published will be given as group means with no individual names given. All data related to

personal information will be kept in a locked cabinet in Room 400F accessible only to Donna Ryland for 5 years or until data are published whichever comes first. The University of Manitoba Research Ethics Board(s) and a representative(s) of the University of Manitoba Research Quality Management/Assurance office may also require access to your research records for safety and quality assurance purposes. All data will be shredded after the time has expired.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release researchers, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time, and/or refrain from answering any questions you prefer to omit, without prejudice or consequence. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. This study is being conducted by Dr. Michel Aliani, [REDACTED]

This research has been approved by the Joint-Faculty Research Board of Ethical Review at the University of Manitoba. If you have any concerns or complaints about this project, you may contact the above-named person or the Human Ethics Secretariat [REDACTED]

Participant's Name (Please Print)

Participant's Signature	Date		
Telephone # _____		E-mail	Address

Researcher and/or Delegate's Signature _____ Date _____

Delegate's contact information:

[REDACTED]

Appendix E- Questionnaire for Sensory evaluation

QUESTIONNAIRE

This information will be kept strictly confidential.

1. Have you participated on sensory evaluation panels before?

Yes____ No ____ If yes,

a) What product(s) did you evaluate?

b) Was training part of the evaluation procedure? Yes ____ No ____

If yes, indicate for which product(s).

2. Are you allergic to any food products? Yes ____ No ____

If yes, note them below.

3. Are there any foods specifically, or food flavors and textures generally, that you would prefer not to evaluate?

Thank you very much for completing this questionnaire.