

Effect of Micronization on Selected Volatiles of Chickpea and Lentil flours
and Sensory Evaluation of Low Fat Beef Burgers Extended with these
Micronized Pulse Flours

By

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ABSTRACT

The effect of micronization (at 130 and 150 °C) as a potential heat treatment to reduce 'beany' aroma and flavor of cooked chickpea (*Cicer arietinum*) and green lentil (*Lens culinaris*) flours was investigated. A simultaneous distillation solvent extraction method was developed to extract key volatile compounds with potential contribution to 'beany' aroma and flavor notes in micronized pulse flours and analyzed by gas chromatography-mass spectrometry. Concentrations of volatile compounds such as pentanol, hexanal, 2-hexenal, hexanol, heptanal, furan-2-pentyl, 2-octenal, nonanal, 2,4 decadienal, and 2,4-undecadienal were significantly ($P<0.05$) decreased with micronization. Low fat burgers fortified with 6% micronized chickpea and green lentil flours showed significantly higher acceptability for aroma, flavor, texture, color and overall acceptability ($p<0.05$) compared to non-micronized samples in a consumer acceptability test with 101 consumers. In addition, fatty acid analysis of burgers showed burgers containing micronized pulses had higher level of linoleic and linolenic acid content.

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LIST OF ABBREVIATIONS

AHA American Heart Association

ANOVA Analysis of Variance

APO Asian Productivity Organization

ARD Alberta Agriculture and Rural Development

CBD Cooked Burger Diameter

CBF Common Bean Flour

CBT Cooked Burger Thickness

CBwt Cooked Burger Weight

CDC Crop Development Centre

CGC Canadian Grain Commission

CGPF Corn Germ Protein Flour

CO Control Burger

DMB Deoxy Myoglobin

DOA Dissolved Oxygen Analysis

EFSA European Food Safety Authority

FACT Food Action Rating Scale

FAO Food and Agriculture Organization

FCR Farm Cash Return

GC-FID Gas Chromatography Flame Ionization Detector

GC-MS Gas Chromatography Mass Spectrometry

IR Infra-Red

LOX Lipoxygenase

LRI Linear Retention Indices

MCP130 Micronized Chickpea at 130°C

MCP150 Micronized Chickpea at 150°C

MGL130 Micronized Green Lentil at 130°C

MGL150 Micronized Green Lentil at 150°C

MMB Met Myoglobin

MUFA Mono Unsaturated Fatty Acid

NIST National Institute of Standards and Technology

OMB Oxymyoglobin

PLS Partial Least Square

RBD Raw Burger Diameter

RBT Raw Burger Thickness

RBwt Raw Burger Weight

RCP Raw Chickpea Flour

RGL Raw Green Lentil Flour

RT Retention Time

SDE Steam Distillation/Solvent Extraction

SFA Saturated Fatty Acid

SPME Solid Phase Micro-Extraction

WHC Water Holding Capacity

1. Introduction

The term “pulses” refers to dry, mature, and edible seeds of leguminous plant that are harvested primarily for their seeds and are used as human food and animal feed (2003). Codex Alimentarius Commission uses “pulse” to refer solely to dry seeds of legume plants and separates them from oil seeds that are primarily harvested for their oil content (Abu-Ghannam & Gown, 2011). With this definition, fresh green beans, peas and oil seeds are excluded from pulse seeds.

Canada is one of the main pulse crop producers and exporters in the world with Saskatchewan producing a major part of Canada’s lentils (*Lens culinaris*) and chickpeas (*Cicer arietinum*). Pulse production in Saskatchewan has increased substantially during the past decade (Saskatchewan Ministry of Agriculture, 2011a) producing about 2.6 million tonnes of peas, 1.5 million tonnes of lentils, and 400 tonnes of chickpeas in 2009 making it Canada’s leading producer of these pulse crops among the Prairie Provinces (Saskatchewan Ministry of Agriculture, 2011a). In the same year, Saskatchewan exported \$1.165 billion worth of lentils and \$42 million worth of chickpeas, which accounted for 97% and 75% of the total Canadian exports, and 66% and 6% of the world’s exports of lentils and chickpeas, respectively (Saskatchewan Ministry of Agriculture, 2011a).

Although Canada is the major exporter of pulses in the world, Canadian pulse consumption is as low as a cup of cooked pulses per week with a median of 0.6 cups per week (Faye, 2010). This is much lower than the dietary pulse recommendation by the American Heart Association (AHA, 2012) which recommends 4-5 servings of pulses, nuts and seeds per week. AHA defines one serving as ½ cup cooked dry beans or peas (Lichtenstein et al., 2006). Canada’s Food Guide also recommends a regular intake of ¾

cup (175 ml) cooked pulses (one serving) as a meat alternative to reduce saturated fat in the diet. Health organizations promote regular pulse consumption because they are a valuable source of nutrients and their regular intake is associated with decreased risk of diabetes and coronary heart disease (Leterme, 2002; Mudryj, Yu, Hartman, Mitchell, & Lawrence, 2012; Papanikolaous & Fulgoni, 2008).

Pulse seeds are rich sources of dietary protein, complex carbohydrates, vitamins, minerals, and fiber (Wang & Daun, 2004) which are all required for human health. Pulse legumes still remain the main source of dietary protein for a large segment of the world's population, particularly in the Middle East (Government of Western Australia, 2010), India (Agriculture and Agri-Food Canada, 2009a) and African Countries (Akibode, 2011). Recent studies on health effects of dietary pulses drew the attention of health organizations to look at pulses not only as a source of dietary nutrients but as a functional food (McCrorry, Hamaker, Lovejoy, & Eichelsdoerfer, 2010; Nothlings et al., 2008; Pittaway, Robertson, & Ball, 2008; Tovar, Granfeldt, & Bjorck, 1992). For instance, researchers through a longitudinal, cohort study of 10,449 participants who were followed for 9 years, showed a positive association between dietary intakes of 80 g per day of pulses with reduced risk of all causes of death (Nothlings et al., 2008). This effect could be due to the fact that dietary pulses reduce glycemic effect after consumption (Tovar et al., 1992). These results are in agreement with those obtained by other researchers (Pittaway et al., 2008) who investigated the effect of adding 728 g of canned chickpea per week to the regular diet of 45 participants for 12 weeks, on total blood cholesterol, LDL cholesterol, and fasting insulin. A significant decrease in each of these markers was observed. These effects are due to the soluble and insoluble fiber content of

the pulse seeds which not only help to decrease LDL cholesterol and total cholesterol, but normalize the gastrointestinal function by increasing fecal bulk and small chain fatty acids within the intestine. Mudryj, Yu, Hartman, and Lawrence (2012) examined the effect of pulse consumption on macro- and micro-nutrient intake in Canadian adults over the age of 19 years. The study was carried out by examining the cross-sectional data (n=20156) from the Canadian Community Health Survey _Cycle 2.2 year 2004 which was conducted by Statistics Canada. The respondents were Canadian adults of different age groups from ten provinces within Canada (Mudryj et al., 2012). The survey was conducted using a 24 hours dietary recall to assess the usual dietary intake and eating patterns of Canadian adults residing in different provinces. The results of the study indicate that only 13% of Canadians consume pulse legumes as part of their regular diet, the highest consumption being 294g/day (standard error 40g). The study revealed that the highest consumer of pulses had higher intake of macro- and micro-nutrient intake. The estimated average requirement for some micronutrients including thiamin, vitamin B6, folate, Fe, Mg, P, and Zn were met for individuals with the highest level of pulse consumption. Such studies contribute towards the benefits of daily dietary pulse consumption and/or their incorporation into the production of high protein food products.

Alberta Agriculture and Rural Development (ARD, 2010) in partnership with Alberta Pulse Growers Commission and Pulse Canada conducted a survey through Ipsos Reid (a marketing research firm) about pulse awareness and factors influencing pulse consumption (ARD, 2010). The survey company conducted 1,100 interviews with adult Canadians, ages 18 and over, in Edmonton and Toronto. Ninety-four percent of respondents born and raised in Canada didn't consume pulses. Based on this survey 20%

of the participants consumed no pulses in the past six months, while 60% consumed one or two types of pulses less than once a month and 20% consumed pulses on a weekly basis. The most frequent reasons given for not eating pulses were that people didn't like them; they didn't like the flavor or taste, weren't aware of how to cook them and never even attempted to cook or eat them. Those who answered simply didn't like pulses in a following question indicated that they didn't like texture or taste or didn't know how to cook them. Among pulse consumers, younger adults, 18 to 34 year of age, consume 1.1 cup per week which is significantly lower compared to older participants 35 to 54 years of age consuming 1.3 cups per week. Some of the factors influencing the utilization of pulses, based on this survey, were flavor, texture, and lack of knowledge about cooking methods.

The objectionable flavor in food by pulses is partially due in part to volatile compounds that are perceived by the olfactory system and also due to water soluble compounds which are dissolved in saliva after consumption (Kilcast, 1996). However, some chemical compounds that are taken through the mouth are also perceived through the sense of smell (Kilcast, 1996) and therefore alter the flavor of the consumed food. Based on Kilcast's (1996) definition of flavor, sense of smell plays an important role in sensing the taste of food. In fact, combination of odor perception through the retro-nasal route, and taste perception defines the flavor perception of any given food. Therefore, analysis of volatiles contributing to 'beany' aroma may be a useful approach to better understand the 'beany' aroma and flavor formation.

It has been suggested that the 'beany' aroma and flavor of pulse seeds are mediated by activity of lipoxygenase (LOX) isozymes (Rackis, Sessa, & Honig, 1979). LOX is known

to be involved in the reaction between linoleic acid Z1-Z2-pentadiene units and molecular oxygen, followed by degradation of formed hydro-peroxide to secondary products such as aldehyde and ketones (Baysal & Demirdoven, 2007). Chickpea and green lentil seeds contain 6% and 1.1% of lipids, respectively, (Wang & Daun, 2004) with high concentrations of ω -3 and ω -6 fatty acids providing active sites for LOX activity (EC 1.13.11.12) isozymes. The accumulation of volatile organic compounds generated by LOX activity may be responsible for the 'beany' aroma and flavor formation and causing reduced quality of pulse products (Baysal & Demirdoven, 2007; Rackis et al., 1979).

Lipoxygenases, like most enzymes, are sensitive to temperature and pH (Baker & Mustakas, 1973) and therefore heat treatment methods may be effective in decreasing their activity.

Micronization is a technique used to increase the internal temperature of food samples through increased exposure to electromagnetic radiation in the infra-red region for a short period of time (Sharma, 2009). Micronization may significantly decrease LOX isozymes activities lowering the concentration of key volatile compounds responsible for 'beany' aroma and flavor and ultimately increase acceptability of cooked pulses.

To evaluate the effect of micronization treatment on the key volatile compounds, aroma compounds need to be first extracted, isolated, concentrated and finally identified and quantified using a gas chromatography-mass spectrometry (GC-MS) system. A Steam Distillation/Solvent Extraction (SDE) is one of the methods that usually employed to prepare the volatile extracts prior to GC-MS analysis. In a typical SDE process steam distillation and solvent extraction steps are simultaneously performed using a Likens and

Nickerson apparatus (Chaintreau, 2001). This extraction procedure mimics the cooking process in that pulses are boiled in water generating volatiles that are subsequently condensed using a cooled water condenser enabling their collection into an organic solvent. This combination of isolation and concentration of volatile compounds decreases the operating time and since the volume of solvent unremittingly recycle through the system therefore decreases the amount of solvent needed for volatile extraction (Chaintreau, 2001).

Although the gold standard of analyzing volatile flavor/off flavor compounds in food products is GC-MS, the sense of smell still remains a critical discriminator in the evaluation of aroma and flavor (Lawless, 1991). Food flavor research requires initial instrumental analysis of food aroma and flavor compounds which is then cross-referenced against the human sense of smell (Lawless, 1991). Therefore, to study the effect of micronization on utilization of pulse flour as ingredient in formulation of minced meat products such as low-fat beef-burgers, a consumer acceptance test is critical to evaluate the aroma, taste and texture of the new formulated meat products. Consumer acceptance testing along with instrumental analysis in this study help to shed light on how new pulse processing techniques can improve the quality of cooked pulses as a whole or when used as innovative ingredients such as in the formulation of meat extenders.

2. Literature review

2.1. Pulses

The word “Pulse” in Greek means thick soup and refers to the dry seed of leguminous plants that are used as human food and animal feed (Riahi & Ramaswamy, 2003). The Codex Alimentarius Commission defines pulses as dry seeds of leguminous plants such as lentils, chickpea, pea, cow pea, and bean variety. The above definition eliminates oil seeds harvested for their oil and green beans and peas which are consumed fresh and counted as vegetable (FAO, 1989).

2.1.1. Chickpea and lentil

Chickpeas (*Cicer arietinum L.*) and lentils (*Lens culinaris*) are considered pulse crops which are harvested mainly for their dry seeds (Ramaswamy & Riahi, 2003). Unlike pea which is used as animal feed, lentil and chickpea are used only for human consumption. These crops are important for Canadian economy and they are grown to improve financial return to producers. They contribute to sustainability of food systems and agriculture.

Lentil and chickpea need little or no nitrogen fertilizer due to their nitrogen fixing ability. This is beneficial for crop rotation as it leaves nitrogen behind following the harvest further decreasing the level of pathogens within the soil and controls the spread of crop disease (Johnston, Miller, & McConkey, 1998). Lentil and chickpea like other pulse crops require less soil and water input for crop production compared to cereal grains. These two pulse crops have a high production in the world and are used as food staples in

many countries (Agriculture and Agri-Food Canada, 2009a; Akibode & Maredia, 2011; Pulse Canada, 2007; Shanmugasundaram, 2000).

Chickpea and lentil have been classified as part of the Fabaceas plant family or in other word, legumes (Chavan, Kadam, & Salunkhe, 1987). There are different classes of chickpea and lentil plants which are produced in the world commercially. The two classes of chickpea most commonly produced in Canada are Desi (85% of world chickpea production) and Kabuli (15% of world chickpea production) (Al-Issa, 2006; McVicar et al., 2007). The seeds of chickpea plants come in short, inflated pods which contain only two seeds (McVicar et al., 2007). Although the seeds come in different shapes, colors and sizes, most typically have a small beak which is the site for expanding roots and the surfaces are creased and grooved (McVicar et al., 2007).

Classification and grading of lentil seeds is based on visual inspection of coat and cotyledon color as well as by the size of the seeds, following grading guide regulations specified by the CGC (Canadian Grain Commission, 2012). Lentil seeds can be classified into two categories according to their size: *large seed* when 1000 seeds weigh more than 50 grams or *small seed* when 1000 seeds weigh less than 45 grams (Saskatchewan Ministry of Agriculture, 2010). Two main categories of lentil are red and green based on cotyledon color. The majority of lentils cultivated in Canada have a green surface and yellow cotyledon and can be subdivided into three classes: *large green*, *medium green*, and *small green* (Saskatchewan Ministry of Agriculture, 2010). Red lentil is also grown in Canada but in much lower quantities than the green type. Research at the Crop Development Centre (CDC) within the University of Saskatchewan has developed lentil and chickpea cultivars targeted for specific Canadian agronomic environments (Der,

2010). Contrary to usual green lentil varieties, which require early seeding and are late maturing, thus becoming more vulnerable to harsh weather conditions, the CDC lentil varieties are early maturing, high yielding, and herbicide tolerant (Saskatchewan Ministry of Agriculture, 2010). CDC Sedley (large green), CDC Improve CL (large green), CDC Meteor (medium green), CDC Impress CL (medium green), CDC Milestone (small green) and CDC Viceroy are some of the green lentil varieties produced by the CDC. The large-seeded green lentil type is the dominant lentil crop produced in Western Canada (Saskatchewan Ministry of Agriculture, 2010). CDC ChiChi (Kabuli), CDC Diva (Kabuli), CDC Frontier (Kabuli), CDC Xena (Kabuli), Amit (B-90), CDC Cabri (Kabuli), CDC Desiray (Kabuli), Dwelley (Kabuli), Evans (Kabuli) are some improved chickpea cultivars produced by Crop Development Centre.

Both lentil and chickpea are dicotyledonous legume seeds which are in the market sold either as whole seeds or split. Furthermore, the abovementioned pulse crops represent a great part of the Farm Cash Return (FCR) in Canada and have a great impact on Canada's economic situation. Chickpea and lentil in 2009 represented 53% of total FCR amounting to \$1.7 billion within Canada (Agriculture and Agri-Food Canada, 2009b)

2.1.2. Pulse production in Canada

Lentil and chickpea cultivation dates back to the origin of agriculture (Al-Issa, 2006). They were first cultivated in South West Asia and Turkey about 7000 BC (Saskatchewan Ministry of Agriculture, 2011a). In the past decade, India, Canada, Turkey, Australia, Nepal, United States and Bangladesh were the top producers of lentil in the world (Al-

Issa, 2006; Saskatchewan Ministry of Agriculture, 2011a). According to the FAO, in 2005, world production of chickpea and lentil was 9.17 and 4.03 million tons from 11 and 4 million hectares (Al-Issa, 2006). Canada's chickpea and lentil production accounted for 1% and 29.5% of world totals (Al-Issa, 2006). Overall world export of chickpea between 1996 and 2003 was between 0.5 to 1 million tonnes (McVicar et al., 2007). FAO reported that Canada was the fifth leading chickpea exporting country after Mexico, Myanmar, Australia, and Turkey (McVicar et al., 2007). In 2004, Canada accounted for 9.7% and 31.9% of 333.3 and 497.3 million US dollars of the total world chickpea and lentil export value (Al-Issa, 2006). Saskatchewan was Canada's major contributor of chickpea and lentil production (Saskatchewan Ministry of Agriculture, 2011b). The overall acres devoted to chickpea cultivation in Saskatchewan has increased 29 fold from 1996 (6,000 acres) to 2005 (172,000 acres) (McVicar et al., 2007). As a result, Canada became one of the top producers of chickpea and lentil worldwide with exports to almost 150 different countries (Saskatchewan Ministry of Agriculture, 2011b). About 75% of pulses produced in Canada are exported to other countries and only 25% are used for domestic consumption (Saskatchewan Ministry of Agriculture, 2011b). Lentil and chickpea, unlike other pulse crops, are merely cultivated for human consumption.

2.1.3. Chickpea (Kabuli) and green lentil nutritional quality

Pulse seeds are considered food source with high nutritional value. Pulses are high in protein, digestible and indigestible carbohydrates, polyphenols and some minerals (Silva-Cristobal, Osorio-Diaz, Tovar, & Bello-Perez, 2010). Canadian grown Kabuli type chickpea and green lentil contain 24.4 and 26.3g protein, 45 and 41g starch, 5 and 8.1g

fiber, 5.9 and 1.1g fat (all values are per 100 g dry matter), respectively as shown in Table 2-1 (Wang & Daun, 2004). In comparison, an Australian variety of chickpea and lentil contain 21.5 and 24g protein, 11.9 and 18g of fiber, 5 and 0.9g of fat. The variation in macro- and micronutrient compositions is caused by the seed characteristics, geographic origin and environmental factors. As an example the range of iron content in green lentil grown in Canada is 5.4 – 11.4mg/100g while Australian green lentil contains 4.3 – 341.5mg/100g, the level of mineral composition is shown in Table 2-2.

Table 2-1 Proximate composition Canadian green lentil and chickpea Kabuli

Composition (g/100g dry matter)	Green lentil	Chickpea
Protein (N x 6.25)	21.3 – 30.2	17.9 – 30.8
Starch	41.9 – 48.5	38.2 – 43.9
Soluble fiber	4.5 – 7.4	3.0 - 5.7
Insoluble fiber	7 – 9.5	4.2 – 7.7
Lipid	1.0 – 1.3	5.5 – 6.9

Green lentil (n=36), chickpea Kabuli type (n= 12), (Wang & Daun, 2004)

Table 2-2 Mineral composition of Canadian Green lentil and chickpea Kabuli type

Minerals (mg/100 g)	Green lentil	Chickpea
Calcium	48.4 – 97.0	80.5 – 144.3
Iron	5.4 – 11.4	4.3 – 7.6
Potassium	550.8 – 1286.5	816.1 – 1580.1
Magnesium	104.1 – 167.1	152.9 – 212.8
Phosphorus	260.3 – 725.8	294.1 – 828.8
Zinc	2.9 – 5.9	3.6 – 5.6
Folic acid (µg/100g) n=8	100.0 – 217.0	153.8 – 486.5

Green lentil (n=36), chickpea Kabuli type (n=12), (Wang & Daun, 2004)

Chickpea and lentil contain high amounts of protein and minerals, but their bioavailability is affected by anti-nutritional factors such as protease inhibitors, alpha galactosides, phytate, saponin, tannin and polyphenols (Silva-Cristobal et al., 2010). Protease inhibitors and phytate are able to bind to the key digestive enzymes like trypsin,

chymotrypsin, pepsin and amylase (Muzquiz & Wood, 2007). Binding inhibits the functionality of these enzymes and alters the macronutrients' digestion. Phytate is also able to bind protein, starch and mineral directly and decrease the digestion and absorption of these nutrients. The presence of tannins (polyphenol compounds) also decreases protein digestibility, but unlike the tannin content found in tea, coffee and wine it has no significant effect on iron digestibility (Muzquiz & Wood, 2007). Silva-Cristobal et al., (2010) showed that lentil and chickpea have high polyphenol and anthocyanin content $3.09 \pm 0.2 \text{ mg/g}$, $36.2 \pm 0.6 \text{ mg/g}$ and $0.72 \pm 0.2 \text{ mg/g}$, $14.9 \pm 0.6 \text{ mg/g}$ respectively, with antioxidant capacity in the soluble polyphenol fraction.

As mentioned, lentil and chickpea are low in fat ranging from 1.0–1.3% and 5.5-6.9% (w/w) respectively, the majority being unsaturated fatty acids according to Table 2-1 (Wang & Daun, 2004). Green lentil and chickpea contain two major physiologically active fatty acids, linoleic and linolenic acid. About 51.20% and 2.69% of the total fatty acid in chickpea are linoleic and linolenic acids while 44.38% and 14.15% of the total fatty acid content of green lentil are linoleic and linolenic acids as it is shown in Table 2-3 (Wang & Daun, 2004). Linolenic and linoleic acid structure include the Z1,Z2-pentadiene moiety which is an active site for lipoxygenase (LOX) isozymes activity which are abundant in lentil and chickpea seeds (Hilbers, Finazzi-Agro, Veldink, & Vliegthart, 1996; Hilbers, Kerkhoff, Finazzi-Agro, Veldink, & Vliegthart, 1995; Robinson, Wu, Domoney, & Casey, 1995; Sanz, Perez, & Olias, 1994; Sanz, Perez, Rios, & Olias, 1992). Polyunsaturated fatty acids are susceptible to degradation and produce small volatile compounds with 'beany' aroma characteristics (Eskin, Grossman, Pinsky, & Whitaker, 1977; Rackis et al., 1979).

Table 2-3 Fatty acid composition of green lentil and chickpea Kabuli type

Fatty acid (% in lipid)	Green lentil (Mean)	Chickpea (Mean)
SFA	17.84	12.29
MUFA	22.94	33.50
Oleic acid (C18:1)	20.86	32.56
Linoleic acid (C18:2)	44.38	51.20
Linolenic acid (C18:3)	14.15	2.69

Lentil (n=36), chickpea: (n=12), (Wang & Daun, 2004)

Most of the anti-nutritional factors in pulses can be removed or inactivated using a variety of food processing techniques. For instance, LOX isozymes and protease inhibitors are heat labile and therefore any heating technique can be effective in decreasing their activity.

2.1.4. Pulse consumption in Canada

People in developed countries eat pulses because they are vegetarians (Dietitians of Canada, 2010), have a health problem such as high cholesterol or diabetes and are required to follow a specific diet (Jenkins et al., 1981; Leterme, 2002; Tovar et al., 1992), are trying to maintain a healthy weight using a nutrient dense food product (McCrary et al., 2010; Mudryj et al., 2012), are looking for the benefits of a healthy diet (ARD, 2010; Faye, 2010; Leterme, 2002) , or are focused on the environmental benefits (Faye, 2010).

Based on the survey conducted by Alberta and Rural Development (ARD, 2010), pulse consumption among Canadian adults is low (ARD, 2010). Health organizations such as Dietitian of Canada, Health Canada, and American Heart Association (AHA) are

promoting pulse consumption for the Canadian population through a set of guidelines. AHA suggests consumption of 4-5 servings of pulses, nuts and seeds per week with counting ½ cup cooked pulse variety as a serving (AHA, 2010) and Health Canada's Food Guide (CFG) recommends pulse consumption as often with the definition of one serving as ¾ cup of cooked pulse.

ARD conducted a survey to evaluate factors influencing Canadian behavior toward pulse consumption. The results of the survey by ARD (2010) suggest that 20% of Canadians are not pulse consumers and the non-consumers are mostly young adult age between 18-35 year-old, with a strong relation between vegetarian diet and pulse consumption.

Based on the outcome of the ARD (2010) survey, 5 different groups of people were identified according to their attitude toward pulse consumption. These groups were classified as informed champions, unexposed reachable (23%), forgetful supporters (23%), health driven persuadable (22%), and disinterested unreachable (not pulse consumer). The three middle groups have potential to become part of a regular cooked-pulse consumer group through increased awareness of the health and environmental benefits as well as through the introduction of different ways to include pulses in their diet (ARD, 2010).

Ipsos Reid also interviewed 230 south Asian immigrants who lived in Canada less than 20 years to assess the high pulse consumer immigrants' change of attitude toward pulse consumption over time (ARD, 2010). Pulse legumes are an important part of traditional diet among South Asians. This population is counted as heavy pulse consumers who are interested in a variety of pulse and pulse products. Moreover, the ARD survey results suggest that the majority of South Asian immigrants, who were considered as high pulse

consumers, had an average pulse intake of 2.5 cups/week and a median of 2 cups/week (ARD, 2010). Assessing pulse consumption over time shows the length of stay in Canada had no effect on the level of pulse consumption among this group, however, a transition was observed from more ethnic pulse foods to those which were processed or canned such as hummus (ARD, 2010). The observed change was attributed to food marketing and distribution which had a strong influence on dietary food patterns.

2.1.5. Factors affecting pulse consumption

Nutritional qualities of pulses, urbanization, income, consumer preferences are just some of the factors driving pulse consumption in many countries. Consumption pattern of pulses as part of a regular diet is steadily growing in developed countries. A major contributing factor is increased consumer education and awareness with respect to nutritional quality and health benefits of pulse consumption. Food labels, grocery stores, magazines, books and internet are among the important sources of education for consumers with family and friends contributing to this aspect the most. Knowing that pulses are a very good source of protein and fiber this information drives consumers to add pulses to their diet. According to the ARD (2010) survey the two principle reasons for eating pulses were the health benefits and taste followed by them being a high source of protein and fiber.

Pulses nutritional quality and health benefits motivate consumers to add pulses in their daily diet but being unfamiliar with preparing pulse based foods, lack of ease of preparation and digestive discomfort are some of the barriers for their consumption

(ARD, 2010). Lack of knowledge about “what exactly pulse legumes are” and “preparation methods” accounted for major barriers in pulse consumption among Canadians. The ARD (2010) survey shed light on this matter by evaluating the factors effecting pulse consumption by individuals. For non-consumers (216) taste was the most important factor while for light consumers (666) and moderate to heavy consumers (218) health benefits, a high protein source, taste, and fiber content were the most important factors. Convenience and ease of preparation were also pointed out as important factors affecting pulse consumption. Vegetarianism and digestive discomfort had the least effect on choosing pulses as part of one’s daily diet (ARD, 2010). Cost did not show any significant effect on choosing pulses as part of a regular diet despite the well-known statement about pulses being a “poor man’s diet.”

Furthermore, general reasons quoted for not eating pulses were “Don’t like them” (comprising a majority of the responses), “Don’t like the flavor and texture”, “Don’t know how to cook and prepare them”, and “Inconvenient and too much work for preparation” (ARD, 2010). Individuals who responded that they didn’t like pulses indicated that it was due to the flavor and texture. Flavor and taste are documented as the most important factors affecting any type of food consumption (Clark, 1998). It is also the first bite, that assesses taste, aroma, and flavor which are then quickly evaluated by the individual leading to the decision of whether to continue to eat the food or not (Glanz, Basil, Maibach, Goldberg, & Snyder, 1998).

2.2. Lipoxygenase activity as source of 'beany' aroma

2.2.1. Lipoxygenase activity in pulse seeds

'Beany' or unpleasant odors or tastes in pulses is a major problem for the food industry and it is caused by oxidative degradation of unsaturated fatty acids (Rackis et al., 1979). There is strong evidence that 'beany' aroma and flavor of pulse seeds is mediated by activity of lipoxygenase (LOX) isozymes (Iassonova, Johnson, Hammond, & Beattie, 2009; Rackis et al., 1979). Plant tissues usually contain several non-heme iron containing LOX isozymes catalyzing di-oxygenation reaction of polyunsaturated fatty acids to produce hydroperoxide (Siedow, 1991). Increased levels of LOX during germination, wounding, and pest problems indicate the importance of LOX isozymes at different stages of plant development and physiological defence mechanisms through production of physiologically active compounds (Hilbers et al., 1995). LOX is known to be involved in the reaction between Z1-Z2-pentadiene units in polyunsaturated fatty acids such as linoleic and linolenic acid and molecular oxygen, which results in the formation of hydroperoxide followed by degradation to secondary products such as aldehydes and ketones (Baysal & Demirdoven, 2007).

LOX isozymes molecular structure and characteristics are broadly studied in soybean (Baysal & Demirdoven, 2007). Four isozymes have been isolated in the soybean with difference in optimum pH, substrate and products. The two most occurring are LOX-1 and LOX-2. Soybean LOX-1 catalyzes oxidation of free polyunsaturated fatty acids to produce 9- and 13-hydroperoxides in ratio of 1:9 with the optimum pH of 9.0. Soybean LOX-2 is active at pH of 6.8 and catalyzes the peroxidation of free or bonded polyunsaturated fatty acids to form the two types of peroxides with the ratio of 1:1 with

the ability to co-oxidate carotenoids (Baysal & Demirdoven, 2007). LOX isozymes in a variety of plants can be categorized based on characteristics of soybean LOX isozymes; Type-1 which is active at alkaline condition and only catalyze free fatty acids and type-2 that is active at neutral pH condition and act on triglyceride and free fatty acids (Baysal & Demirdoven, 2007).

So far several isozymes with different physiochemical properties have been identified in chickpea and lentil seeds (Hilbers et al., 1996; Sanz et al., 1992). CL-1 and CL-2 (two active forms of chickpea LOX isozymes) differ in pH optima and the oxidized products are formed after oxidation of linolenic acid (Sanz et al., 1992). CL-1 has characteristics of type-2 LOX, while CL-2 is more considered as type-1 LOX with alkaline pH. Lentil seeds also contain similar types of LOX isozymes as chickpea (Hilbers et al., 1995). C1 and C2 LOX have been identified in lentil seeds, however, each type of LOX consists of a few different isozymes which searchers have not been able to isolate (Hilbers et al., 1995). Lentil seeds LOX isozymes showed two different pH optima of 6.5 and 9 with different substrate preferences (Hilbers et al., 1995). These isozymes are present at various concentrations in chickpea and lentil seeds having various enzymatic activities depending upon surrounding factors. Chickpea and green lentil seeds, containing 6% and 1.1% lipid as shown in Table 2-3 (Wang & Daun, 2004), offer relatively high concentrations of ω -3 and ω -6 fatty acids providing active sites for activity of LOX isozymes. The concentration of the two key 13- and 9- hydroperoxides and the volatile produced after degradation of them depends on the pH condition and type of substrates (free polyunsaturated fatty acid or triglyceride bond polyunsaturated fatty acid). The hydroperoxides can be further degraded into carbonyl compounds (C-6 to C-9 aldehydes

and alcohols) (Fauconnier & Marlier, 1997). The accumulation of produced volatile organic compounds generated by LOX activities is responsible for the “beany” aroma and flavor and has deleterious effects on the quality of pulse foods (Baysal & Demirdoven, 2007; Rackis et al., 1979).

Pre-treatment of pulses is a common practice in the food industry and can provide nutritional and organoleptic benefits (Walker & Kochhar, 1982). Several heat treatment methods are currently used for pre-treatment of pulse seeds and cereal grains including boiling (Siegel & Fawcett, 1976), radio frequency (McCroory et al., 2010) energy (Ahmed, Malek, Ahmed, Rahman, & Juni, 2011; Guo, Wang, Tiwari, Johnson, & Tang, 2010) and infra-red radiation known as micronization (Sharma, 2009). Micronization has been shown to significantly decrease anti-nutritional properties and improve protein quality (Khattab & Arntfield, 2009) and decrease beany characteristic of pulse seeds (Sharma, 2009).

2.2.2. Micronization

Micronization is a technique used to increase the internal temperature of food samples by exposing them to electromagnetic radiation in the infra-red region for a short period of time increasing the internal temperature of food items (Sharma, 2009). During micronization absorbent material such as pulse or cereal grains are exposed to electromagnetic radiation in the infrared (IR) region. Infra-red radiation is between visible light and radio waves. Radiation with longer wavelengths has less energy than radiation with shorter wavelengths. IR radiation has longer wavelengths than visible light and travels at the speed of light and has wavelengths between 0.75 and 400 μm . Infrared

radiation covers a wider spectrum which can be divided into three regions: near-infrared with the shortest wavelength (0.75-1.4), medium region (2-4 μm), and far infrared (4-400 μm). Infrared wavelengths of 1.8 – 3.4 μm , which fall into the medium region, are used for micronization techniques. The source of energy for micronization is a heated ceramic tile to temperatures of 750°C-930°C. These heated tiles produce the IR radiation necessary for micronization. IR energy acts directly on the food and does not influence the air temperature within the work environment. During micronization, the electromagnetic waves initiated from ceramic tiles hit the food particles. Food molecules absorb this radiation and start to vibrate and generate heat which in turn causes an increase in the water vapour pressure inside the seeds. This causes the food particles to swell and soften and starch molecules become gelatinized. IR technology has many benefits for food industries such as short processing time with high rates of heat transfer to food particles, low energy consumption and food products with increased nutritive quality (Khattab & Arntfield, 2009; Sharma, 2009).

2.2.3. Micronization and nutritive value of pulses

Research efforts have primarily focused on using micronization technology to increase nutritive value, digestibility of carbohydrates and amino acids as well as to reduce anti-nutritional factors in cereal grains and pulses for animal feed (Huang, Sauer, Pickard, Li, & Hardin, 1998; Igbasan & Guenter, 1997). Igbasan & Guenter (1997) showed that micronization is beneficial in decreasing anti-nutritive substances such as trypsin inhibitor and hemagglutinin in field peas as feed for laying hen. They also reported the possibility of structural changes in protein and starch content due to heating treatment

thus making animal feed more digestible. Denaturation of protein is also a reason for inactivation of anti-nutritional factors (Arntfield et al., 1997). The same results were reported by Huang et al. (1998) that micronization processing of barley improved amino acid and energy utilization of barley in young pigs. Recent attempts have been made to study the effects of micronization on the cooking quality, anti-nutritional factors, and protein quality of pulse seeds including lentils, peas, chickpeas and beans (Arntfield et al., 1997; Bellido, Arntfield, Cenkowski, & Scanlon, 2006; Khattab & Arntfield, 2009). Arntfield et al (1997) examined the effect of tempering seeds and level of moisture on micronized lentil quality. They reported that high moisture content seeds result in softer texture which is explained by better gelatinization of starches in lentil seeds during micronization. Arntfield et al (1997) also reported that micronization had a slight effect on lentil color. Bellido et al. (2006) reported the same results for micronization of navy and black beans. Higher moisture contents of the bean during micronization are attributed to softer textures of the cooked beans. Khattab et al (2009) examined the effects of micronization and other treatments such as water soaking, boiling, roasting, microwave cooking, autoclaving, and fermentation on anti-nutritional content of cowpea, pea, and kidney bean. The anti-nutritional factors examined in this study were tannin, phytic acid, trypsin inhibitors and oligosaccharides. Micronization showed a decreased level of oligosaccharides in pulse seeds, yet the effect was less pronounced compared to autoclaving and fermentation. Micronization like soaking and roasting showed a reduction of 32-38% in phytic acid content of abovementioned pulses. This effect can be related to a water tempering step used in micronization and to the solubility of phytate in water (Khattab & Arntfield, 2009). Unlike phytate, trypsin inhibitors are heat sensitive

and inactivated with different cooking methods. Khattab and Arntfield (2009) reported 88.8-94.4% reduction in trypsin inhibitor by micronization heating of selected pulse seeds. The soaking step of micronization also could have a role in reduction of trypsin inhibitor as it has been reported that 11% enzyme activity reduction occurs with long soaking times at room temperature (Khattab & Arntfield, 2009).

2.2.4. Effects of micronization on 'beany' aroma and flavor

LOX isozymes are active components of pulse seeds and mediate di-oxygenation of polyunsaturated fatty acids to hydroperoxide (Eskin et al., 1977; Siedow, 1991). LOXs are major contributors of off-aroma in legumes and limit utilization of legumes as food ingredients (Rackis et al., 1979). In many studies these isozymes have been implicated as the main cause of undesirable aroma which have been described as dusty, musty, earthy, green grassy and 'beany' (Vara-Ubol, Chambers, & Chambers, 2004). The off-aroma is developed by an accumulation of organic volatile aldehydes, alcohols and ketones from the degradation of linoleic and linolenic acids (Rackis et al., 1979).

LOX isozymes are heat and pH sensitive (Baker & Mustakas, 1973). A soy protein concentrate prepared with zoetrope extraction along with steam heating can be free of off-flavor and aroma (Wolf, 1975). Iassonova et al. (2009) showed that heat treatment of defatted soy flakes with soy bean oil, followed by Dissolved Oxygen Analysis (DOA) decreased the level of formed hexanal, 1-octen-3-ol, pentylfuran, octanone, octenal and nonenal compared to unheated soy flakes used as control. The aforementioned compounds are reported as 'beany' aroma compounds (Vara-Ubol et al., 2004).

Iassonova et al. (2009) showed the formed chemicals are related to the activity of the LOX isozymes and the activity can be controlled by heating process.

Zilic, Sobajic, Kresovic and Vasic (2010) have evaluated the effect of dry extrusion at 100, 125, 140°C, micronization at 100, 125, 140 and 150°C, microwave roasting for 1(57°C), 2(88°C), 3(108°C), 4(121°C), 5(132°C) minutes and autoclaving at 120°C for 10, 20 and 30 minutes on LOX activities in two different soy bean cultivars. The authors reported that all types of heat treatment decreased the activity of soy bean cultivar LOX isozymes, with the highest decrease in extrusion and micronization at 100°C. Exception to these results was the microwave heating for 1 minute with temperatures reaching 57 to 60°C, which lead to increased LOX activity (Zilic et al., 2010). The result of this study is in agreement with Baker and Mustakas (1973). They reported that heating soy bean at 100°C for 15 minutes inactivated lipoxygenase, but inactivation occurred within 15 min at 50°C only with addition of acid or base (Baker & Mustakas, 1973). A full-fat flour of dehulled soy bean with good flavor was successfully prepared by dry heating at 100°C or steaming (Mustakas et al., 1969). LOX isozymes are heat labile and can be inactivated by heat treatment; therefore micronization can improve the characteristics of legume flour. However, factors such as heating temperature in regard to the type of seed also should be monitored to achieve complete enzyme inactivation and optimal decrease in 'beany' aroma.

2.2.5. Volatile compounds contribute to 'beany' flavor

Utilization of legume flour as an ingredient in a meat prototype depends on the characteristics of the flour. 'Beany' flavor and aroma limit the acceptability of legume

flours as extenders in meat products (Rackis et al., 1979). In order to evaluate the volatile compounds causing beany flavor in pulse flour one needs to first determine the cause of the problem, then determine what type of compounds are involved and finally how to measure the amounts in which they are present within the sample.

The presence of volatile compounds such as aldehydes, ketones, and alcohols has been associated with 'beany' aroma and off-flavor in legumes seeds (Rackis et al., 1979). For instance, small alcohols such as iso-pentanol, hexanal, and heptanol are considered the source of green bean-like odor and characteristic in soybean (Arai, Koyanagi, & Fujimaki, 1967). Vara-Ubol et al. (2004) assessed the sensory properties of chemical compounds that have been associated with 'beany' flavor and aroma in the literature shown in Appendix A. They designed experiments to define the 'beany' characteristic and odour description of 19 different chemical compounds which have been reported as 'beany' in literature with the help of five trained panellists. They evaluated a wide range of different bean samples in order to define the 'beany' aroma. The panellists examined chickpea, soy bean, soy milk, tofu as well as a set of different beans that were canned, cooked, dried, or frozen. The panel described the 'beany' characteristic as a combination of musty/earthy or musty/dusty with other characteristics described as green/pea pod, nutty or brown. They further evaluated a set of chemicals in the head space of legumes that had been characterized as 'beany' and were not toxic if inhaled in relation to their concentration. They reported that some volatile compounds such as hexanal, hexanol, and pentyl-2-furan which have been associated with 'beany' aroma in the literature did not show 'beany' odour at any level of concentration. Instead, other volatile compounds such as 3-methyl-1-butanol and pentanal at 1 ppm, acetophenone, 1-octen-3-one, and 3-

isopropyl-2-methoxypyrazine at 10 ppm, and 2,4-heptadienal and 1-octen-2-ol at 100 ppm were shown to impart beaniness (Vara-Ubol et al., 2004). A description of beany aroma with definition and a list of these compounds which has been adapted from Vara-Ubol is shown in Table 2-4.

Table 2-4 Chemical compounds were reported that exhibit no ‘beany’ aroma in Vara-Ubol (2004) study

<i>Chemical compounds</i>	<i>Odor characteristics</i>
<i>Hexanol</i>	<i>Musty/earthy, peanut hull, perfumy, chemical</i>
<i>Propanal</i>	<i>Sweet or sour aroma, yeast/pungent/chemical aroma</i>
<i>Pentanal</i>	<i>Musty/earthy, sour aroma, sweaty, cheesy, chemical</i>
<i>Hexanal</i>	<i>Green/pea pod, rancid, sour, chemical aroma</i>
<i>Octanal</i>	<i>Waxy, sweet, fruity, soapy aroma</i>
<i>Trans-2-hexenal</i>	<i>Green grass, cherry, almond</i>
<i>Trans 2-octenal</i>	<i>Musty/earthy, waxy, spicy</i>
<i>Trans-2-nonenal</i>	<i>Musty/dusty, green/grass, sweet, floral, chemical aroma</i>
<i>Trans,trans-2,4-decadienal</i>	<i>Heated oil, floral</i>
<i>2-pentyl furan</i>	<i>Musty/earthy, mushroom like, perfume, floral</i>

Blagden and Gilliland (2006) reported methanol, acetaldehyde, ethanol, and hexanal as the main volatile compounds contributing to ‘beany’ characteristics of soy milk. They considered that the ‘beany’ characteristics of the aforementioned volatile were related to a combination of them rather than from the individual compounds themselves. Bott and Chambers (2006) studied the effect of combinations of volatile organic compounds associated with ‘beany’ aroma and flavor. They examined combinations of chemicals that had been evaluated by a trained sensory panel described by Vara-Ubol (2004) and reported ‘beany’ or ‘non-beany’ aroma compounds (Bott & Chambers-IV, 2006). They found that combinations of ‘beany’ and ‘non-beany’ compounds produce more intense ‘beany’ aromas compared to each individual chemical compound. For instance, 1-octene-3-ol when combined by hexanal imparts higher ‘beany’ intensity compared to 1-octene-3-

ol aroma intensity. Based on reports from Vara-Ubol (2004) 1-octen-3-ol, 1-octen-3-one, 3-methyl-1-butanol and acetophenone impart 'beany' aroma as a single compound and hexanal, pentanal, *trans*-2-hexanal, *trans*-2-octenal, *trans*-2-nonenal, *trans-trans*-2,4-nondienal and *trans, trans*-2,4-decadienal showed no beaniness at any concentration. A flavor and food matrix interaction study conducted by Adhikari, Hein, Elmore, Heymann and Willott (2006) hypothesized that the presence of two or more different flavor compounds in a food matrix can either enhance or suppress the perception of that volatile compound compared to single aroma compounds. The authors hypothesized that the interaction between two or more different aroma compounds and the interaction of aroma compounds within the food matrix determine the perception of that compound. They evaluated three different flavor compounds diacetyl, decalactone and hexanal in two different food matrices: water and skim milk (Adhikari et al., 2006). For the purpose of the study, 12 trained panelists evaluated the threshold of each flavor compound either individually, a combination of two flavors, or a combination of three flavors in two different food matrices. The authors found that diacetyl had two-fold higher threshold in skim milk than water, while presence of decalactone decreased its threshold in two flavor settings. In addition, diacetyl showed higher threshold when both decalactone and hexanal were present. Therefore, a specific volatile compound that contributes to beany characteristics may exhibit different aroma characteristics in the presence of other aroma compounds and food matrix (Adhikari et al., 2006). For instance, hexanal enhances the 'beany' attribute of 1-octene-3-ol (Bott & Chambers-IV, 2006) and a combination of methanol, acetaldehyde, ethanol and hexanal, which are the main volatile compounds in soymilk, are considered to be involved in the flavor of soy milk, not each component

individually (Blagden & Gilliland, 2006). Volatile aroma compounds can interact with components of food matrix such as fat, protein or carbohydrates. The interaction determines the volatility of each flavor and if they reach the retro-nasal cavity (Adhikari et al., 2006).

2.2.6. *Instrumental analysis of aroma*

The sensory evaluation and aroma analysis may be paired with instrumental techniques. The most widely used instrument for aroma and volatile studies is gas chromatography (GC) coupled with mass spectrometry (MS). GC-MS is a technique which allows the identification and quantification of volatiles. However, prior to GC-MS analysis, volatile compounds need to be extracted and concentrated using an appropriate extraction method.

2.2.7. *Simultaneous Distillation/solvent Extraction (SDE)*

To eliminate the possible matrix effect on aroma analysis sample preparation is necessary. One of the methods for volatile extraction is simultaneous distillation solvent extraction (SDE) methods (Likens & Nickerson, 1964). This method can be utilized to perform two steps of isolation and concentration at the same time. SDE technique is based on both solubility and volatility of chemical compounds of interest (Chaintreau, 2001). Either solubility-based or volatility-based techniques each have their drawbacks if used solely for volatile extraction. Solubility-based techniques, such as solvent extraction, leads to co-extraction of non-volatile and other components of food matrix

whereas volatility-based techniques, such as solid phase micro-extraction, isolate only highly volatile compounds with low concentration (Chaintreau, 2001).

The SDE method allows for isolation of volatile compounds through distillation while concentrating the isolated compounds in the solvent of choice (Chaintreau, 2001). The device for this method is called a Likens and Nickerson apparatus which was first designed to isolate and evaluate the aroma of hop oil (Likens & Nickerson, 1964).

Sample (in water) and organic solvent are heated to their boiling point separately in two flasks at different temperatures, each attached to one arm of the apparatus. The volatile generated from the sample is carried to the upper arm of the apparatus by water vapor pressure and then condenses on the cold finger surface while being extracted by the condensed organic solvent as illustrated in Figure 2-1. Both this solvent and the extracted volatile return to the original organic solvent flask while water returns to the sample flask (Chaintreau, 2001). Co-condensation of water vapor containing the volatile, and solvent vapor, and contact between these two phases on the surface condenser provides optimum conditions to exchange the volatile between two phases (Chaintreau, 2001).

The cold water that moves through the cooling condenser is also a critical factor in preventing solvent evaporation and loss of the volatile compounds (Moldoveanu & David, 2002). Fundamentally, SDE is a steam distillation followed by a liquid-liquid solvent extraction. This method takes advantage of volatile compound solubility in both water and organic solvent.

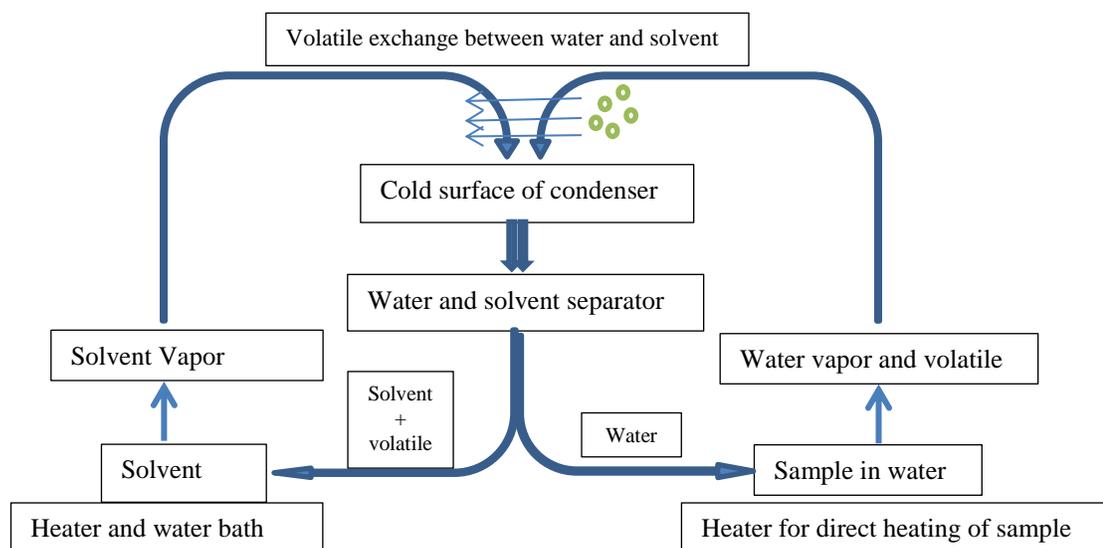


Figure 2-1 Schematic diagram of SDE

Some volatiles are developed or released during cooking which may contribute to beany characteristic. In SDE method, the sample boils in water during both distillation and extraction steps which resembles the cooking process of pulses (Chaintreau, 2001). 1-octene-3-one was detected in volatile aroma of cooked mushroom boiled in water after 15 minutes with SDE method, while the concentration increased 3 fold after 30 minutes to 1 hour of boiling (Picardi & Issenberg, 1973). The volatile analysis of raw mushroom extracted by vacuum assisted SDE at 45-49°C did not show any trace of 1-octene-3-one which indicates the effect of cooking on production of some volatiles.

Organic volatile compounds have a higher affinity to the organic solvent during the extraction process (Chaintreau, 2001). However, some water during extraction may enter the solvent flask either as a molecule of water attached to volatile compounds or a few drops of water at the time of adding the remaining solvent in the separator part of the apparatus to the solvent flask. Water should be removed from the extract prior to organic

solvent concentration and especially before introducing the volatiles onto the GC-MS as these instruments are highly sensitive to water molecules. Water is usually removed in two steps of freezing followed by using anhydrous salt. Freezing the extract causes water in the extract to form crystals and by changing the flask, the water crystals remain in the original flask. The final step involves solvent evaporation by using nitrogen evaporator (Barra et al., 2007; Cadwallader, Tan, Chen, & Meyers, 1995; Lee, Suriyaphan, & Cadwallader, 2001; Madruga, Elmore, Dodson, & Mottram, 2009).

Another factor that can help the recovery of volatiles from sample matrix is through using salt in the sample/water mixture. Salting out reduces the solubility of some polar organic compounds in water (Moldoveanu & David, 2002).

Factors affecting the optimum time of extraction in SDE method depend upon the type of food matrix being extracted as well as the total lipid content as foods having higher lipids require longer extraction times (Chaintreau, 2001). A general rule is 1 to 2 hours for non-fat samples and longer times requiring optimization for higher fat foods. Bouseta and Collin (1995) optimized the time and condenser temperature for a mixture of compounds usually found in honey. The results showed that between 30 to 45 minutes had the highest analytes recovery with an optimum condenser temperature of -5°C for most of the analytes. The exceptions were benzaldehyde and two other terpenes which required condenser temperatures of -10°C (Bouseta & Collin, 1995).

A blank extraction is usually carried out after optimization of all the experimental conditions to eliminate the possibility of contaminants coming from distilled water and/or organic solvent (Chaintreau, 2001). The blank experiment consists of all experimental extraction with the omission of the food sample.

An advantage of SDE is that it uses very small volumes of solvent due to the recycling nature of the Likens-Nickerson apparatus. This is important since the use of high quality grade solvents is very expensive and solvent disposal is then minimal (Chaintreau, 2001). The Likens-Nickerson apparatus is simple to use and highly efficient once all factors such as cooling, heating temperatures for solvent and sample as well as sample preparation are optimized. The technique is widely used to study a range of aroma volatiles, including those of meat (Cadwallader et al., 1995; Madruga et al., 2009), meat products (Ansorena, Gimeno, Astiasaran, & Bello, 2001; Xie, Sun, & Wang, 2008), fruits (Matthews & West, 1993; Sinyinda & Gramshaw, 1998), herbs (Yu, Wu, & Ho, 1993), and beer (Likens & Nickerson, 1964). However no studies have been carried out to evaluate volatile off-flavor of micronized pulses or overall volatile profiles of micronized pulse seeds using SDE.

2.3. Beef burger

Burgers are one of the most consumed fast food items containing high salt, high calories, and high fat (Samil, 2000). Burgers are popular among university and college students as well as on family menus (Nelson, Story, Larson, Neumark-Sztainer, & Lytle, 2008). In addition, half of the meat consumed in industrial countries comes in the form of processed meat such as sausages, burgers and meat pies (Kearney, 2010). Ground beef patties and beef burgers have been consumed by many people regardless of their age and ethnicity. In 2005, Americans consumed an average of 6.5 kg ground beef per capita in the form of hamburgers or meatballs in restaurants or fast food stores. This value represents 60% of beef that is eaten away from home (Davis & Lin, 2005). In 2007,

American consumption of hamburger was about 15.1 Kg per capita (American Meat Institute, 2009). Despite all health-related alarming data and publicities against consumption of red meat and red meat products such as hamburger, they remain a popular meal among North Americans. Considering the high consumption of beef burgers, with high levels of animal fat (about 30%) and the prevalence of chronic diseases in North America, there is increased demand for healthier burger prototypes.

2.3.1. Low fat beef burger

Commercially produced beef-burgers' fat content can vary between 10 to 30%.

According to the Canadian Food Inspection Agency (CFIA) (2012), low fat beef burgers (extra-lean and lean) are allowed to have 10-17% fat compared to medium and regular beef burgers with 23-30% fat. Meat fat is an important factor in meat flavor and other sensory characteristics of beef-burgers and beef patties (Mallika, Prabhakar, & Reddy, 2009). Reducing the fat content leads to a firm, rubbery texture, bland, dry and dark burger after cooking (Keeton, 1994). On the other hand, high fat content leads to higher cooking loss compared to its low fat counterpart (Huffman, Egbert, & Frobish, 1990).

Although there is a high demand for low-fat beef-burgers from health conscious consumers and from the food industry, burgers with less juiciness, rubbery texture and bland flavor won't be accepted by consumers (McDonagh, Troy, Desmond, & McDermott, 2004; Resurreccion, 2003). Researchers in Alabama Agricultural Experiment Station at Auburn University conducted a research project to develop minced meat products with 95% lean meat content and very low fat content (5–10%) which would be acceptable to consumers (Huffman et al., 1990). They reported that the higher

fat level was associated with more cook loss. In addition the consumer study of burgers with different fat levels (5, 10, 15, 20, 25%) showed that fat levels of 15 to 20% are more desirable compared to low fat levels of 5-10% (Huffman et al., 1990). In addition, burgers with 15 to 20 % fat were juicier compared to 5%. Considering the results of the above project, although the addition of small amounts of hydrolyzed vegetable protein (0.13%) improved the overall acceptability of low-fat beef burgers, the addition of whole pulse flour can add fiber and enhance the nutrient content. Therefore, it is beneficial to develop an alternative natural ingredient, such as chickpea and lentil flour, to replace the fat while increasing the palatability of the low-fat beef-burger. It has been well established that addition of protein isolate, carbohydrate, or water can partially replace fat in minced meat products and improve flavor, texture and other physical properties of the low-fat burgers (Kassem & Emara, 2010; Keeton, 1994; Mallika et al., 2009). There is a need to study other plant-based ingredients such as pulse legume flour to replace part of the red meat content of comminuted meat products.

2.3.2. Meat binder (extender and filler)

Extenders are non-meat sources of proteins such as soy bean protein or pulse flour whereas fillers are non-meat substances with high level carbohydrates that are used as binders in meat products (FAO, 2012). Using filler and extender substances in comminuted meats products is a common practice in the meat industry in order to reduce prices. Adding these non-meat substances also can improve the water holding capacity, texture, binding, and juiciness in meat products (Dzudie, Scher, & Hardy, 2002). Plant sources of extenders include peas, beans, lentil and chickpea, all of which contain fibers (Wang & Daun, 2004). Meat products fortified with fiber are a new line of healthy foods.

Fiber also contributes to some functional properties such as water binding, fat retention, better flavor and softer texture in low-fat meat products (McDonagh et al., 2004).

2.3.3. Utilization of pulse flour in low fat meat product

The pulse consumption survey conducted by Ipsos Reid revealed that dietary cooked pulses are low among young adults compared to older individuals (ARD, 2010). By adding pulse flour to popular food items such as burgers and bologna, two goals can be met. The first is to decrease saturated fat in the diet and second to increase the level of pulse consumption amongst the younger generation.

Increased interest in both healthy lifestyles and foods demands that food products be produced having low fat and calorie contents. Ironically, consumers are still desiring juiciness, and flavor of meat and meat products of which it is the increased fat content that contributes to this (Mallika et al., 2009).

Food producers use different technology to partially replace meat in meat products with low fat content to maintain sensory characteristics of products as well as improve their nutritional quality (Malika, 2009). Considering the health benefits of pulses, the pulse industry has focused on new marketable products for healthy, nutritious pulse flour or whole seeds (Agriculture and Agri-Food Canada, 2009b).

According to Food and Drug Regulations (B.14.074), extended meat products should contain no less than 16% protein and no more than 25% fat (Food and Drug Regulations, 2013). Low fat ground beef (90% lean meat and 10% fat) contains 21.43/100g protein (USDA, 2012), while Canadian green lentil and Kabuli chickpea provide 26.3 and 21.4

/100g (W/W) protein (Wang & Daun, 2004). Pulse flour increases the yield of meat products, and in contrast to burgers extended with rice flour or bread crumbs, the nutritional quality of burgers remains high (Pearson & Gillett, 1996). Similar results have been reported by Moharram and colleagues (1987) that beef burgers containing 25% faba bean or chickpea have higher protein content than beef burgers extended by rice flour (Moharram et al., 1987). In addition Modi, and colleagues (2003) studied the effect of adding 8% legume flours (soya bean, Bengal gram, green gram, and black gram) roasted and unroasted to low fat buffalo meat burger. They showed roasted legume flour significantly increased the cooking yield of products in some of the legumes (green gram and black gram) compared to products containing unroasted legume flours. They also showed all burgers with added legume flours had a high acceptability even after 4 months of storage. Previous research has successfully developed beef burgers with 35, 42.5, and 50% pulse-to-meat ratio using twenty three different types of pulses including a variety of beans, chickpea, lentil, and peas (Holliday, Sandlin, Schott, Malekian, & Finley, 2011). They prepared the pulses by soaking, drying, and passing through a grinder. Weight loss, decrease in diameter, and color of the patties were instrumentally evaluated, however, the acceptability of beef-patties with added pulses wasn't evaluated by consumers. There is a possibility that the taste and flavor of such products will be objectionable to consumers. Still beef burgers with added rice flour or bread crumbs have high acceptability (Kurt & Kilincceker, 2012). There is a need to study the effect of different processing of pulses on acceptability of meat products with added pulse flours.

2.3.4. Effect of micronization on sensory characteristics of burgers with pulse flour

The effect of micronization on sensory properties of beef-burger with added micronized green and red lentil flour has been documented (Der, 2010). Using micronized lentil flour as a binder decreased juiciness of beef-burgers compared to the no-binder control and non-micronized lentil binder. Non-micronized lentil binder beef burger was juicier than control with no-binder. The author has reported no difference in flavor intensity and aroma of beef-burgers with micronized and non-micronized lentil flour, however, in terms of overall acceptability of flavor the micronized lentil binder had a significantly higher score (Der, 2010). In addition, micronization of binders showed a positive effect on tenderness of beef-burgers (Der, 2010). In this study the color of the burgers wasn't part of a sensory evaluation. The effect of micronization on lentil color has been evaluated in different studies (Arntfield et al., 1997; Der, 2010). The change in color of the lentil after micronization also may have an effect on color of raw and cooked beef-burgers with lentil flour binder. In addition, the color of raw and cooked burger is very important in market value and consumer acceptability of these products. Therefore, a darker green lentil with more redness and less yellowness after micronization at 170°C (Arntfield et al., 1997) could change the color of beef-burgers to darker color in raw and cooked states.

2.3.5. Flavor

Flavor is one of the most important factors in quality and acceptability of cooked pulses and food products containing pulse ingredients (ARD, 2010). Belitz, Grosch, and Schieberle (2009) defined 'flavor' as the combination of taste, odor and textural

characteristics of consumed food. According to Belitz et al. (2009) compounds contributing to taste are soluble in water and perceived by taste buds located on the tongue while compounds contributing to aroma are volatile at room temperature and perceived by odor receptors located in the olfactory epithelium. Aroma perception is considered to play a major role in flavor formation than taste (Belitz et al., 2009). There are only five primary taste sensations sour, sweet, salty, bitter, and umami while a large number of flavors exist in different food items. A combination of aroma and taste will make the large number of flavors possible (Belitz et al., 2009).

The generation of undesirable 'beany' flavor in some legumes and vegetables has been attributed to the enzymatic oxidation and degradation of polyunsaturated fatty acids to their volatile carbonyl compounds (Eskin et al., 1977; Rackis et al., 1979). Specific volatile compounds such as hexanal, hexanol, 2-pentylfuran and 3-hexenal have been identified as oxidation products of linolenic and linoleic acid (Eskin et al., 1977; Rackis et al., 1979). Food legumes containing high levels of unsaturated fatty acids are susceptible to oxidation rancidity (Rackis et al., 1979). Over 50% of fatty acids in chickpea and lentil seed is linoleic and linolenic acids as shown in Table 2-3 (Wang & Daun, 2004). Activity of LOX during storage or after milling of chickpea and lentil seeds may influence the development of 'beany' aroma making them undesirable for consumption limiting their utilization in formulation of food products. Total and/or partial inactivation of LOX may play a major role to decrease the formation of key flavor volatiles contributing to 'beany' aroma and improving the quality of pulse seeds.

Pretreatment of legumes to decrease the 'beany' characteristic has been explored in several different studies (Okaka & Potter, 1979; Zilic et al., 2010). Soaking in acidified

water (pH 2 and pH 6) followed by blanching in steam at 100°C was used to produce cowpea powder however, soaking in water with pH 4 followed by blanching was less effective (Okaka & Potter, 1979). A sensory panel compared a combination of acidification and blanching to acidification alone showed a significant decrease in 'beany' attribute of the legume. The presence of multiple LOX isozymes with different pH optima in pulse seeds may explain these results (Baysal & Demirdoven, 2007; Okaka & Potter, 1979).

Although heating treatment is more effective in reducing 'beany' compounds compared to other processing methods such as crushing and soaking (Iassonova et al., 2009), reducing 'beany' aroma in pulses depends on the type and duration of the heating method. For instance, microwave heating for one minute causes a sudden increase in LOX activity, while after 2 or 3 minutes the level of activity decreases (Zilic et al., 2010). Reaching temperatures of 100°C, either in micronization or extrusion techniques, has been demonstrated to be effective for decrease LOX activity (Zilic et al., 2010). Lv, Song, Li, and Guo (2011) demonstrated a gradual decrease in LOX activity after processing soy milk using hot water blanching (80-100°C) and grinding for 2, 4, 6, 8 and 10 minutes. In addition, the gradual decrease in LOX activity with processing was correlated with a decrease in level of 'beany' aroma compounds (Lv et al., 2011). Overall, time, temperature and pH are the main factors to be considered for an effective inactivation of LOX enzymes.

Micronization is one of the methods which has been applied to legumes in order to prevent formation of 'beany' aroma (Zilic et al., 2010). Micronization had been effective for inactivation of LOX isozymes in different pulse seeds (Dillard, Henick, & Koch,

1960). Given the fact that similar enzyme activities exist in chickpea and lentil seeds, it can be hypothesized that similar heat treatments may be effective for decreasing 'beany' aroma of chickpea and lentil flours. However, blanching and heating methods may also cause the loss of nutrients (Okaka & Potter, 1979). During micronization procedure seeds are exposed to intensive heat waves and temperature is increased ($>100^{\circ}\text{C}$) in less than a minute which may effectively decrease LOX activity. Analytical instruments such as gas chromatography mass spectrometry (GC-MS) and sensory evaluation approaches may be used in combination to study the aroma formation in food systems. Extraction of volatile compounds from food products may be both selective and challenging. A number of techniques have been reported to isolate volatile compounds in chickpea and lentil seeds. Of these, headspace techniques including solid phase microextraction (SPME) (Lasekan, Juhari, & Pattiram, 2011) and Tenax TA (Lovegren, Fisher, Legendre, & Schuller, 1979; Rembold, Wallner, & Singh, 1989) have been reported. Headspace methods suffer from being extremely selective due to the chemical nature of fibers and Tenax and their sensitivity to different classes of volatiles. In addition, they are time consuming as each extraction may be injected only once on a GC-MS system. In the present study a Steam Distillation/Solvent Extraction (SDE) method was employed to extract and isolate volatile compounds from pulse flours. In this method, samples of pulse flour are dispersed in water and heated to boiling point (100°C). Volatile compounds are extracted gradually during the cooking process. SDE has been used to extract volatile compounds from a variety of pulses and legumes including lima beans, common beans, lentils, mung beans, soybeans and split peas (Lovegren et al., 1979).

Since the pH and availability of polyunsaturated fatty acids (linoleic and linolenic acids) in pulses may affect the formation of volatile aroma compounds that contribute to beany flavor, free fatty acids analysis and the pH measurements of pulse flours in raw state were also conducted.

2.3.6. Sensory quality of the new formulated meat products

Food industries have focused on producing healthier versions of beef burgers without sacrificing the sensorial properties which are familiar to people. Fat in minced meat products plays an important role in sensory properties such as preserving aroma, flavor, juiciness, and tenderness after cooking (Huffman et al., 1990). Addition of binders and extenders in burger formulation is a common practice in meat industries and can improve sensory properties of burgers (FAO, 2012). Addition of other ingredients instead of fat should not compromise the nutrient content of burgers or cause any problems with sensory properties. For instance, adding rice bran in beef burgers should not exceed 3% due to its low protein content. Lentil and chickpea flours nutritionally are good candidates for meat extenders. However, adding pulses with anti-nutritional factors and active components may also have effects on the quality of the meat products. The presence of enzymes such as those from the LOX family can cause fatty acid oxidation and add 'beany' aroma to meat products (Rackis et al., 1979). Other sensory considerations are texture, color, and juiciness of the final products which can also be affected by addition of pulse flour.

It is important to process lentils and chickpeas prior to utilization as ingredients in burger production due to higher carbohydrate and protein digestibility of cooked pulses (El-Faki, Venkatarama, & Desikachar, 1984; Jood, Chauhan, & Kapoor, 1989). Micronization, among other processing methods, can improve the bioavailability of nutrients in pulse seeds and inactivate enzymes which leads to minimizing beaniness and off-flavor (Dzudie et al., 2002; Igbasan & Guenter, 1997; Khattab & Arntfield, 2009; Okaka & Potter, 1979; Zilic et al., 2010).

Any newly formulated food product must meet consumer expectations (Molnar, 1995a). Change in the original formulation may alter chemical, physical, and sensory characteristics and change the original product quality (Molnar, 1995a). These changes can be determined by quality evaluation with appropriate measuring tools and by correlating the results with definitions of certain food characteristics that are perceived through sensory evaluation. Some of these perceived sensations include color, texture, and flavor with the latter being a combination of odor and taste (Molnar, 1995b). It is clear that instrumental analysis is needed to quantify these changes along with evaluation by human senses.

Color of food is an important factor for consumers, and can influence the acceptability of food products. Consumers usually expect a color based on their memory of a specific food (Molnar, 1995a). For instance, a raw burger with reddish color is more acceptable than a brownish color. A desirable color motivates people to purchase or consume a food product, and like or dislike it (Molnar, 1995a; Molnar, 1995b). Although human eyes are able to distinguish changes in food color there is still a need for a modern quantitative instrument such as a Spectrocolorimeter to measure color of food quantitatively.

Texture of food can be related to the sense of touch as well as vision. Texture is described as roughness, smoothness, rubbery feeling which is perceived by mouth or eye (Molnar, 1995b). Physical aspects of burgers, such as water holding capacity, can be correlated to chewiness, hardness, or juiciness of the cooked burger. A rubbery texture could be the result of lower fat and water holding capacity (Mallika et al., 2009). The tenderness of meat products is very important in quality determination and can be measured instrumentally. One of the most accepted instruments for determining tenderness is the computerized Warner-Bratzler shear device. The results of shear force are highly comparable with consumer acceptance of meat products (McKenna, 2013).

Flavor of food during consumption is perceived through the sense of taste and smell while other senses such as hearing and vision may also play a role (Molnar, 1995b). Flavors are perceived through interaction of the sense of taste compounds and odor compounds (Molnar, 1995b). The perceived taste and aroma sensation of food can be correlated with isolated aroma active compounds from food which have been quantified by instrumental analysis. Usually, among hundreds of volatiles in food, only several major compounds play a significant role in desirable or unwanted flavor. Instrumental quantification of these volatiles can be correlated to consumer acceptability testing using a measure of liking or disliking of the new products.

Among all physical properties of any food products, appearance, color, and texture are the most important determinants of food quality (Molnar, 1995a). Adding a new line of ingredients in minced meat products with reduced fat may change the sensory attributes of beef-burgers such as color, flavor, texture, juiciness, or cooking properties. From a producer's point of view, water holding capacity, weight loss, shrinkage, and cost are

factors that should be considered too. For instance, addition of 6% lentil flour as binder to low fat beef-burger (10% fat) reduced shrinkage of burgers after cooking, and improved juiciness and tenderness (Der, 2010). However, increase in off-flavor was reported when low-fat burgers were extended with 6% and 12% non-micronized lentil flour (Der, 2010)

The quality of fresh meat products depends on two interrelated factors including water holding capacity (WHC) and pH (Gault, 1985; Huff-Lonergan & Lonergan, 2005). The tenderness of meat products after cooking is vastly related to the ability of the myofibrils to retain this moisture within cells and between the myofibrils and membrane (Huff-Lonergan & Lonergan, 2005). The shrinkage of myofibrils leads to release of moisture as exudates (Huff-Lonergan & Lonergan, 2005). Therefore, WHC is an important factor for both fresh meat products as well as meat products after cooking.

In this study the physical and chemical changes in low-fat beef burgers were measured instrumentally and by using an untrained consumer panel. Finally, in order to determine the influence of selected quality parameters on acceptability of newly formulated burgers, a PLS model was developed and applied to the data collected. The correlation and visual presentation of these measurements can help to produce new innovative meat products with functional properties and acceptable sensory characteristics.

2.4. Consumer acceptance testing

Consumer acceptance testing is carried out to evaluate the liking of sensory attributes of food products such as aroma, flavor, texture, and appearance (Moskowitz, Beckley, & Resurreccion, 2006). This test is usually conducted when part of the ingredients are

replaced by other substances to determine if the quality of the product has changed and will be accepted once introduced into the market. The combination of all sensory attributes such as flavor, aroma, texture, and appearance has a correlation with consumer decision making (Schutz, 1965). This effect is measured by using a Food Action Rating (American Meat Institute Fact Sheet) Scale which was introduced by Schutz (1965). This method has been developed based on the consumers' attitude toward the specific food product, sensory attributes and the action that might be taken as a result of the attitude (Lawless & Heymann, 2010). The aforementioned scale uses a 9-point sequential set of statements regarding the frequency of consumption of the specific food products (Lawless & Heymann, 2010). It is reported that FACT rating is correlated to a hedonic scale (like or dislike rating) while it is more sensitive to differences in food sensory characteristics than the hedonic scale (Schutz, 1965).

The consumer testing provides valuable information with minimal expense and time (Stone & Sidel, 2004). Consumer acceptance testing uses a small, untrained panel containing usually 50 to 100 people, to measure the level of preference and liking of food products in a carefully monitored environment. This eliminates any distraction and other confounding factors which might have an impact on sensorial properties of food during evaluation (Moskowitz et al., 2006). Tests take place in a well-ventilated room that has air constantly exhausted to the outside which eliminates odor carryover from previous sessions. Each booth is equipped with incandescent lights that illuminate from above and provide shadow-free light. The temperature and humidity of the booth during evaluation must be kept at an acceptable range (Vaclavik & Christian, 2008). In consumer testing, participants measure the acceptance of new food products by assigning a number on a

scale of 1 to 9 based on their sensory experience (Moskowitz et al., 2006). Sensory evaluation has been defined by the Sensory Evaluation Division of the Institute of Food Technologists as follows (Stone & Sidel, 2004): “*Sensory evaluation is a scientific discipline used to evoke measure, analyze and interpret reaction to those characteristics of foods and materials as they are perceived by the senses of sight, smell, touch and hearing*”.

Evaluators play an important role in sensory testing. To draw a reliable and unbiased result the evaluators must be qualified for the test. For example, there is a possibility that some of the evaluators do not like the products to begin with, therefore, they won't be able to qualify for the food product and if they do, the results won't be meaningful (Stone & Sidel, 2004). In order to solve this problem, participants must sign a form prior to the test indicating they have no food allergies and no objection or dislike toward the food to be evaluated. A copy of the form is presented in appendix D. Moreover, other important information can be extracted from a consumer test by looking at demographic information. Collecting data about age, gender, and eating habits can be used to understand the consumer behavior toward specific food characteristics (Miquelim, Behrens, & Lannes, 2008). In addition, inspecting demographics data can help to understand consumer acceptance of specific food products (Stone & Sidel, 2004).

The sensory rating of food products by participants could be influenced by factors which may have no relation to the product tested and are solely related to the differences between subjects (Stone & Sidel, 2004). To take these differences into account, the statistical model participants must be taken as random effects.

Sensory evaluation of the burgers with 6% micronized and non-micronized lentil and chickpea flour was based on a consumer acceptance test using an untrained panel. The test was conducted to estimate which sensory characteristics have an effect on the consumer response. Untrained participants can detect changes in sensorial properties of newly developed products such as aroma, flavor, and texture compared to a control. In addition, this test can determine if addition of chickpea and lentil flour or micronization treatment are able to enhance the sensory quality of low-fat beef burgers.

2.5. Hypotheses and objectives

2.5.1. Hypotheses

The hypotheses of this project were:

- 1) Micronization of pulse flours such as lentil and chickpea significantly decreases the LOX activity as measured by concentration of key volatile compounds contributing to ‘beany’ aroma and flavor.
- 2) The overall changes in the concentration of these volatile compounds is sufficient to improve the acceptability of low-fat beef burgers containing micronized lentil and chickpea flours compared to those with added non-micronized flours.
- 3) The micronization temperature has an impact on the final acceptability of the low-fat beef-burgers.

2.5.2. Objectives

The objectives of this study were threefold:

- 1) To develop a simultaneous distillation solvent extraction (SDE) method coupled with gas chromatography mass spectrometry (GC-MS) to collect and quantify the key volatiles involved in the formation of “beany” aroma and flavor in chickpea and green lentil flours
- 2) To investigate the effect of two levels of micronization (at 130 and 150 °C) on selected key volatiles in chickpea and green lentil flours and also on selected physical parameters such as color, water holding capacity, shrinkage, cook loss and texture on low fat beef burgers
- 3) To evaluate the acceptability of micronized chickpea and green lentil flours added as binders to low fat beef burgers using a consumer acceptability test

2.1. Study design

Volatile compounds extracted from micronized and non-micronized chickpea and green lentil flours using SDE method. The extracted volatile were analyzed by GC-MS (3 replicates). Extracted lipid from chickpea and green lentil flour were analyzed for fatty acid composition using GC-FID (6 replicates). In addition pH of pulse flour was measured in 4 replicated.

Low fat beef burgers with added non-micronized and micronized chickpea and lentil flours were used to extract lipid and methylate fatty acids (six replicates). The extracted fatty acids were analyzed by GC-FID (six replicates) to assess the impact of utilization of micronized pulse flour in the low-fat beef burger fatty acid profile. The results were compared to the low-fat beef burger with no binder as control (Co). In addition, the pH

and water holding capacity of raw burgers were measured in six and four replicates respectively.

Cooked burger sensory properties such as shrinkage, drip loss, cooking loss, texture and color (duplicate for raw and cooked burgers) were measured instrumentally.

A consumer acceptability test was used to assess the overall acceptability of low fat beef burgers with added non-micronized and micronized chickpea and lentil flours compared to beef-burger with no binder as control.

A four-ways ANOVA and one-way ANOVA were conducted to for consumer acceptability testing and physico-chemical parameter of micronized and non-micronized pulse flours and low-fat beef burgers containing pulse flours (SPSS, version 19).

Correlations between selected 'beany' volatile compounds, pH value of raw burger, selected polyunsaturated fatty acids, and overall acceptability as well as acceptability of aroma, flavor, texture and appearance of cooked low-fat beef burgers was performed using a partial least square (PLS) analysis (XLSTAT, 2012.1.01).

3. Material and Methods

3.1. Reagents and chemicals

The following chemicals were used: 1,2-dichlorobenzene, pentane chromasolv (HPLC grade), sodium chloride (BioReagent, suitable for cell culture, >99.5% titration), sodium sulfate anhydrous, diethyl ether chromasolv (HPLC grade), chloroform ($\geq 99\%$), methanol (>99%), hexane, potassium hydroxide, calcium chloride, and boron trifluoride (BF₃). 0.5N methanolic KOH is prepared in the lab prior to saponification and methylation All chemicals were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

3.2. Effect of micronization treatment at 130 and 150°C on selected volatile compounds in lentil and chickpea flour

3.2.1. Pulses (Lentil and chickpea)

Green lentil (variety Eston, small green, 2009 crop year) and chickpea (small seed Kabuli type, 2009 crop year) were supplied by the Crop Development Centre, College of Agriculture and Bioresources, University of Saskatchewan in March 2011 and April 2012. Pulse seeds were commercially cleaned and dehulled by Saskcan Pulse Trading Inc. (Regina, Saskatchewan).

3.2.2. Micronization

Green lentil and chickpea seeds were micronized by InfraReady Products Ltd. (Saskatoon, SK), using a small laboratory micronizer (Model A 156379-B0, FMC Syntron® Bulk Handling Equipment, Homer City, PA). The micronizer system was

composed of a gas heating stainless steel burner with specially designed ceramic tiles (Model type R 1603-2 PAT, Rinnai, Japan) as source of heating. The pulse seeds were micronized by moving on a vibrating belt causing each seed to rotate and be exposed all around the surface to the infrared radiation coming from the infrared lamp. Dry seeds were micronized to the surface temperature of 130°C to 150°C without tempering with water. Micronized seeds and control (non-micronized chickpea and lentil) were milled using a Cyclone Lab Sample Mill belt-drive (UDY Corporation, Fort Collins, CO) to less than 0.5mm particle size and vacuum-packed into polyethylene bags and shipped to St. Boniface Research Centre for further analysis. Upon arrival, all sample bags were wrapped in aluminum foil to reduce light exposure and stored at 4°C.

3.2.3. Extraction of volatile compounds from pulse flour by SDE method using Likens and Nickerson apparatus

The Likens & Nickerson apparatus is designed to allow a simultaneous steam distillation/solvent extraction of volatile compounds from a wide range of sample matrices. As shown in Figure 3-1 and Figure 3-2 the apparatus is composed of a condenser jacket, a cold finger and two distillation arms at different levels of apparatus. The top of the cold finger is fitted with a ground glass joint on top of the condenser jacket, and the two pieces of glassware are connected through an 8mm flexible rubber tube (Thermo Fisher Scientific, Ottawa, Canada). Chilled water (4°C) is pumped (Laguna Pond Water, Statuary Pump 2 214 GPH, Des Moines, IA, US) through the system continuously. The ice water bath, made in a 2 liter heavy-duty polypropylene beaker (Nalgene® Polypropylene Griffin, Thermo Fisher Scientific, Ottawa, Canada), and the fountain

pump system were checked every 15 minutes to remove water and refill with ice to maintain the cold temperature throughout the duration of the extraction.

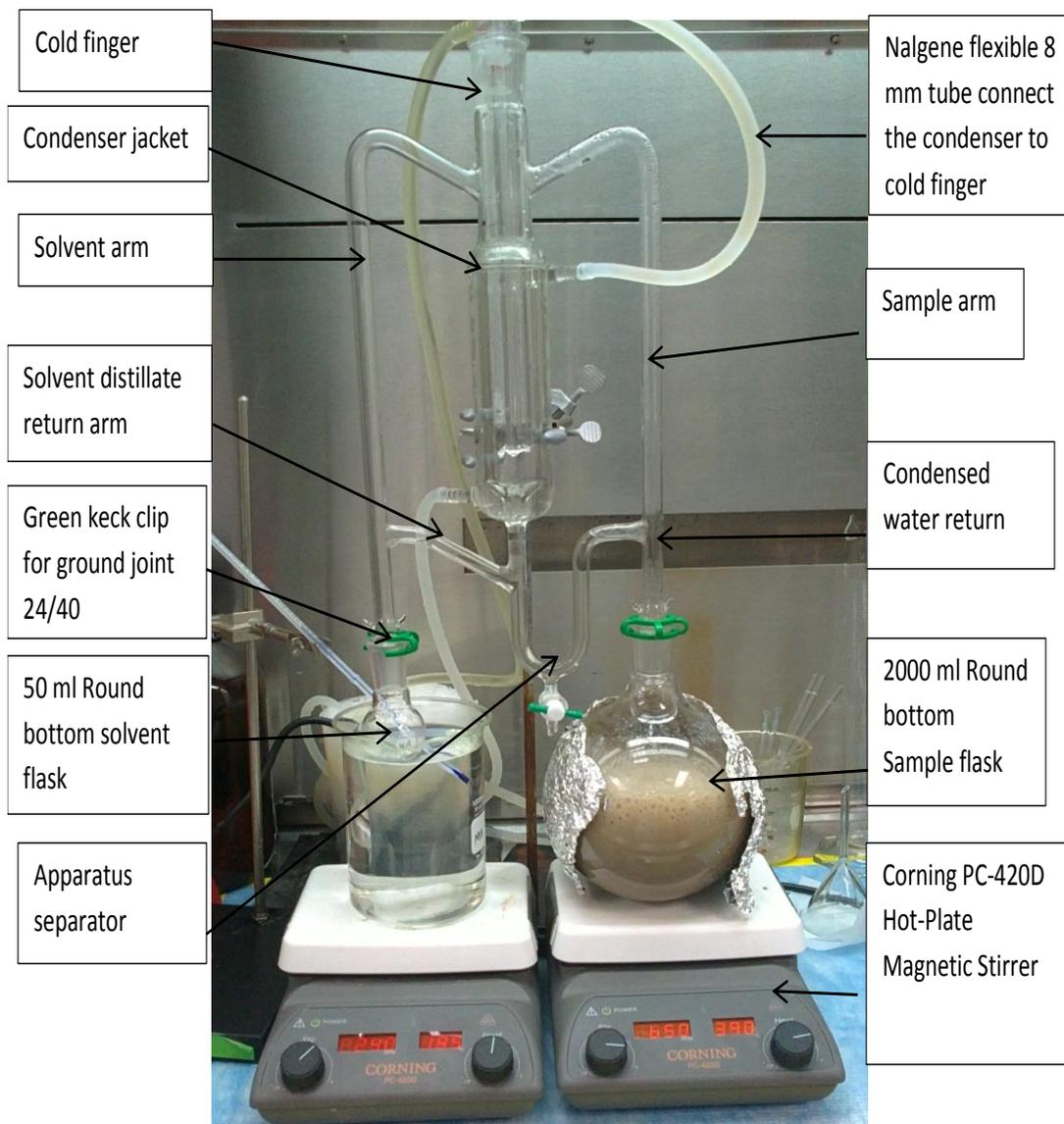


Figure 3-1 volatile extraction of green lentil using Likens and Nickerson Apparatus

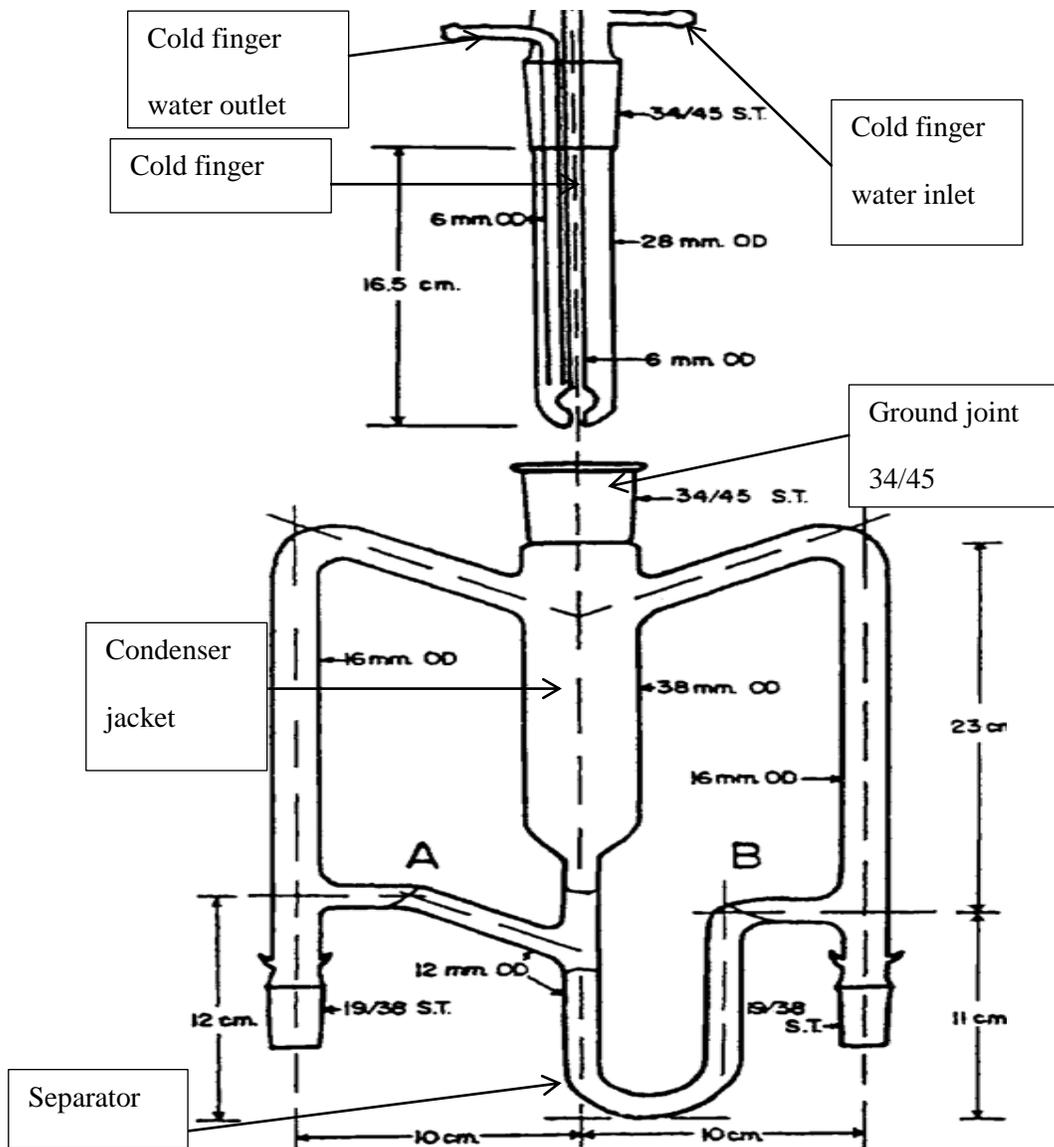


Figure 3-2 Scale drawing of Likens and Nickerson apparatus, reproduced by permission (Source: New method for isolating hop oil in brewing product is published in Journal of the American Society of Brewing Chemists (Likens & Nickerson, 1964), The dimensions are in millimeter and centimeter.

A total of 100g of pulse flour and 80g NaCl were measured using an analytical balance (Denver Instruments Analytical balance, model SI-234, Bohemia, NY 11716, US) and mixed with 1200mL purified water (Barnstead E-Pure, Thermo Scientific, Dubuque,

Iowa, US) in a 2L Pyrex beaker (Fisher Scientific, Nepean, Ottawa, Canada). The mixture was stirred with a glass rod to break any clumps and poured into a 2L Pyrex round bottom flask. An internal standard (100µg of 1,2-dichlorobenzene in methanol (1000µg/mL) was added to the mixture at the end of sample preparation. The slurry of pulse flour in water was stirred and boiled in a 2L Pyrex round bottom flask (Fisher Scientific, Nepean, Ottawa, Canada) using a hot plate equipped with a magnetic stirrer (Corning PC-420D, Corning Incorporated, Corning, New York, US) and was connected to the right arm glass ground joint (24/40). The organic extraction solvent was boiled in a 50mL Pyrex round bottom flask (Fisher Scientific, Ottawa, Canada) using a 58-60°C water bath which was also heated using a hot plate. The temperature of both water baths for the solvent extraction and cooling system were monitored throughout the extraction procedure. The volatile compounds from the sample were steam distilled through the right arm and extracted by solvent vapor. The solvent and sample vapors were condensed on the surface of the condenser and solvent extraction of organic volatiles were performed (pentane: diethyl ether, 5:1 v/v, total volume = 18mL) by forming a liquid film on the condenser surface. Condensed organic solvent and water were separated based on their density in the separator part of the apparatus. Low density solvent and high density water are returned to their corresponding original flasks.

The volatile extraction was started when the condensation became visible at the upper part of the sample arm. The 100g pulse flour slurry was extracted for 120 minutes with a mixture of 15mL distilled pentane and 3mL diethyl ether. During the extraction the sample temperature was maintained at 100°C and solvent temperature maintained at 58-60°C. After cooling to ambient temperature (15 minutes), the extract was collected and

stored at -20°C for 12 hours. During this step, excess water in solvent extract was found as ice crystals and easily removed by transferring the extract to a clean 50mL flask. The remaining solvent was dried over anhydrous sodium sulfate. Finally, in order to enrich the volatiles within the extract, the resulting solution was concentrated to 1mL using a nitrogen evaporator (Organomation Associates Inc., MA 01503-1699, US). The resulting concentrate was transferred and stored in a 2mL crimped cap vial (Agilent, Mississauga, Ontario, Canada). Triplicate extractions and duplicate GC-MS injections from each extract were carried out for each pulse flour sample.

3.2.4. Optimization of SDE parameters

SDE parameters were evaluated to increase recovery of extracted volatiles and to minimize the loss of volatiles due to thermal reaction. Parameters that were considered included volume of solvent, time, and boiling temperatures. Sample weight in the dry state also played a significant role in the final recovery of volatiles from the sample. During the volatile extraction, heated pulse flour thickens in boiling water as starch granules absorb water and coagulate. The thickness of the mixture is directly associated with the overall amount of pulse flour and water. Preliminary experiments were designed to determine the sample size giving the best chromatographic results. Figure 3-3 shows the typical chromatograms of volatiles extraction of 100g and 200g of non-micronized green lentil flour. More volatiles were obtained when 100g of green lentil flour was used. More water steam may be generated with the batch containing the smaller amount of flour. Therefore, a ratio of 100g pulse flour to 1200mL distilled water was chosen for our study.

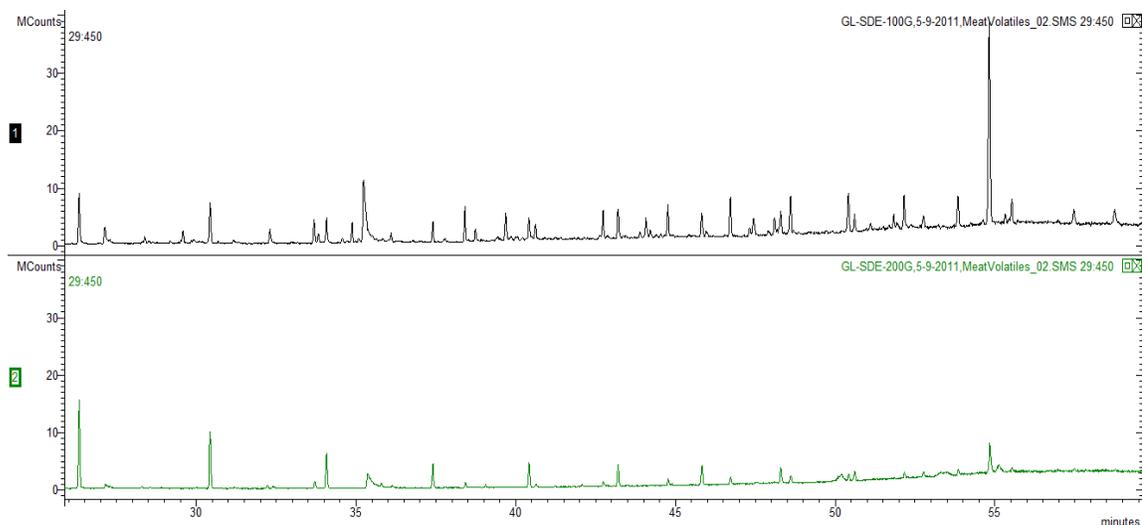


Figure 3-3 Extraction of volatile from 100 and 200g of green lentil flours dispersed in 1.2L of water.

Extraction time, volume of solvent and water and their temperatures were also optimized prior to the commencement of the study. Extraction time was determined based on previously described methods (Madruga et al., 2009). Sample matrix is a major contributing factor in evaluating extraction times as food matrices rich in lipids require longer times than samples with low lipid content (Chaintreau, 2001). A wide range of extraction times have been tested in different studies on a variety of sample matrices for instance orange juice, oil fried garlic, lobster meat, fermented sausage, and French beans (Ansorena et al., 2001; Barra et al., 2007; Cadwallader et al., 1995; Matthews & West, 1993; Yu et al., 1993). The 3 hours and 4 hours were used as time of extraction for garlic and lobster tail. Chaintreau (2001) suggested that 1-2 hours of extraction time is sufficient for a non-fat sample matrix. Given the low lipid content of pulse flours, a two hours extraction time was used in our study.

Heating the solvent mixture (pentane-diethyl ether) over 60°C resulted in less solvent recovery. In addition, it has been reported that high solvent temperatures are associated with decreased extraction efficiency, while increased sample temperatures leads to decreased time of volatile isolation (Chaintreau, 2001). For this study it was concluded that 2 hours of extraction followed by 15 minutes of cooling using a solvent bath temperature of 58°C and a sample temperature of 100°C were suitable for sufficient volatile extraction from cooked pulses. The amount of sample, distilled water, solvent and volume of water and solvent for filling up the separator were determined based on the time needed for equilibrium to be established in the system.

3.2.5. *Gas chromatography- mass spectrometry (GC-MS)*

GC-MS analysis of the extracted volatiles from pulse flours was performed using a 450 gas chromatograph instrument (Agilent Technologies, Walnut Creek, CA, US) 240MS/4000 Mass Spectrometry (Agilent Technologies, Walnut Creek, CA, US). An aliquot (1µl) of extract was injected using an auto-sampler in splitless mode at 250°C. A VF-5ms low bleed/MS fused-silica capillary column (5% phenyl/95% PDMS, 30m×0.25mm I.D., 0.25µm film thickness, FactorFour, Varian) was used for all analyses. A filament delay of 3 minutes was used at the start of each run. The oven temperature was programmed using an initial temperature of 40°C (held for 5 min), which was increased to 220°C at a rate of 4°C/min and held again for 5 minutes with a total run time of 55 min. Helium was used as the carrier gas at rate of 1mL/min.

The ion source was operated in electron ionization (EI) mode using a voltage of 70 eV. The MS scanned from 40 to 540m/z in full scan mode using a scan average of 4 micro-scans (1.75 seconds/scan). Distilled pentane was used for the needle wash in between each injection. One blank extraction was used for each set of injections. The blank was defined as one extraction using all components of the extracting method except sample.

3.2.5.1. *Semi-quantification*

The GC-MS data were analyzed using the MS Workstation software (Varian, version 6.9.3). Semi-quantification was performed by calculating the ratio of compound peak area to internal standard peak area. Peaks were identified by matching their mass spectra with mass spectra of authentic compounds analyzed and reported in the NIST library (MS Search Version 2.0). To provide additional information for their identification, the linear retention indices (Schindler et al.) were calculated for these compounds (Van Den Dool & Dec. Kratz, 1963). LRI values are obtained by calculating the relative retention time of that compound to a retention time of a homologous series of n-alkane standards (C8-C22, 40µg/mL hexane) analyzed under similar operating conditions. A list of alkanes and retention time and linear retention indices can be found in Table 3-1. Values were then entered into the following equation:

$$\text{LRI} = 100 * (\text{RT compound} - \text{RT alkane}_n) / (\text{RT alkane}_{n+1} - \text{RT alkane}_n) + \text{LRI}_n$$

The calculated LRI of compounds based on LRI and RT of series of alkane standard are shown in Table 3-2. Calculated LRI of compounds were compared to those reported in literature or analyzed from authentic compounds in the LRI library (University of

Reading). The semi quantitation of compounds was carried out by comparison of their peak areas with that of 1,2-dichlorobenzene (internal standard).

Table 3-1 Retention time and LRI values of n-alkane standards (C8-C20)

Compounds Name	RT	LRI
Octane	5.543	800
Nonane	9.709	900
Decane	14.015	1000
Undecane	18.073	1100
Dodecane	21.861	1200
Tridecane	25.364	1300
Tetradecane	28.687	1400
Pentadecane	31.808	1500
Hexadecane	34.752	1600
Heptadecane	37.59	1700
Octadecane	40.195	1800
Nonadecane	42.743	1900
Eicosane	45.145	2000

Table 3-2 List of volatile compounds in cooked lentil and chickpea flours with potential contribution to ‘beany’ aroma and flavor

Compounds Name	RT (min)	Calculated LRI	NIST LRI	U of R LRI	Quan Ion
1_Pentanol	4.539	< 800	761	754-761	55
Hexanal	5.573	801	806	787-802	43
2_Hexenal	7.682	851	814	848-862	55
Hexanol	8.51	871	860	862&865	41
Heptanal	9.812	902	905	898-902	55
Furan_2_Pentyl	13.591	990	977	988-992	81
1,2-dichlorobenzene	15.27	1031	1040	-	146
Octanal	14.203	1004.6	1005	1002&1005	41
2-Octenal	16.444	1060	1059&1062	1059-1062	55
Undecane	18.042	1099	1100	1100	41
Nonanal	18.271	1105	1104	1104-1109	67
2,4-Decadienal	25.271	1297	1270	1295&1319	81
Tridecane	25.356	1299.759	1300	-	57
2,4-Undecadienal	26.08	1322	1319	-	81
Hexadecane	34.72	1599	1600	1600	57

RT: Retention time (the period from start of injection to the top of the eluting peak)

LRI: Linear retention indices

U of R: University of Reading LRI database

3.3. Fatty acids analysis of micronized and non-micronized pulse flours

3.3.1. Lipid extraction and fatty acid analysis of pulse flours

Fat content was extracted by chloroform–methanol (2:1 v/v ratio) according to the Folch method (Folch, Les, & Sloane-Stanley, 1975). Lentil flour (1g) and chickpea flour (0.5g) (micronized and non-micronized) was weighed using an analytical balance (Denver Instruments Analytical Balance, model SI-234, Bohemia, NY, 11716, US) and placed into a 50 mL Pyrex heavy-duty centrifuge tube (Sigma-Aldrich, Oakville, Canada), vortexed for 2 minutes with 20 mL chloroform-methanol and 4 mL 0.025% CaCl₂, and centrifuged in an Allegra™ 6R Centrifuge (Beckman coulter, Indianapolis, USA) for 15

minutes at 3000×g. The lower layer containing the pure lipid extract was transferred to a clean 20 mL screw cap test tube (Sigma-Aldrich, Oakville, Canada) and the solvent evaporated under nitrogen using an N-EVAPTM111 nitrogen evaporator (Organomation Associates Inc., Berlin, MA, USA). A 10mg per mL solution was prepared by adding an appropriate volume of chloroform-methanol (1:1, v/v) to the pure extracted lipid (based on weight of the lipid). One mL of this solution was transferred to a clean screw cap test tube and solvent was evaporated under the nitrogen evaporator. Saponification and methylation steps were performed using 0.5N methanolic KOH and BF₃ in methanol (14% w/w boron trifluoride) and the sample was heated at 110°C in a sand bath for 1 hour and 1 ½ hour, respectively, for each step. The top organic layer, completely dried under nitrogen gas, was reconstituted in 1 mL of hexane and after flushing with nitrogen gas was capped tightly in a 2 mL GC vial, and stored at -20°C for further analysis.

3.3.2. Gas chromatography and flame ionization detector (GC-FID)

The methylated fatty acid samples extracted from pulse flour were separated on a Varian WCOT Fused Silica CP-SELECT FAME column (100m×0.25mm diameter; 0.25µm film thicknesses; Varian Canada Inc., Mississauga, Ontario) using a Varian 450 GC coupled with a Flame Ionization Detector. The column was operated at 100°C for 2 min after which the temperature was ramped up to 175°C at 25°C/min, and held for 30 min. The temperature increased again to 220°C at 15°C/min, was held for 10 min and then raised to 240°C at 20°C/min and held for 11 min. The samples were run with a 10:1 split ratio and a column flow of 1.8 ml/min was used with a total run time of 60 min for each sample. Samples were quantified using an external standard calibration made using fatty acids

standard (Nu-check high purity series of saturated and unsaturated fatty acids, Funakoshi Co. Ltd., Tokyo, Japan). Six replicate lipid extractions and fatty acid separations were performed for each treatment.

3.4. Pulse flour pH measurement

The method used is described in the European Pharmacopoeia, 4th edition International-Starch-Institute (1999). The pH meter (Accumet Basic AB15/15+, Fisher Scientific, Ottawa, ON, Canada), was calibrated to pH 4 to 7 prior to measurement. The pH of pulse flour was measured by stirring 5g of pulse flour with 25mL of purified distilled water for 60 seconds. The mixture stood for 15 minutes so that solid particles could settle at the bottom of the 50mL Pyrex beaker (Fisher Scientific, Nepean, Ottawa, Canada). pH was recorded in the water phase by dipping the glass electrode into the sample for 2 min or until the pH reading was stabilized. Four replicates were prepared for each flour type and for each 4 readings were recorded. The electrode was washed with distilled water in-between each measurement.

3.5. Evaluation of sensory properties of low-fat beef burger containing micronized lentil and chickpea flours

3.5.1. Meat

Beef as boneless outside rounds mainly biceps femoris (Canada AAA grade) is obtained by University of Saskatchewan from Centennial Food Ltd., Saskatoon, Sk. All

subcutaneous fat and inter muscular fat were removed and used as the fat source for burger formulation.

3.5.2. Pulses

Chickpea and lentil samples which used as binder in low-fat beef burgers were supplied by Crop Development Centre, College of Agriculture and Bioresources, University of Saskatchewan.

3.5.3. Low fat beef-burger preparation procedure

Low-fat beef burgers for this experiment were prepared at the University of Saskatchewan as follow. Lean beef and fat trimmings were ground separately through a kidney plate (Biro Grinder, model AFMG-24, The Biro MFG Co, Marblehead, Ohio, USA), with lean beef put into a large tumbler (Glass Vacuum Tumbler, model VSM-150, H. Glass D-4790 Paderborn Ob, Frankfurt), and tumbled until mixed. Lean meat and fat were each ground through a 3/8" plate (Biro grinder, model AFMG-24, the Biro MFG Co, Marblehead, Ohio, USA). The temperature of the meat was kept less than 4°C through all procedures of making the burgers using strained ice water. A fat test was conducted on both the lean meat and trimmed fat using an HFT 2000 Rapid Fat Analyzer (Data Support Co, Encino, Ca, USA). The amount of fat needed for formulation of burgers with 10% fat content was calculated using Pearson Square. Eighty-two percent ground meat, 10% fat, 0.9% salt and 6% pulse flour were measured for different batches and mixed separately using a large mixer bowl (Berkel Ba-20 Mixer, Berkel Co.,

Countryside, IL, USA) and 11.1% cold water was added to the mixture slowly throughout. Except for the control batch which contained 88% lean meat and no binder, the burgers contained 82% lean meat. Each batch of burgers passed through a 1/8” grinder plate and the equipment was rinsed with cold water in-between batches. Burgers were made using a Hollimatic Pattie machine (Hollimatic Pattie Former, model Super 54, Hollimatic Corporation, Countryside, IL., USA) at the University of Saskatchewan pilot plant. Each batch of burgers was processed in a randomized manner. Burgers were lined with patty paper and frozen at -30°C. Each batch of burgers were packaged in polyethylene bags and labeled and stored at -20°C in Human Nutritional Sciences in Winnipeg, MB for further sensory and instrumental analysis. The formulation of the seven burger samples is given in Table 3-3.

Table 3-3 Formulation of low-fat beef burgers extended by pulse flour

Sample	Lean Beef %	Binder %	Water %	Salt%	Fat %	Total
Control (Co)	88	-	11.1	0.9	10	100
Green lentil (RGL)	82	6	11.1	0.9	10	100
Green lentil (MGL-130)	82	6	11.1	0.9	10	100
Green lentil (MGL-150)	82	6	11.1	0.9	10	100
Chickpea (RCP)	82	6	11.1	0.9	10	100
Chickpea (MCP-130)	82	6	11.1	0.9	10	100
Chickpea (MCP-150)	82	6	11.1	0.9	10	100

3.6. Low-fat beef burger with added pulse flour fatty acid analysis

3.6.1. Beef burger lipid extraction and fatty acid analysis

To obtain a homogenized sample to represent the fat content of the whole sample, burgers were processed in a Hamilton Beach Chef Prep food processor (Sears, Canada) for 1 minute or until the ground meat looked completely uniform. Ground meat (0.5g)

was weighed into a 50mL centrifuge tube and homogenized with 4mL 0.025% CaCl₂ for 30 second using a Polytron (PT 2100 Polytron, Kinematic, Switzerland). Homogenized samples were extracted with chloroform-methanol (2:1, v/v) by vortexing for 1 minute. The aqueous and organic layers separated completely after centrifugation at 2000 rpm for 12 min. The chloroform layer was transferred into a weighed 20mL screw cap test tube and the organic solvent was evaporated under a gentle nitrogen flow. Pure lipid was weighed again using an analytical balance and lipid dissolved in chloroform-methanol (1:1, v/v) to a 10mg/mL concentration. A two-step saponification and methylation was performed by heating the sample at 110°C (for 1 hour and 1 ½ hour) with 1.5mL 0.5N methanolic KOH and 1.5 mL BF₃ in methanol (14% W/W boron trifluoride) for the first and second step, respectively. Purified water (1mL) was added to the sample after cooling to room temperature, stoppered, vortexed (1 min) and centrifuged at 1300 rpm for 12 minutes. The organic layer was transferred to a 2mL GC vial and chloroform was completely evaporated in a nitrogen evaporator and 1mL hexane was added to the vial.

3.6.2. Temperature programing for GC-FID to separate and quantify burger sample fatty acid content

Extracted fatty acids from low-fat beef-burgers with added micronized and non-micronized pulse flour were separated, identified and quantified by GC-FID using the same instruments, procedures, and temperature programming as for fatty acids in the micronized and non-micronized pulse flour section 3.3.2.

3.7. Physiochemical analysis of raw low-fat beef burger with added pulse flour

3.7.1. pH of raw beef-burger with micronized and non-micronized pulse flour

The pH was measured based on the method described by Troutt et al. (1992). Raw burger samples were prepared by blending 10 g of raw burger with 100 mL of distilled water using a Hamilton Beach Chef Prep food processor (Sears, Canada) for 1 minute. The fat and meat fiber were removed by straining the mixture through a mesh and collecting the liquid in a measuring cup. The mixture was stirred and poured into a small beaker for pH measurement. The pH of the liquid was recorded by dipping the glass electrode of the bench top pH meter (Accumet Basic AB15/15⁺, Fisher Scientific, Ottawa, ON, Canada) with accuracy of ± 0.01 pH into the mixture. The pH meter calibrated to pH 4 to 7 prior to measurements. Each reading was recorded after 2 minutes. Four replicates were prepared for each sample and three readings were recorded for each preparation. The glass electrode was washed with distilled water between each measurement.

3.7.2. Water holding capacity of raw burger

A swelling and centrifuge technique was used to determine water holding capacity (WHC) of raw burgers with added pulse flour (Wardlaw, McCaskill, & Acton, 1973). In a 50mL weighed centrifuge tube (Corning graduated centrifuge tube, Thermo Fisher Scientific Inc., Ontario, Canada) 10g of raw burger and 15mL 0.6 M NaCl solution were vortexed for 1 minute. The mixture was centrifuged for 15 min at 3000×g using an Allegra™ 6R bench top centrifuge. Supernatant was removed and its volume and

weight recorded. The amount of solution retained by the meat sample was reported as WHC. Four replicates were performed for each burger sample.

3.8. Low-fat beef-burger instrumental and sensory evaluation

3.8.1. Cooking method

Baking pans were prepared prior to cooking by lining with aluminum foil and labeling them with the 3 digit random numbers chosen for the 7 burger samples. Four Electric Range ES510 Control ovens (Electrolux Home Products Inc., 2004, Electrolux Canada Corp., Mississauga, Ontario) were preheated to 290°C (by choosing the broil option) for 30 min prior to cooking. Frozen burgers were removed from the -20°C freezer, the patty papers were removed and randomly distributed on the allocated pans with the same 3 digit codes, based on the predetermined randomization for each set of samples. The burgers were cooked from frozen which is recommended by commercial manufacturers. Temperatures of the burgers were checked 6 min from the start of cooking using a digital thermometer (Traceable Full-Scale Plus Thermometer, 2006, Control Company, Friends Wood, Texas, Accuracy $\pm 1^\circ\text{C}$) placed in the center of the burger. Patties were flipped once when the central temperature reached 53 to 55°C and were cooked for an additional 4 min. Burgers with central temperatures less than 53°C were left in the oven for another 2 minutes. Cooked burgers were removed at 11 minutes checked for temperature and determined to be finished cooking when the central temperatures reached 71-74°C as recommended by health authorities (Health-Canada, 2010). They were wrapped in heavy-duty aluminum foil and placed in Styrofoam containers to keep warm for consumer

testing or placed on a cooling rack for further analysis. All cooking procedures were carried out in the Weston Sensory and Food Research Center, University of Manitoba, Winnipeg, Manitoba.

3.8.2. Instrumental evaluation of color of low-fat beef- burgers by Spectrocolorimetry

3.8.2.1. Raw burger color evaluation

Color measurements (L^* , a^* , and b^* values) for raw burgers were carried out on the surface of raw burger samples using a HunterLab MiniScan XE Spectrocolorimeter; Model 4500L (Hunter Associates Laboratory Inc., West Virginia, USA) with xenon flash as a light source. The light source produces an intense full spectrum white light within visible range of 400nm to 700 nm. Prior to color measurement, the Spectrocolorimeter was standardized on the CIE color system by using a white tile. Following an 18-hour cold thaw in an air tight plastic container at 4°C, raw burgers were removed and exposed to air one by one every 2 min and thereafter in order for meat to bloom for 30 min. Blooming refers to the turning of meat color from purple to red, which results from exposure of the myoglobin to oxygen resulting in oxy-myoglobin (Rentfrow, Linville, Stahl, Olson, & Berg, 2004). A spectrally pure glass was used to help level uneven surfaces and prevent meat pieces and juice entering the opening. Four burgers from each sample group were evaluated for CIE $L^*a^*b^*$ color components. The surface of each sample was scanned four times in several areas at right degree angle to get more representative results.

3.8.2.2. *Cooked burger color evaluation*

Burgers were cooked as described in section 3.8.1. Cooked burger color was evaluated using a HunterLab MiniScan XE Spectrocolorimeter; Model 4500L (Hunter Associates Laboratory Inc., West Virginia, US) with large area view. Prior to color measurement, the Spectrocolorimeter was standardized on CIE color system by using a white tile. The CIE (L* a* and b*) color values were measured at the surface of cooked burgers at room temperature (23 - 24°C). A spectrally pure glass was used on the surface of cooked burgers to help level uneven surfaces and prevent meat pieces and juice entering the opening. The glass was washed and dried using powder free soft fabric between samples. Four replicates of each sample were prepared and each burger scanned 4 times at a right degree angle across the surface in different area to account for variation within treatments.

3.8.3. *Weight loss, cooking loss, shrinkage, drip loss*

Burger samples were cooked as described in section (3.8.1) to the internal temperature of 71-74°C. For cook and drip loss, only one burger was placed on each rack (38.1cm×25.4cm×1.3cm height) in a foiled pan (43.2cm×27.9cm×1.9cm height) after being weighed on a Precision Electronic Balance (A & D Company Ltd., Toshima-Ku, Tokyo). Thickness and diameter of the burgers were measured using a Digital Caliper (Control Company, Friends wood, Texas, USA). After cooking, the drip-loss was collected on aluminum foil and was calculated by the mass difference of aluminum foil before and after cooking. Cooked burger weight and diameter were measured and recorded. Burger

cook-loss (%) was determined by difference between raw and cooked burger weight as follows.

$$\% \text{ Cook-loss} = [(\text{RB wt} - \text{CB wt})/\text{RB wt}] \times 100$$

Where:

RB wt = Raw Burger weight

CB wt = Cooked Burger weight

The reduction in the diameter of the burger (% shrinkage) was determined (8 replicates) with the following equation:

$$\% \text{ Shrinkage} = \{[(\text{RBT}-\text{CBT}) + (\text{RBD}-\text{CBD})]/(\text{RBD}+\text{RBT})\} \times 100 \text{ (Ibrahim, Salma et al. 2011)}$$

Where:

RBT = Raw Burger Thickness

CBT = Cooked Burger Thickness

RBD = Raw Burger Diameter

CBD = Cooked Burger Diameter

3.8.4. Shear force measurement of low-fat beef burgers extended with micronized lentil and chickpea flour

Burgers were cooked as for sensory and cook loss from frozen state and spread out on a rack to cool to room temperature (23-25°C). The temperature was checked in the middle of the burger before cutting. The oval edge of burgers was removed to make it square (50 × 50mm) using a sharp knife. Each square was cut into strips of 50 × 10 × 10mm. The result was 4 slices of 1cm thick and 5cm long. The texture of samples was measured with a materials testing machine (Lloyd Instruments Limited, Fareham, Hants. UK) equipped with a 100N load cell (Shea AEP, Model No. TS-100N, Bresimar Group, Portugal). TESTLOOP™-C software (Lab Integration Inc., Oakville, ON, Canada) was used to

measure shear force of cooked burgers. A V-Shape Warner Bratzler shearing blade with the thickness of 1mm and a spacer with a 2mm thick gap for the V-shape cutting blade to slide through and cut the strip of burger in the middle as shown in Figure 3-4.

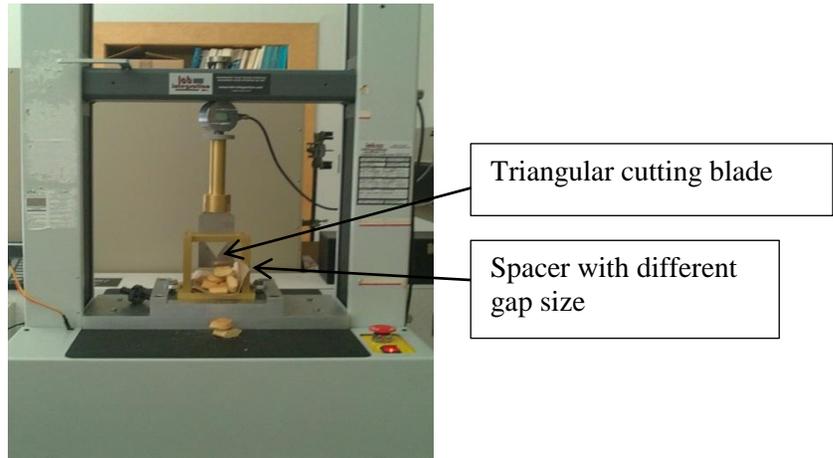


Figure 3-4 Warner Bratzler shear force attachment

The shear force for each piece from four burgers was recorded in Newton (N).

3.8.5. Consumer evaluation test

3.8.5.1. Sample preparation

One or two burgers from each of seven treatments were cooked for each sensory session depending on the number of participants for the session. The burgers were assigned to four ovens in a randomized manner. Burgers were cooked from frozen as described in cooking section (3.8.1) prior to each sensory evaluation session based on the pre-planned timing and to an internal temperature of 71 -74°C. Cooked burgers immediately were cut into 6 pieces, placed in 60mL coded plastic portion cups (Solo Cup Co., Lake Forest, Ill.,

USA), capped and served immediately. Preliminary sample cooking and preparation was performed to standardize the cooking and delivery time.

3.8.5.2. Volunteer recruitment

One hundred and one untrained participants, ages 18 and over, were recruited from the university staff and students. The largest portion of this population was between ‘18-34 years old’, because the sensory testing was carried out at the university which is more populated with young adults. The most important criteria for volunteers were that they didn’t have any allergies and objection to consuming burgers and they were able to spend thirty minutes to evaluate the products. All participants, 71 female and 30 male, completed a questionnaire to confirm the absence of allergies and were asked to sign a consent form which is shown in appendix D prior to the session. Upon the completion of the sensory evaluation, all volunteers received an honorarium. The procedures for the consumer acceptance study were approved by the Joint-Faculty Human Research Ethics Board at the University of Manitoba. A copy of approval certificate is available in appendix C.

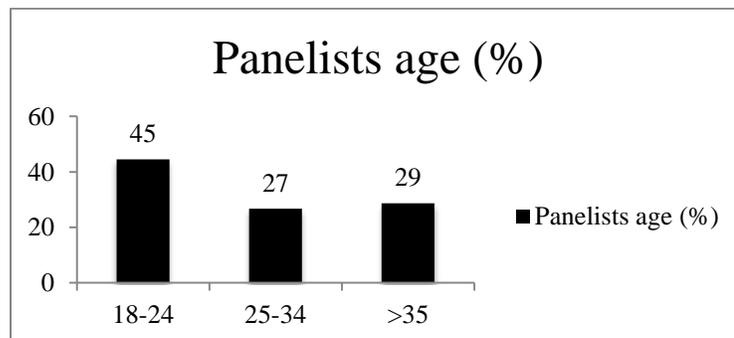


Figure 3-5 Age distribution of consumer test participants (n=101)

3.8.5.3. *Testing area*

The consumer test took place in the Weston Sensory Research Center located in the Human Nutritional Sciences Department, University of Manitoba. The sensory evaluation center was located across from the food preparation laboratory facility (where the samples were cooked in randomized manner and prepared for the evaluation). This setting allowed samples to be served hot from the oven and prevented odors from cooking samples interfering with the evaluation. Consumers were seated in individual testing booths equipped with computerized sensory evaluation software (Sensory Integrated Management System, Morristown, N. J., 2011) under bright incandescent light. The sensory area was furnished, hygienic and cleaned using odorless sanitation materials. Rooms were well ventilated to eliminate any odors, and temperature was kept between 22- 24°C.

3.8.5.4. *Sample delivery and sensory evaluation*

The same 3-digit randomization number on each batch of burger samples was used to code 60 mL plastic portion cups for blind-coding the samples. The sample design included the randomized order of presentation of samples within each session. Assessors were asked to take the sample as the code appeared on the screen of the computer and between each sampling to drink room temperature filtered water in amounts as required to eliminate taste carry over.

Participants were also guided through the evaluation procedure orally prior to residing in a sensory booth and were provided with written instructions as well. A set of seven samples each about 12g (maximum of 100g for all seven samples) was delivered to evaluators through small fitted sliding doors referred to as sample pass-through doors with no contact from food servers to minimize distraction as shown in Figure 3-6. A jug of room temperature, filtered water was provided to cleanse the palate between each sample evaluation.

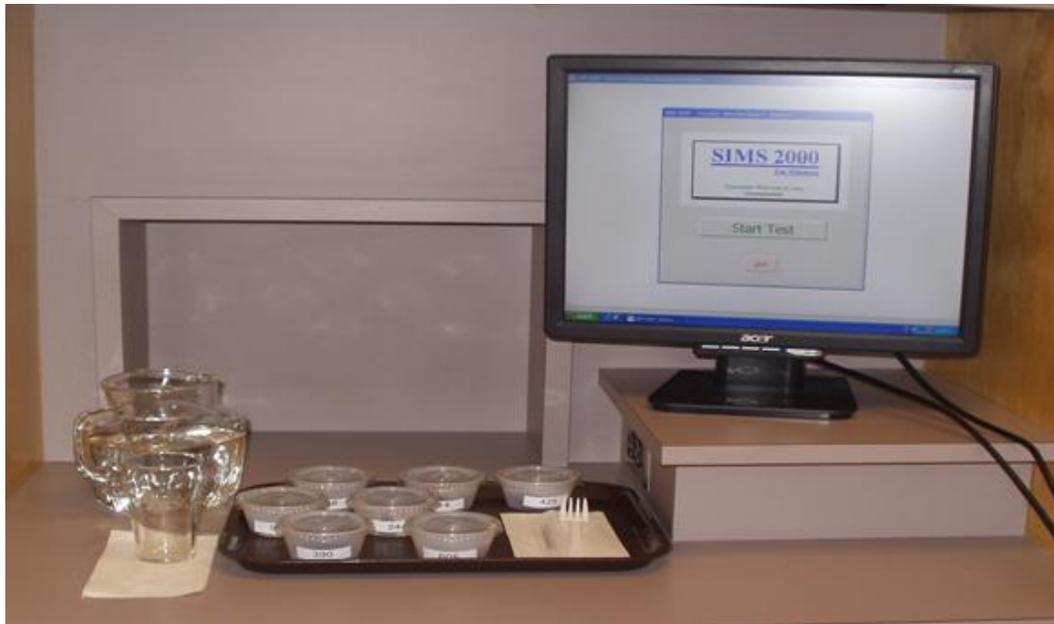


Figure 3-6 Sensory evaluation booth set up

The samples were rated on the acceptance of aroma, appearance, flavor, texture, and overall acceptability. The test was used to evaluate how much each sample was liked based on a 9-point hedonic scale (Stone and Sidel, 2004) (where: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). The samples were also rated for frequency of eating by using a Food Action (FACT)

scale (Schutz, 1965). Participants selected one of the following options: 9 = I would eat this every opportunity I had; 8 = I would eat this very 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 it on an 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. Information was collected regarding gender, age, frequency of eating pulses such as chickpea, lentil, and beans, and frequency of eating beef burgers. The participants were instructed to first open the lid, take two or three short sniffs and determine how much they liked or disliked the aroma of the sample. They were also asked to look at and taste the sample and rate the appearance, flavor, and texture of the sample. A University of Manitoba bookstore gift card was offered to each participant after completing the session.

All data were recorded anonymously and all personal information will be kept in a locked cabinet for 5 years or until the data are published before being shredded.

3.8.5.5. *Serving and testing order*

During each experimental session, assessors evaluated seven samples at once without condiments in one thirty minute session. Samples were presented to the panel on a tray containing toothpick, fork, and napkins. Participants evaluated the samples in a randomized order according to a plan generated by the sensory programming software.

3.8.5.6. *Statistical analysis*

One way Analysis of Variance (ANOVA) was performed using SPSS software program (version 19) to analyze the data obtained for volatile concentrations, fatty acid analysis of pulse flour and low-fat beef burgers extended by pulse flour, pH, drip loss and weight loss, Spectrocolorimetry, texture from different treatments. Tukey's multiple comparison test was used where the assumption of homogeneity was valid to determine mean treatment differences where significant ($P < 0.05$). Games Howell tests were used when the assumption of homogeneity was not valid.

For consumer acceptability a four-way ANOVA was conducted with presence of micronization (μ), type of pulse (T), gender (G) and age group (A) as fixed effects using SPSS (version 20) software as described in section 4.1.5.

Partial Least Square regression (PLS) is used to map consumer acceptability of low-fat beef burgers extended with micronized pulse flour with a set of volatile compounds which contributing to beany aroma, pH, texture, color parameter and selected fatty acids.

4. Results and discussion

4.1. Pulse flour volatile and chemical analysis

4.1.1. Pulse flour volatile analysis

In this study, the level of selected ‘beany’ aroma compounds in micronized and non-micronized small green lentil and Kabuli chickpea to the surface temperature of 130°C and 150°C were evaluated and the results were compared to those of non-micronized counterparts. Volatile compounds with potential contribution to ‘beany’ aroma in Kabuli type chickpea flour (13 compounds) and in green lentil flour (10 compounds) were identified (Figure 4-1). These compounds were among those previously reported as ‘beany’ aroma compounds. List of these compounds, their sensory attributes and food source are shown in APPENDIX A. The LRI of each compound was calculated using a series of analogous n-alkane standards (C8-C20) is shown in Table 3-1. Subsequently, each compound was identified by comparing its mass spectra and LRI with spectra of known compounds in the NIST library and published papers APPENDIX B.

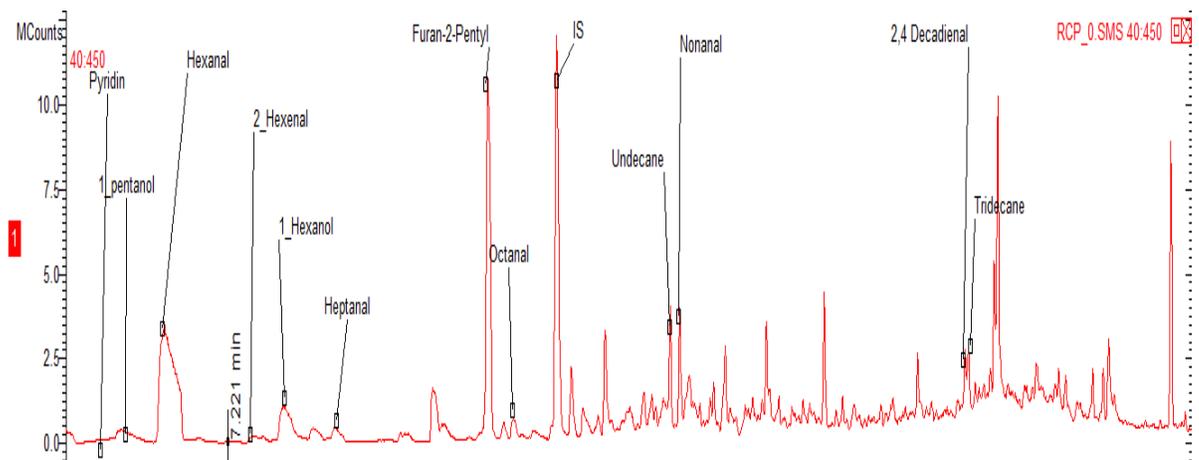


Figure 4-1 A chromatogram of volatile compounds extracted from non-micronized chickpea flour

Significantly lower concentrations of 'beany' volatile compounds were detected in lentil flour compared to chickpea. This may be due to the lower lipid content in lentil compared to chickpea resulting in reduced substrate concentration for LOX enzymes. A significant decrease in concentration of following 'beany' aroma compounds hexanal, 2-hexenal, heptanal, furan2-pentyl, and undecane was obtained when chickpea was subjected to micronization to 130°C surface temperature. However micronization to the surface temperature of 130°C had no significant effect on decreasing any of selected volatile compounds in green lentil. Micronization at 130°C decreased the following compounds in chickpea: 1-pentanol, hexanal, 2-hexenal, 1-hexanol, heptanal, furan 2-pentyl, and 2-octenal, by 43%, 39%, 37%, 31%, 37%, 41%, 23% respectively compared to non-micronized chickpea. Micronization at 150°C decreased the same volatile compounds by 62%, 44%, 70%, 74%, 37%, 49%, 51%, and 32% compared to non-micronized chickpea flour.

Table 4-1 Volatile compounds contributing to beany aroma and flavour in micronized and non-micronized chickpea

Beany Volatile Compounds	Sensory Attribute Based on literature †	LRI	Chickpea Kabuli			Leven test Significance	F-value & Significance ANOVA
			RCP	MCP130	MCP150		
1-pentanol	Green beany	<800	11.94 ^a (1.35)	6.77 ^{ab} (2.90)	4.51 ^b (3.22)	NS	F(2,8)=8.6 *
Hexanal	Beany, grassy, green odor	801	146.88 ^a (19.12)	88.67 ^b (28.17)	81.62 ^b (29.06)	NS	F(2,8)=7.6 *
2-Hexenal	Beany, grassy, green odor	851	2.92 ^a (0.58)	1.83 ^b (0.38)	0.89 ^b (0.42)	NS	F(2,8)=17.9 **
1-Hexanol	Beany, grassy, green odor	871	56.39 ^a (5.99)	38.79 ^a (4.18)	14.82 ^b (11.64)	NS	F(2,8)=25.3 ***
Heptanal	Beany	902	5.08 ^a (1.01)	3.20 ^b (0.76)	3.23 ^b (3.75)	NS	F(2,8)=6 *
Furan-2-pentyl	Beany, grassy, green odor	990	116.33 ^a (9.30)	68.16 ^b (22.69)	59.20 ^b (20.91)	NS	F(2,8)=11.3 **
Octanal	Painty oxidized	1005	3.86 (0.72)	2.59 (0.78)	2.64 (1.11)	NS	F(2,8)=2.4 NS
2-octenal	Green beany	1060	9.33 ^a (0.35)	7.19 ^{ab} (1.99)	4.57 ^b (2.29)	NS	F(2,8)=7.6 *
Undecane	Green	1099	14.89 ^a (0.59)	3.32 ^b (0.38)	1.80 ^c (0.89)	NS	F(2,8)=430 ***
Nonanal	Green beany	1105	15.38 (1.43)	11.47 (3.74)	10.49 (4.94)	NS	F(2,8)=2 NS
2,4-Decadienal	Beany	1297	8.64 ^a (1.17)	6.67 ^{ba} (3.24)	2.17 ^b (2.58)	NS	F(2,8)=7.7 *
Tridecane	Beany	1300	1.14 ^b (0.21)	0.96 ^b (0.09)	2.34 ^a (0.62)	*	F(2,8)=12.8 **
2,4-Undecadienal	Overall beany with other volatile	1322	59.48 ^a (6.51)	45.01 ^{ab} (19.93)	17.44 ^b (20.19)	NS	F(2,8)=6.8 *

RCP: raw chickpea (n=4), MCP130: micronized chickpea at 130°C (n=3), MCP150: micronized chickpea at 150°C (n=4), All means are ± standard deviation, Value for each volatile with different letters (a, b for row) are significantly different $\alpha = 0.05$, † literature cited for each compound is reported in Appendix A, NS not significant, * <0.05, ** < 0.01, *** < 0.001

Table 4-2 Volatile compounds contributing to beany aroma and flavor in micronized and non-micronized green lentil

Beany Volatile Compounds	Sensory Attribute Based on literature †	LRI	Green Lentil			Leven test Significance	F-value & Significance ANOVA
			RGL	MGL130	MGL150		
Hexanal	Beany, grassy, green odor	801	43.54 (16.40)	49.21 (26.36)	9.65 (1.48)	NS	F(2,8)=4.3 NS
2-Hexenal	Beany, grassy, green odor	851	3.51 ^a (1.48)	3.24 ^{ab} (1.56)	0.33 ^b (0.07)	NS	F(2,8)=6.0 *
1-Hexanol	Beany, grassy, green odor	871	19.59 ^a (7.25)	14.01 ^{ab} (8.20)	1.39 ^b (0.51)	NS	F(2,8)=6.5 *
Heptanal	Beany	902	0.60 (0.12)	0.60 (0.26)	0.37 (0.04)	NS	F(2,8)=1.9 NS
Furan-2-pentyl	Beany, grassy, green odor	990	16.50 (5.63)	17.53 (10.35)	7.89 (1.18)	NS	F(2,8)=1.8 NS
Octanal	Painty oxidized	1005	0.55 (0.16)	0.59 (0.24)	0.48 (0.05)	NS	F(2,8)=0.3 NS
2-octenal	Green beany	1060	0.04 ^b (0.01)	0.20 ^a (0.09)	0.15 ^{ab} (0.03)	*	F(2,8)=6.0 *
Undecane	Green	1099	1.15 (0.44)	1.45 (0.65)	0.95 (0.19)	NS	F(2,8)=0.9 NS
Nonanal	Green beany	1105	2.80 (0.8)	3.25 (1.67)	2.25 (0.53)	NS	F(2,8)=0.6 NS
Tridecane	Beany	1300	1.37 (0.57)	1.79 (0.52)	1.02 (0.28)	NS	F(2,8)=2.0 NS

RGL: raw green lentil (n=3), MGL130: micronized green lentil at 130°C (n=3), MGL150: micronized green lentil at 150°C (n=3). All means are ± standard deviation, Value for each volatile with different letters (a, b for row) are significantly different $\alpha = 0.05$, † literature cited for each compound is reported in Appendix A, NS not significant, * <0.05, ** < 0.01, *** < 0.001

As it is shown in Table 4-1 hexanal, 2-hexenal, heptanal, furan-2-pentyl, and undecane significantly decreased by micronization to 130°C while 1-pentanol, hexanal, 2-hexenal, hexanol, heptanal, furan-2-pentyl, 2-octenal, undecane, 2,4-undecadienal, and 2,4-undecadienal significantly decreased by micronization of chickpea to 150°C. The results indicated that micronization of chickpea to the surface temperature of 130°C and 150°C significantly influences the concentration of ‘beany’ aroma of chickpea and may be considered as an effective method to reduce the activity of LOX isozymes. However, it

should be noted that the differences between the effects of both levels of micronization in chickpea were statistically significant only for the following volatiles: 1-hexanol, undecane, and tridecane. The aforementioned volatiles are formed by oxidation and degradation of polyunsaturated fatty acids. These results indicate that micronization at 130°C can be used for processing chickpea and will be efficient to totally or partially reduce the beany aroma compounds.

Table 4-1 and Table 4-2 summarize all the compounds contributing to 'beany' aroma and flavor of chickpea and lentil and their concentrations. Three compounds including 1-pentanol, 2,4-decadienal, and 2,4 undecadienal were not detected in green lentil. Based on these results, micronization at 130°C had no significant effects on the concentration of volatile aroma compounds in green lentil compared to control flour. It must be noted that a 100 fold reduction in LOX activity has been reported in micronized green lentil at 135°C compared to non-micronized green lentil in previous study on green and red lentil (Der, 2010). Therefore, a significant reduction in the selected volatile compounds was expected after micronization of green lentil to surface temperature of 130°C. Based on the results reported in Table 4-2, micronization at 130°C had no impact on these volatile compounds and only hexanal and 2-hexenal were significantly reduced when green lentil seeds were micronized to surface temperature of 150°C. Although, micronization treatment to surface temperature of 130°C should decrease the concentration of volatile compounds which are formed by LOX activity, it should be considered that the lentil seeds in the present study were micronized in a dry state, while a tempering step was part of procedure in the study done by Der (2010). A higher micronization temperature such as 150°C, tempering the seeds prior to heating or longer time of micronization could be

effective to reduce LOX activities in green lentil. Micronization effect on physico-chemical characteristics of pulses depends on the seeds type, moisture content, surface temperature, and time of micronization (Sharma, 2009).

One of the major compounds with significant contribution to beany aroma and flavor is 1-pentanol (1 ppm in propylene glycol) (Vara-Ubol et al., 2004). At concentrations > 100 ppm, 1-pentanol showed sweat-like and barnyard manure aromas (Vara-Ubol et al., 2004). Lv et al. (2011) described the aroma of 1-pentanol as alcoholic wine and reported this aroma as being an objectionable aroma in acceptability of soy milk. This compound was found in untreated chickpea flours in our study while micronization treatment at 150°C significantly decreased its concentration. In addition, 1-pentanol has been reported in high concentration in unprocessed chickpea (Rembold et al., 1989) but not in roasted chickpea (Lasekan et al., 2011). Therefore, a heating treatment may be responsible for reduction of the level of 1-pentanol in chickpea.

Hexanal and heptanal in food legumes are usually produced by enzymatic or auto-oxidation of polyunsaturated fatty acids (Chitsamphandhvej, Phakdee, & Thanasan, 2008). Hexanal which has been reported as 'beany' in many studies showed no 'beany' aroma at any concentrations when tested as a single aroma (Vara-Ubol et al., 2004).

Hexanal aroma has been defined as a green/pea pod, rancid, sour aromatic or chemical like aroma in previous studies (Vara-Ubol et al., 2004) and is considered the most potent aroma in soymilk (Lv et al., 2011). However, it has been reported that hexanal with other 'beany' aroma compounds such as 1-octene-3-one intensifies the 'beany' attribute of that compound (Bott & Chambers-IV, 2006).

It has been reported that volatile carbonyl compounds concentration in lentil are lower than those for other bean varieties and split peas, while the level of alcohol compounds such as pentanol and hexanol are higher (Lovegren et al., 1979).

4.1.2. Pulse flour fatty acid analysis

The fatty acid composition of non-micronized and micronized chickpea and lentil are presented in Table 4-3. The values are reported as percentage of total fatty acid. Non-micronized green lentil, micronized green lentil at 130°C and 150°C contained 45.06 ±0.34, 45.00±0.1, and 45.13±0.15% linoleic and 11.66±0.13, 11.44±0.03, and ±11.50±0.08% linolenic acids respectively while chickpea contains 61.46±0.23, 61.72±0.14, and 61.67±0.14% linoleic acid and 2.48±0.02, 2.49±0.01, and 2.62±0.02% linolenic acid. Although the level of lipid in chickpea and lentil is low the majority of fatty acid in the lipid portion is polyunsaturated fatty acids, belonging to the ω-6 and ω-3 series with physiological activity and health benefits. On the other hand conjugated double bonds in ω-6 and ω-3 fatty acids provide an active site for activity of LOX isozymes and make chickpea and lentil susceptible to deterioration.

Table 4-3 Effect of micronization on fatty acid content of chickpea and lentil (% of total fatty acid)

Fatty Acid	RCP	MCP130	MCP150	RGL	MGL130	MGL150	F-value & significance
C14:0	0.16 ^b (0.00)	0.16 ^b (0.00)	0.16 ^b (0.00)	0.32 ^a (0.00)	0.32 ^a (0.00)	0.32 ^a (0.00)	F(5,34)=5900 ***
C15:0	0.07 ^b (0.00)	0.07 ^b (0.00)	0.08 ^b (0.00)	0.15 ^a (0.00)	0.15 ^a (0.00)	0.14 ^a (0.00)	F(5,34)=3169 ***
C16:0	11.07 ^b (0.09)	11.06 ^b (0.05)	10.94 ^b (0.02)	14.45 ^a (0.15)	14.53 ^a (0.13)	14.39 ^a (0.09)	F(5,34)=2124 ***

Fatty Acid	RCP	MCP130	MCP150	RGL	MGL130	MGL150	F-value & significance
C16:1	0.16 ^a (0.00)	0.16 ^a (0.00)	0.16 ^a (0.00)	0.07 ^b (0.00)	0.07 ^b (0.00)	0.07 ^b (0.00)	F(5,34)=2793 ***
C17:0	0.08 ^b (0.00)	0.08 ^b (0.00)	0.08 ^b (0.00)	0.12 ^a (0.00)	0.12 ^a (0.00)	0.12 ^a (0.00)	F(5,34)=1921 ***
C17:1	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	0.07 (0.00)	F(5,34)=3.6 NS
C18:0	1.32 ^a (0.01)	1.31 ^a (0.01)	1.27 ^c (0.01)	1.32 ^a (0.02)	1.32 ^a (0.01)	1.30 ^b (0.00)	F(5,34)=20 ***
C18:1(9)	20.10 ^b (0.14)	19.93 ^b (0.03)	19.94 ^b (0.07)	21.96 ^a (0.18)	22.17 ^a (0.15)	22.18 ^a (0.09)	F(5,34)=565 ***
C18:1(7)	1.09 ^a (0.01)	1.08 ^a (0.00)	1.08 ^a (0.01)	0.88 ^b (0.01)	0.88 ^b (0.01)	0.87 ^c (0.00)	F(5,34)=2054 ***
C18:2(6)	61.46 ^a (0.23)	61.72 ^a (0.14)	61.67 ^a (0.14)	45.06 ^b (0.34)	45.00 ^b (0.13)	45.13 ^b (0.15)	F(5,34)=11830 ***
C19:1	0.02 (0.00)	0.01 (0.01)	0.02 (0.00)	0.02 (0.01)	0.02 (0.01)	0.03 (0.00)	F(5,34)=4.6 NS
C18:3(3)	2.48 ^c (0.02)	2.49 ^c (0.01)	2.62 ^b (0.02)	11.66 ^a (0.13)	11.44 ^a (0.03)	11.50 ^a (0.08)	F(5,34)=32715 ***
C20:0	0.54 (0.01)	0.53 (0.01)	0.51 (0.00)	0.56 (0.01)	0.55 (0.00)	0.54 (0.00)	F(5,34)=111 NS
CLA 9,11	0.04 (0.01)	0.02 (0.04)	0.04 (0.02)	0.02 (0.01)	0.02 (0.02)	0.03 (0.01)	F(5,34)=1.16 NS
C20:1(15)	0.04 (0.00)	0.05 (0.02)	0.04 (0.01)	0.03 (0.01)	0.04 (0.01)	0.04 (0.01)	F(5,34)=3.7 NS
C20:1(12)	0.02 ^b (0.00)	0.02 ^b (0.01)	0.02 ^b (0.00)	0.17 ^a (0.00)	0.17 ^a (0.00)	0.17 ^a (0.00)	F(5,34)=3311 ***
C20:1(9)	0.38 ^c (0.01)	0.38 ^c (0.00)	0.37 ^d (0.00)	0.68 ^a (0.01)	0.68 ^a (0.00)	0.66 ^b (0.00)	F(5,34)=11165 ***
C20:2(6)	0.08 (0.00)	0.07 (0.03)	0.11 (0.03)	0.28 (0.28)	0.28 (0.25)	0.35 (0.21)	F(5,34)=2.7 NS
C20:3(6)	0.01 (0.01)	0 (0)	0.01 (0.01)	0.03 (0.00)	0.02 (0.01)	0.02 (0.00)	F(5,34)=14.5 NS
C22:0	0.36 ^c (0.01)	0.35 ^c (0.00)	0.35 ^c (0.00)	0.51 ^a (0.01)	0.51 ^a (0.00)	0.49 ^b (0.00)	F(5,34)=3360 ***
C22:1	0.02 ^b (0.00)	0.02 ^b (0.01)	0.02 ^b (0.00)	0.16 ^a (0.00)	0.16 ^a (0.00)	0.16 ^a (0.01)	F(5,34)=1556 ***
C20:5(3)	0.06 (0.04)	0.07 (0.04)	0.07 (0.01)	0.05 (0.01)	0.06 (0.01)	0.05 (0.00)	F(5,34)=1.04 NS
C22:2(6)	0.14 ^b (0.06)	0.14 ^b (0.06)	0.14 ^b (0.01)	0.71 ^a (0.53)	0.72 ^a (0.27)	0.71 ^a (0.21)	F(5,34)=8.4 ***
C22:3(3)	0.17 ^c (0.02)	0.16 ^c (0.02)	0.17 ^c (0.00)	0.40 ^a (0.01)	0.39 ^a (0.00)	0.38 ^b (0.00)	F(5,34)=983 ***
C24:1	0.01 ^b (0.02)	0.01 ^b (0.01)	0 ^b (0)	0.01 ^b (0.00)	0.06 ^a (0.01)	0.05 ^a (0.00)	F(5,34)=51 ***
C22:5(3)	0.03 ^c (0.02)	0.00 ^c (0.01)	0.01 ^c (0.01)	0.09 ^a (0.00)	0.09 ^a (0.00)	0.08 ^b (0.00)	F(5,34)=145 ***

Fatty Acid	RCP	MCP130	MCP150	RGL	MGL130	MGL150	F-value & significance
C22:6(3)	0.02 (0.01)	0.02 (0.02)	0.02 (0.00)	0.01 (0.01)	0.01 (0.01)	0.003 (0.01)	F(5,34)=2.4 NS
SUMSFA	13.59 ^b (0.12)	13.57 ^b (0.10)	13.42 ^b (0.06)	17.52 ^a (0.21)	17.58 ^a (0.16)	17.39 ^a (0.11)	F(5,34)=2030 ***
SUM MUFA	21.93 ^b (0.18)	21.74 ^b (0.12)	21.74 ^b (0.1)	24.18 ^a (0.23)	24.39 ^a (0.19)	24.36 ^a (0.12)	F(5,34)=648 ***
SUM ω -6	61.69 ^a (0.30)	61.93 ^a (0.23)	61.93 ^a (0.19)	46.08 ^b (1.16)	46.01 ^b (0.66)	46.21 ^b (0.57)	F(5,34)=6510 ***
SUM ω -3	2.76 ^c (0.10)	2.74 ^c (0.08)	2.88 ^b (0.04)	12.20 ^a (0.16)	12.00 ^a (0.06)	12.02 ^a (0.10)	F(5,34)=31239 ***

RCP: raw chickpea (n=5), MCP130: micronized chickpea at 130°C (n=6), MCP150: micronized chickpea at 150°C (n=6), RGL: raw green lentil (n=6), MGL130: micronized green lentil at 130°C (n=6), MGL150: micronized green lentil at 150°C (n=6). All means are \pm (standard deviation), Value for each fatty acid with different letters (a, b, c, d in the same row) were significantly different $P \geq 0.05$, NS not significant, * <0.05, ** < 0.01, *** <0.001

The results show micronization had no significant effect on linoleic acid content of chickpea and lentil flour after heat processing compared to non-micronized flour. However, micronization at 150°C caused a significant increase in total ω -3 and linolenic acid in chickpea while no change was observed in lentil linolenic acid content at any level of micronization. Linolenic acid with three double bonds is more unstable and susceptible to auto-oxidation compared to linoleic acid and oleic acid (Shahidi & Zhong, 2005). In contrast to the result of our study, micronization of soybean at 140°C decreased linoleic acid content by 8.5% with higher decrease in linolenic acid (Zilic et al., 2010). Heating treatment by micronization is far more intense than autoclaving and extrusion vapor pressure (Zilic et al., 2010). Higher decrease of linolenic acid has been reported with micronization (14-38%) than dry extrusion (6-12%) (Zilic et al., 2010). The moisture level of seeds during heat treatment may have an effect on degradation of

polyunsaturated fatty acids. The results show that micronization of lentil and chickpea (at dry state) had no effect on total saturated, mono-unsaturated and ω -6 fatty acids, while a significant increase in total ω -3 was observed only in chickpea and not in lentil. Although there was no difference observed in saturated and mono-unsaturated fatty acid after micronization in this study, Zilic et al. (2010) reported an increased oleic acid and stearic acid after micronization due to transition from linoleic acid with two double bonds to a more stable state with one double bond or the saturated state. The results of fatty acid analysis also revealed a difference in the concentration of each class of fatty acids between species. Chickpea had significantly higher ω -6 class of fatty acids while lentil was higher in saturated, mono-unsaturated, ω -3 fatty acids. Although an intense heat treatment is important for digestibility and bioavailability of legumes' macro- and micro-nutrients; it also may decrease the nutritional quality of pulse legumes (Zilic et al., 2010). Tempering prior to micronization may have a protective effect against degradation of ω -3 polyunsaturated fatty acids due to increase the seed moisture content and vapor pressure.

LOX is known to have a significant role in 'beany' volatile formation of legumes (Baysal & Demirdoven, 2007; Rackis et al., 1979). The main substrate for LOX is linoleic and linolenic acid; peroxidation and degradation of linoleic and linolenic acids are the source of volatile carbonyl compounds (Baysal & Demirdoven, 2007; Rackis et al., 1979).

Although a positive correlation has been reported between LOX-1 and LOX-2 activity and hexanal and pentanal and the of level of oleic, linoleic, and linolenic acids with pentanal and hexanal in buckwheat noodles; the result of present study did not confirm these correlation (Suzuki et al., 2010).

4.1.3. Pulse flour pH analysis

The results for pH measurements are summarized in Table 4-4. The pH of chickpea and lentil without micronization is 6.12 and 6.33 respectively. CL-1 in chickpea and C1 (pH optimum 6.5) in lentil have been reported to have a soybean LOX-2 type characteristic with pH optimum of 6.8 with the ability to act on both triglyceride and free fatty acids (Baysal & Demirdoven, 2007; Hilbers et al., 1995; Sanz et al., 1994). Although there are LOX enzymes in chickpea and lentil with soybean LOX-1 characteristics and pH optimum of 9, the most active LOX in both chickpea and lentils are CL-1 and C1 with pH optimum of <6.5 which can act on the polyunsaturated fatty acid content of pulse seeds. It has been noted that after micronization the level of ‘beany’ aroma compounds has been alleviated in both types of pulse flour, however these compounds have not been eliminated completely which indicate the possibility of other pathways for ‘beany’ aroma formation other than the enzymatic pathway.

Table 4-4 pH measurement of micronized and non-micronized lentil and chickpea

Compounds	pH of pulse flour
RCP	6.12 ^d ±0.03
MCP130	6.14 ^d ±0.01
MCP150	6.23 ^c ±0.03
RGL	6.33 ^b ±0.02
MGL130	6.34 ^b ±0.01
MGL150	6.39 ^a ±0.01
F-value & Significance	F(5,23)=576, ***

RCP: raw chickpea (n=4), MCP130: micronized chickpea at 130°C (n=4), MCP150: micronized chickpea at 150°C (n=4), RGL: raw green lentil (n=4), MGL130: micronized green lentil at 130°C (n=4), MGL150: micronized green lentil at 150°C (n=4), All means are ± (standard deviation), Value for pH measurements with different letters (a, b, c, d in the same column) are significantly different, $\alpha \geq 0.05$, NS not significant, * <0.05, ** < 0.01, *** < 0.001

4.2. Low-fat beef burger chemical and sensorial analysis

4.2.1. Raw low-fat beef burger fatty acid analysis results and discussion

This part of the study was done to determine the impact of micronized pulse flour in beef burger formulation on the concentration of different types of fatty acids in beef burger samples with 10% fat content. Data for the fatty acid profile of burgers are presented in Table 4-5. The predominant type of fatty acid in all types of burgers was mono unsaturated fatty acid followed by saturated fatty acid. It has been reported that A high level of monounsaturated fatty acid provides softness and palatability (Smith, Gill, Lunt, & Brooks, 2009). The fatty acid composition of beef depends on the animal feed and breed (Smith et al., 2009). Grain fed cattle has a higher concentration of monounsaturated fatty acids due to increased activity of tissue stearoyl-CoA desaturase (SCD) (Smith et al., 2009). However, an increase in unsaturated fatty acids contributes to decreased shelf life and increased off-odor formation (Rhee, 2000). The saturated fatty acid content of burgers with chickpea and lentil was $38.2\pm 0.8\%$ and $39.0\pm 0.5\%$, respectively, with palmitic acid (C16:0) being the major saturated fatty acid ($25.3\pm 0.5\%$ and $25.6\pm 0.3\%$) followed by stearic acid (C18:0) ($8.4\pm 0.2\%$ and $9.0\pm 0.3\%$) and myristic acid (C14:0) ($2.9\pm 0.2\%$ and $2.9\pm 0.1\%$). No significant differences were observed in levels of myristic and palmitic acid after micronization of lentil, while a significant decrease in stearic acids were noted in burgers with micronized lentil at 150°C . Longer chain saturated fatty acids, such as arachidic (C20:0) and behenic (C22:0) acids, had concentrations less than 0.1% with a slight decrease in behenic acid in burgers with micronized lentil at 150°C compared to the non-micronized lentil flour. The major monounsaturated fatty acid was oleic acid (C18:1) having $42.68\pm 1.17\%$ in burgers with no binder followed by C16:1

(6.65±0.22%) and C14:1 (1.65±0.14%). The level of monounsaturated fat was not affected by micronization treatment in pulse flour as reported in section 4.1.2.

Table 4-5 Effect of micronization on fatty acid content of low-fat beef-burgers extended with chickpea and lentil (% of total fatty acid)

	Co	RCP	MCP-130	MCP-150	RGL	MGL-130	MGL-150	F-Value & Significance
SUM SFA	39.80 ^a (1.12)	38.2 ^{bc} (0.75)	37.30 ^c (0.54)	37.20 ^c (0.75)	39.03 ^{ab} (0.53)	38.73 ^{ab} (0.65)	39.44 ^a (1.33)	F(6,35)= 8.4 ***
SUM MUFA	53.73 ^a (1.22)	53.0 ^{ab} (0.89)	53.2 ^{ab} (0.54)	52.25 ^b (0.68)	53.4 ^{ab} (0.49)	53.76 ^a (0.57)	52.60 ^b (1.01)	F(6,35)=2.88 *
SUM ω-6	2.76 ^c (0.12)	4.32 ^c (0.40)	4.83 ^b (0.68)	5.82 ^a (0.31)	2.95 ^c (0.13)	2.81 ^c (0.22)	3.22 ^d (0.33)	F(6,35)=66.6 ***
SUM ω-3	0.43 ^c (0.02)	0.41 ^c (0.03)	0.52 ^b (0.05)	0.57 ^a (0.03)	0.45 ^c (0.03)	0.45 ^c (0.04)	0.52 ^{ab} (0.05)	F(6,35)=15.0 ***
C14:0	3.24 ^a (0.22)	2.91 ^{ab} (0.23)	2.74 ^b (0.1)	2.72 ^b (0.06)	2.90 ^{ab} (0.11)	2.99 ^{ab} (0.09)	3.23 ^a (0.42)	F(6,35)=6.12 ***
C15:0	0.52 ^a (0.03)	0.49 ^{ab} (0.04)	0.46 ^b (0.02)	0.46 ^b (0.01)	0.47 ^{ab} (0.01)	0.49 ^{ab} (0.02)	0.52 ^{ab} (0.06)	F(6,35)=4.06 **
C16:0	26.1 ^{ab} (0.70)	25.3 ^{bc} (0.54)	24.66 ^c (0.27)	24.70 ^c (0.34)	25.6 ^{abc} (0.25)	25.7 ^{abc} (0.27)	26.39 ^a (1.20)	F(6,35)=7.04 ***
C17:0	1.04 (0.04)	1.00 (0.04)	0.97 (0.04)	0.97 (0.07)	1.00 (0.02)	1.00 (0.04)	0.99 (0.03)	F(6,35)=2.14 NS
C18:0	8.85 ^{ab} (0.32)	8.38 ^{ab} (0.22)	8.36 ^{ab} (0.21)	8.23 ^b (0.53)	9.01 ^a (0.29)	8.49 ^{ab} (0.47)	8.24 ^b (0.42)	F(6,35)=4.03 **
C20:0	0.06 ^b (0.01)	0.08 ^a (0.01)	0.08 ^a (0.01)	0.08 ^a (0.00)	0.07 ^{ab} (0.01)	0.07 ^{ab} (0.02)	0.06 ^b (0.02)	F(6,35)=5.3 **
C22:0	0.014 ^{bc} (0.004)	0.033 ^a (0.008)	0.032 ^a (0.01)	0.037 ^a (0.002)	0.018 ^b (0.01)	0.011 ^{bc} (0.008)	0.002 ^c (0.006)	F(6,35)=22.4 ***
C14:1	1.65 ^a (0.14)	1.44 ^{ab} (0.10)	1.39 ^b (0.06)	1.36 ^b (0.10)	1.40 ^{bc} (0.05)	1.51 ^{ab} (0.12)	1.66 ^a (0.22)	F(6,35)=5.91 ***
C16:1	6.65 ^a (0.22)	6.38 ^{ab} (0.13)	6.32 ^{ab} (0.25)	6.18 ^b (0.39)	6.35 ^{ab} (0.22)	6.54 ^{ab} (0.33)	6.69 ^{ab} (0.41)	F(6,35)=2.46 *
C18:1(9)	42.68 (1.17)	42.43 (0.86)	42.67 (0.32)	41.98 (0.35)	42.92 (0.33)	42.93 (0.21)	41.61 (1.36)	F(6,35)=2.38 0.05NS
C18:1(7)	2.36 (0.15)	2.38 (0.09)	2.39 (0.08)	2.33 (0.12)	2.34 (0.06)	2.37 (0.08)	2.29 (0.08)	F(6,35)=0.77 NS
C20:1(12)	0.12 (0.003)	0.12 (0.007)	0.12 (0.002)	0.13 (0.003)	0.13 (0.001)	0.14 (0.03)	0.12 (0.02)	F(6,35)=1.5 NS
C20:1(9)	0.25 (0.02)	0.25 (0.01)	0.25 (0.02)	0.25 (0.01)	0.25 (0.01)	0.25 (0.01)	0.22 (0.05)	F(6,35)=1.9 NS
C22:1(12)	0.034 ^a (0.016)	0.023 ^{ab} (0.007)	0.027 ^{ab} (0.006)	0.027 ^{ab} (0.005)	0.018 ^{ab} (0.000)	0.016 ^b (0.009)	0.019 ^{ab} (0.013)	F(6,35)=2.6 *
C18:2(6)	2.24 ^c (0.09)	3.89 ^b (0.35)	4.22 ^b (0.56)	5.16 ^a (0.27)	2.43 ^c (0.09)	2.38 ^c (0.16)	2.68 ^c (0.24)	F(6,35)=88.06 ***
C20:2(6)	0.04 (0.01)	0.03 (0.01)	0.04 (0.003)	0.04 (0.002)	0.04 (0.001)	0.04 (0.004)	0.03 (0.01)	F(6,35)=5.2 *
C20:3(6)	0.15 ^{ab} (0.007)	0.13 ^b (0.01)	0.16 ^a (0.02)	0.17 ^a (0.01)	0.15 ^{ab} (0.008)	0.13 ^b (0.01)	0.14 ^{ab} (0.04)	F(6,35)=5.2 **
C20:4(6)	0.33 ^{bc} (0.03)	0.27 ^c (0.04)	0.40 ^{ab} (0.09)	0.45 ^a (0.04)	0.34 ^{bc} (0.03)	0.26 ^c (0.05)	0.36 ^{ab} (0.06)	F(6,35)=9.98 ***
C18:3(3)	0.31 ^b	0.32 ^b	0.40 ^a	0.42 ^a	0.33 ^b	0.34 ^b	0.40 ^a	F(6,35)=26.3

	Co	RCP	MCP- 130	MCP- 150	RGL	MGL- 130	MGL- 150	F-Value & Significance
C22:5(3)	(0.02) 0.12 ^{ab}	(0.02) 0.09 ^b	(0.03) 0.12 ^{ab}	(0.02) 0.15 ^a	(0.01) 0.12 ^{ab}	(0.02) 0.11 ^{ab}	(0.03) 0.12 ^{ab}	*** F(6,35)=4.2
ω -3/ ω -6	(0.01) 0.155 ^{ab} (0.003)	(0.02) 0.095 ^d (0.005)	(0.03) 0.108 ^c (0.007)	(0.01) 0.098 ^d (0.006)	(0.02) 0.151 ^b (0.005)	(0.02) 0.160 ^a (0.010)	(0.03) 0.162 ^a (0.003)	** F(6,35)=162 ***

The treatments were Co: burgers with no pulse added, RCP: burgers with chickpea flour (n=6), MCP130: burgers with micronized chickpea at 130°C (n=6), MCP150: burgers with micronized chickpea at MCP150°C (n=6), RGL: burgers with raw green lentil (n=6), MGL130: burgers with micronized green lentil at 130°C (n=6), MGL150: burgers with micronized green lentil at 150°C (n=6). All means are \pm (standard deviation), mean values for each fatty acid with different letters (a, b, c, d for row) are significantly different $\alpha \geq 0.05$, NS not statistically significant, * <0.05, ** < 0.01, *** < 0.001

Total omega-6 fatty acid content of burgers was significantly affected by addition of chickpea flour. Compared to control which had 2.76 \pm 0.12% total omega-6 fatty acid, burgers with chickpea flour had significantly higher total omega-6 fatty acid content at 4.32 \pm 0.4%. The results can be explained by the level of omega-6 fatty acid in chickpea flour (61.7 \pm 0.3%) measured in section 4.1.2. Although, there was no significant difference in total omega-6 content of chickpea and lentil flour after micronization, an increase in level of ω -6 fatty acids was observed in burgers with micronized pulses at 150°C. These results suggest that the active components of pulses, such as LOX isozymes, may have effects on fatty acid content of beef burgers. In contrast to ω -6 content of burger with chickpea, the level of total omega-3 fatty acid is low at 0.43 \pm 0.01% with micronization significantly increased its concentration (section 4.1.2). The total ω -3 fatty acid in burgers also wasn't affected by addition of chickpea and lentil flours compared to control, while there was an increase in omega-3 fatty acid observed in burgers with addition of micronized chickpea at 130 and 150°C and lentil at 150°C. The increase in the level of unsaturated fatty acid after utilization of micronized pulse flour indicates that activity of LOX in non-micronized pulse flour may cause the oxidation of

polyunsaturated fatty acids. Another factor that should be considered is the antioxidant activity of the polyphenols content of pulses (Grajales-Garcia et al., 2012; Hernandez-Salazar et al., 2010; Silva-Cristobal et al., 2010). Pulse legumes such as lentil, chickpea, and black bean have been shown to contribute to antioxidant properties (Hernandez-Salazar et al., 2010). Micronization of pulse flour is shown to be an effective way to decrease or eliminate oxidation of poly-unsaturated fatty acids in beef-burgers. To prevent rancid, off-flavor of meat products at freezing temperatures, due to lipid soluble radicals, there is a need to treat meat with an antioxidant (Forell, Ranalli, Zaritzky, Andres, & Califano, 2010).

The shift in saturated and unsaturated fat content of burgers formulated with pulse flour indicates that micronization treatment of chickpea and lentil flour is effective in reducing oxidation activities of LOX isozymes. However, optimum micronization temperature is different between the two types of pulses. Micronization at 130°C is effective to produce functional pulse flour with no oxidizing effect while a higher temperature of 150°C is needed for green lentil flour.

4.2.2. Results of pH analysis and WHC of raw low-fat beef-burgers

The results of pH measurements and WHC behavior for each burger are shown in Table 4-6. Analysis of variance of WHC for the overall swelling property of beef burgers showed no significant differences throughout the different meat systems. Low-fat beef burgers extended with chickpea flour showed significantly higher pH value (5.66 ± 0.01) compared to those with no binder (5.57 ± 0.02). The burgers with micronized chickpea at

130°C showed higher pH (5.75 ± 0.01) compared to non-micronized chickpea (5.66 ± 0.01).

A significant decrease in pH was observed in burgers extended by micronized chickpea at 150°C (5.71 ± 0.01) compared to micronized chickpea at 130°C (5.75 ± 0.01).

Table 4-6 Effect of micronization on pH of low-fat beef-burger extended with micronized and non-micronized chickpea and lentil flours

Sample	pH of Raw Burger	WHC of Raw Burgers
Co	$5.57^e \pm 0.02$	1.14 ± 0.53
RCP	$5.66^d \pm 0.01$	1.35 ± 0.26
RGL	$5.70^{bc} \pm 0.02$	1.20 ± 0.37
MCP-130	$5.75^a \pm 0.01$	1.42 ± 0.15
MCP-150	$5.71^b \pm 0.01$	0.85 ± 0.24
MGL-130	$5.67^d \pm 0.01$	0.73 ± 0.41
MGL-150	$5.69^c \pm 0.02$	1.07 ± 0.46
F-Value & Significance	F(6,77)=152.6 ***	F(6,21)=1.87 NS

The treatments were Co: burgers with no pulse added (Control), RCP: burgers with chickpea flour (n=4), MCP130: burgers with micronized chickpea at 130°C (n=4), MCP150: burgers with micronized chickpea at MCP150°C (n=4), RGL: burgers with raw green lentil (n=4), MGL130: burgers with micronized green lentil at 130°C (n=4), MGL150: burgers with micronized green lentil at 150°C (n=4), All means are \pm (standard deviation), mean values for each treatment with different letters (a, b, c, d,e for column) are significantly different $\alpha \geq 0.05$, NS not statistically significant, * <0.05, ** < 0.01, *** < 0.001

Addition of green lentil flour also increased pH to 5.70 ± 0.01 with a slight decrease by utilization of micronized lentil at 130°C and further increased to 5.69 ± 0.02 with micronized lentil at 150°C. Although the change in pH by utilization of pulse flour was significant theoretically it is not over the normal range of pH 4.5–7 for meat products (Gault, 1985). Harrell et al. (1978) examined the tenderness of beef steers in pH condition between 4.9 and 6.5. In contrast to our results, they showed that there is a linear relationship between improved tenderness and pH condition of meat (Harrell, Bidner, &

Iaza, 1978). They found an increase in sarcomere length and a decrease in shear value with increasing pH along with a decrease in cooking loss percentage (Harrell et al., 1978). The highest measured pH among the low-fat burgers with lentil and chickpea flour was 5.75 ± 0.01 for burgers with micronized chickpea at 130°C followed by micronized chickpea at 150°C . A higher pH of 6.72 ± 0.07 was reported by incorporating 12% soy flour as binder into beef patties, with acceptable texture and juiciness (Ammar, 2012). Moreover, a significant increase ($P < 0.05$) in pH and WHC has been reported for burgers extended with common bean flour at 2.5%, 5%, 7.5%, and 10% compared to the control which contained no extender with no significant difference in WHC with percentage of extender (Dzudie et al., 2002). However, Dzudie et al. (2002) reported a positive linear relationship between the level of extender and pH condition. To increase WHC in meat products, an increase in pH condition is necessary. By increasing the pH condition, the molecules of water will have the ability to bond to the side groups of the amino acids in protein (Sickler, 2000).

pH is also one of the factors which has considerable effects on the rate of lipid oxidation in meat products by protonation of bonded oxygen and formation of superoxide (Aberle, Forrest, Gerrard, & Mills, 2001; Chaijan, 2008). Increases in lipid oxidation by decrease in pH condition in meat products has been reported (Rhee, 2000). Therefore, incorporation of a variety of ingredients in processed meat products may delay the lipid oxidation by increasing pH of the products. Therefore, improving WHC of meat products, through addition of binder, could exert their effects by increasing the pH value.

4.2.3. *Spectrocolorimetry of raw and cooked low-fat beef burger with added micronized and non-micronized chickpea and lentil flour*

4.2.3.1. *Raw burger color evaluation*

Since the color of fresh meat products is one of the most important factors influencing consumer acceptability and purchasing decision (Carpenter, Cornforth, & Whittier, 2001; Wulf & Wise, 1999), the color of raw burgers containing pulse flours was evaluated. The color measurements (CIE L*, a*, b*) of raw samples are shown in Table 4-7. The Spectrocolorimeter results for color differentiation of beef burgers showed that lentil and chickpea flours and micronization treatment affected the a* and b* value for raw beef burgers. The addition of chickpea flour resulted in a higher L* value which translates to a lighter color with a slight but not statistically significant decrease by micronization. This difference between the L* value of the burgers with chickpea flour compared to control (only meat) could be related to the dilution of myoglobin with the addition of bean flour (Dzudie et al., 2002). A significant increase in L* value was reported in raw meat patties extended with chickpea flour compared to green lentil which is in agreement with the result observed in our study (Holliday et al., 2011). In contrast to burgers with chickpea flour, addition of micronized and non-micronized green lentil flour had no significant effect on L* value compared to control burger. The maximum value for L* is 100 which means complete reflection whereas the minimum is 0 which is for black. Positive a* and b* reflect on redness and yellowness respectively.

Table 4-7 Spectrocolorimetry results for raw low-fat beef-burgers extended by pulse flour after 30 minutes blooming time

Pulse Beef-Burger	L*	a*	b*
Control (Co)	36.00 ^b ± 0.91	7.56 ^c ± 0.09	15.13 ^c ± 0.34
RCP	39.41 ^a ± 0.66	11.14 ^d ± 0.29	17.48 ^{cd} ± 0.41
MCP 130 °C	38.95 ^a ± 0.51	14.84 ^b ± 0.32	19.63 ^a ± 0.34
MCP150 °C	38.81 ^a ± 0.97	15.60 ^a ± 0.36	19.91 ^a ± 0.53
RGL	35.94 ^b ± 0.52	11.39 ^d ± 0.59	17.05 ^d ± 0.29
MGL 130 °C	35.46 ^b ± 0.83	14.23 ^c ± 0.40	17.90 ^{bc} ± 0.41
MGL 150 °C	35.90 ^b ± 0.89	14.45 ^{bc} ± 0.50	18.16 ^b ± 0.17
F-Value & Significance	F(6,49)=41 ***	F(6,49)=424 ***	F(6,49)=152 ***

Co: burgers with no binder, RCP: burgers with raw chickpea (n=2), MCP130: burgers with micronized chickpea at 130°C (n=2), MCP150: burgers with micronized chickpea at 150°C (n=2), RGL: burgers with raw green lentil (n=2), MGL130: burgers with micronized green lentil at 130°C (n=2), MGL150: burger with micronized green lentil at 150°C (n=2). All means are ± standard deviation, Value for each treatment with different letters (a, b, c, d, e for column) are significantly different $\alpha \geq 0.05$, NS not significant, * < 0.05, ** < 0.01, *** < 0.001,

Utilization of pulse flour resulted in significant increases in a* value with further increases by micronization. However, there was no difference between the levels of micronization in burgers containing lentil while a significant increase in a* value was observed for burgers with chickpea micronized at 150°C compared to 130°C. A similar effect was observed for b* value in which a significant increase was observed in b* value in burgers containing pulse flour. Micronized chickpea and lentil to 130°C increased the b* value compared to burgers with non-micronized chickpea and lentil, with no statistically significant difference between two micronization treatments. Overall, micronized chickpea increased the lightness, redness and yellowness of raw burger color compared to micronized lentil flour, while non-micronized chickpea only had a significant effect on lightness of raw burgers compared to non-micronized lentil flour

resulting in lighter burgers. Moisture availability may have an effect on gelatinization of pulses leading to formation of richer and darker color (Arntfield et al., 1997)

The redness (a^* value) of meat products is developed as a result of oxygenation of deoxymyoglobin (DMB) pigments to produce oxymyoglobin (OMB) (Claus, 2007). DMB is present in meat as native state of pigment which is characterized by a purplish red color. When the pigment in meat cannot bind oxygen, due to oxidation of the heme iron from ferrous (Fe^{+2}) to ferric (Fe^{+3}), it turns brown and is called met-myoglobin (MMB) (Claus, 2007). Increases in a^* and b^* values in raw burgers containing pulse flour could be due to increased water binding by other components of burgers which can leave the binding site of the pigments unoccupied to bind oxygen molecules.

The increased availability of binding sites of the pigments for oxygen results in more OMB pigment formation. Serdaroglu, Yildiz-Turp, and Abrodimov (2005) reported that addition of 10% lentil and chickpea flour to meatballs resulted in an increased L^* value as a result of myoglobin dilution with no significant effect on a^* and b^* value. In agreement with Serdaroglu et al. (2005), micronized and non-micronized chickpea increased the L^* value of the raw burgers compared to control, while addition of lentil did not modify the lightness of the product. The conflict could be due to the difference in the type of lentils used in these two studies. It is not clear if red or green lentils were used in formulation of the meatballs.

Holliday et al. (2011) reported no significant differences in three color values with addition of 35-50% green lentil and chickpea flour to beef and pork sausage burgers containing 20 and 18% fat compared to controls which were each type of burgers without

extender. However, in agreement with our study, a significant increase in L* value was reported in raw meat burgers containing chickpea flour compared to green lentil.

Der (2010), in agreement with our results, reported no change in L* value with addition of 6% non-micronized and micronized green lentil flour the first day and after storage for seven days. Although, compared to control, burgers with 6% micronized green lentil were reported to have lighter color (Der, 2010), yet there was no significant difference in L* value of burgers with added non-micronized and micronized lentil flour in the present study. The discrepancy in results could be due to absence of tempering steps in the present study.

Based on the results of Spectrocolorimetry micronization was effective in increasing redness and yellowness but not on lightness of raw burgers which indicates micronization treatment can improve the characteristics of pulses as ingredients in minced meat products.

4.2.3.2. Cooked burger color evaluation

Although, addition of chickpea flour increased the L* value and lentil flour had no effect in L* value in raw burgers, cooked burgers with pulse flour showed significantly darker color compared to control (Table 4-8). Micronization showed no effect on the L* value of cooked burger compared to burgers extended with non-micronized pulse flour. This result supports the findings by McWatters and Heaton (1979) on beef burgers extended with 5% steam heated field pea. Cooked burgers containing chickpea had higher redness, while cooked burger containing lentil showed lower redness compared to control. The

results showed that in cooked burgers the color parameters are affected by the type of extender but not the heat treatment. These findings also are supported by the study performed by McWatters and Heaton (1979).

Table 4-8 Spectrocolorimetry result of cooked low-fat beef-burger with micronized and non-micronized chickpea and lentil flours

Pulse Burger	L*	a*	b*
Control (Co)	39.45 ^a ±1.35	5.51 ^c ±0.40	15.91 ^a ±0.62
RCP	35.46 ^b ±1.15	6.23 ^{ab} ±0.07	14.94 ^{bc} ±0.47
MCP 130 °C	35.98 ^b ±0.93	6.44 ^a ±0.31	15.68 ^{ab} ±0.91
MCP 150 °C	35.45 ^b ±0.88	5.96 ^b ±0.12	14.76 ^c ±0.54
RGL	33.28 ^c ±1.02	4.95 ^d ±0.21	12.45 ^d ±0.53
MGL 130 °C	33.28 ^c ±1.72	4.91 ^d ±0.47	13.01 ^d ±0.68
MGL 150 °C	32.39 ^c ±0.98	4.76 ^d ±0.22)	12.64 ^d ±0.60
F-Value & Significance	F(6,49)=32 ***	F(6,49)=44 ***	F(6,49)=43 ***

Co: burgers with no binder, RCP: burgers with raw chickpea (n=2), MCP130: burgers with micronized chickpea at 130°C (n=2), MCP150: burgers with micronized chickpea at 150°C (n=2), RGL: burgers with raw green lentil (n=2), MGL130: burgers with micronized green lentil at 130°C (n=2), MGL150: burger with micronized green lentil at 150°C (n=2). All means are ± standard deviation, Value for each treatment with different letters (a, b, c, d for column) are significantly different $\alpha \geq 0.05$, NS not significant, * <0.05, ** <0.01, *** <0.001

The results of the cooked burger color analysis as shown in Table 4-8 that all cooked burgers with added pulse flour had less yellowness compared to the control except for the chickpea sample micronized at 130°C. Cooked burgers containing lentil showed lower L*, a*, and b* values compared to cooked burgers extended with chickpea flours. This result supports the McWatters' finding that the cooked color is affected by the type of extender not by treatment. The decrease in color parameters of cooked burgers with added pulse flour could also be as a result of two factors: firstly dilution of myoglobin pigments in meat system and secondly milling the pulses leads to degradation of the pigments due to instability of the pigments. Der (2010), in contrast to the results of the present study, reported a significant increase of L* value in cooked low-fat beef burgers

extended with 6% micronized green lentil, while 6% micronized red lentil decreased the a^* value of cooked burger. Control burgers with no binder were darker, less yellow and red than cooked burgers with binder.

4.2.4. Weight loss, cooking loss, shrinkage, and drip loss

Water and oil retention are important functional characteristics of meat products such as sausages and burgers (Sirivongpaisal, 2008). Food matrix interaction with water and oil is important in food flavor and palatability as well as cooking loss and shrinkage of the meat products (Sirivongpaisal, 2008). The total cooking loss, drip loss, and shrinkage of low fat beef burgers extended with pulse flour were significantly lower compared to control burgers as shown in Table 4-9. Cooking loss was $42.10 \pm 2.57\%$ for low fat beef burgers with no binder, while the cooking loss was significantly lower for burgers with pulse flour, ranging from 21.01 to 22.52%. Reducing fat in meat products decreases the particle binding, increasing the cooking loss which leads to smaller size after cooking (Mallika et al., 2009). The cooking loss of burgers containing 6% chickpea was similar to burgers containing 6% green lentil, which indicates the ability of chickpea and lentil to retain fat and moisture in cooked low-fat beef burgers is the same. The same result has been reported by Dzudie et al. (2002), who showed a significant decrease in cooking loss of beef sausages extended by 5, 7.5, and 10% common bean flour (CBF) compared to control with no CBF.

Table 4-9 Effect of utilization of micronized and non-micronized green lentil and chickpea on shrinkage, drip loss, and cooking losses low-fat beef-burger

	Shrinkage %	Drip loss g/100g	Cooking loss g/100g
Control (CO)	21.39 ^a ±1.43	8.02 ^a ±2.61	42.1 ^a ±2.57
RCP	14.84 ^b ±1.25	1.89 ^b ±1.01	22.2 ^b ±3.57
RGL	14.16 ^b ±1.41	2.12 ^b ±0.7	21.51 ^b ±2.17
MCP130	15.21 ^b ±1.79	2.42 ^b ±0.5	22.52 ^b ±2.28
MCP150	14.14 ^b ±1	2.07 ^b ±1.36	22.16 ^b ±1.87
MGL130	13.97 ^b ±1.74	2.49 ^b ±0.7	22.21 ^b ±1.43
MGL150	13.65 ^b ±1.17	1.66 ^b ±1.13	21.01 ^b ±3.47
F-Value & Significance	F(6,49)29.24 ***	F(6,49)=52.03 ***	F(6,49)=69.8 ***

Co: burgers with no binder, RCP: burgers with raw chickpea (n=8), MCP130: burgers with micronized chickpea at 130°C (n=8), MCP150: burgers with micronized chickpea at 150°C (n=8), RGL: burgers with raw green lentil (n=8), MGL130: burgers with micronized green lentil at 130°C (n=8), MGL150: burger with micronized green lentil at 150°C (n=8). All means are ± standard deviation, Value for each treatment with different letters (a, b for column) are significantly different $\alpha \geq 0.05$, NS not significant, * <0.05, ** <0.01, *** <0.001

This effect is through fat or water retention. A higher fat retention of up to 97.9% and cooking yield of 94.4% compared to control patties (51.6%) (with no pea fiber) have been reported by addition of inner pea fiber to high fat ground beef patties (Anderson & Berry, 2001). Higher WHC and lower cook loss were observed in low fat beef sausage extended with 5, 7.5, 10% CBF with no significant difference shown between the percentage of flour added in the Dzudie et al. (2002) study. As previously discussed water and fat retained in raw burger by the components of pulse flour is positively related to juiciness of meat product after cooking in addition to higher yield. Canadian Kabuli chickpea and green lentil were reported to contain 18.7–30.3g/100g dietary fiber (Wang & Daun, 2004). The effect of fiber and other carbohydrates on physical characteristic of burgers is due to their physiochemical properties (de-Almeida-Costa, Keila da Silva Queiroz-Monici, Pissini-Machado-Reis, & de-Oliveira, 2006). The main components of insoluble

dietary fiber mainly in the plant cell wall are cellulose, hemicellulose, and pectin (Heredia, Jimenez, & Guillen, 1995). Cellulose is made up of a series of hydrogen bonded linear polymers that form into microfibrils. Cellulose forms inter spaces and increases surface area by linking to hemicellulose which is made of groups of interlinked polysaccharides (Heredia et al., 1995). The capillary space and high surface area of microfibrils are responsible for trapping water and oil. Moreover, the level of water and oil absorption depends on the length of the fibers (EFSA, 2010). Physical characteristics of non-starch poly-saccharides are mainly related to the size and structure of molecules which in turn are related to the plant source of the fiber (EFSA, 2010). The level of water and oil retention also depends on the source of the fiber. For instance, presence of mucilage and pectin limits the water binding capacity while high concentration of hemicellulose and lignin increases fat absorption (Sosulski & Cadden, 1982). Chickpea contains 2.7% pectin, 5.5% hemi-cellulose, and 2.1% lignin (W/W) while lentil contains significantly lower amount of pectin, hemicellulose, and lignin 1.5%, 1%, and 1.8% (W/W) (Riaz-Khan, Alam, Ali, Bibi, & Khalil, 2007). The result as reported in Table 4-9 indicated there was no significant difference between cooking loss and shrinkage of the burgers extended with 6% chickpea and lentil flour while it is expected to see difference in water and oil absorption.

During cooking, beef burgers shrink due to denaturation of protein and release the water and fat which are trapped inside the meat matrix (Serdaroglu et al., 2005). Loss of water and fat further leads to shrinkage of the burgers. Burgers containing chickpea and lentil flour had the least diameter and thickness reduction compared to control burgers (data not shown here individually), with no significant difference shown with micronization. The

water bonded to the microfibrils through hydrogen bonds will be part of the food matrix after heating or cooking (Pietrasik & Janz, 2010; Riaz-Khan et al., 2007) therefore less drip loss and cooking loss are observed in burgers with lentil and chickpea flour than in burgers with no binder. The same effect can be explained in respect to fat content of meat products extended by chickpea and lentil flour; part of the fat and oil which are bonded to the hydrophobic part of the protein or trapped in the fiber matrix will be retained in the food after heating (Biswas, Kumar, Bhosle, Sahoo, & Chatli, 2011). The results of present study showed utilization of pulse flour increased the yield of low-fat beef burger by decreasing cooking loss. It also helped to maintain the meat product dimension and juiciness. Low-fat beef burgers with no binder had higher cooking loss which led to a small, dry, elastic, and unappetizing burger.

4.2.5. Texture of low-fat beef burgers containing micronized lentil and chickpea flour

Texture is one of the most important sensory qualities of meat especially minced meat products. The textural properties of minced meat products such as burgers and sausages refer to evenness, consistency, and uniformity of the product in the raw and cooked state. The texture of meat products also is evaluated based on the binding of meat particles together and elasticity and hardness of the final product (Coggins, 2012).

Table 4-10 shows that the shear force of burgers with added micronized and non-micronized chickpea and lentil flours significantly ($p < 0.05$) decreased compared to the control. The control burgers were significantly tougher compared to burgers with pulse flour which indicates that pulse flours can improve the textural properties of the burgers. Holliday and colleagues (2011) in agreement with the present study reported that utilization of high fiber pulses such as hydrated lentil and chickpea and different types of

beans at the 35-50% level in formulations of beef burgers resulted in more tender cooked burgers compared to only meat. The same tenderness was also reported for burgers with stabilized rice bran (Holliday et al., 2011). Rice bran is high in fiber similar to dietary pulses. Fibers in the burger matrix bind to water and trap lipid and the lipophilic part of the protein interacts with the lipid part of the food. These interactions cause retention of more water and lipid which leads to more tender and softer cooked burgers compared to cooked low-fat beef burgers with no binder. The moisture and fat which are retained by fiber and protein in the food matrix stay intact through the cooking process. In addition, substitution of part of the meat with pulse flour in burger formulations dilutes the connective tissues and protein myofibrils in the burgers which leads to lower shear force (Dzudie et al., 2002).

Table 4-10 Effect of utilization of micronized and non-micronized green lentil and chickpea on texture of cooked low-fat beef-burger

Cooked burger	Shear Force (N)
Co	10.43 ^a ±1.94
RCP	6.66 ^b ±1.29
RGL	7.63 ^b ±3.06
MCP130	6.22 ^b ±0.77
MCP150	7.09 ^b ±2.11
MGL130	6.42 ^b ±1.86
MGL150	6.42 ^b ±1.21
F-Value & Significance	F(6,105)=9.9 ***

The treatments were Co: burgers with no pulse (Control), RCP: burgers with chickpea flour (n=4), MCP130: burgers with micronized chickpea at 130°C (n=4), MCP150: burgers with micronized chickpea at MCP150°C (n=4), RGL: burgers with raw green lentil (n=4), MGL130: burgers with micronized green lentil at 130°C (n=4), MGL150: burgers with micronized green lentil at 150°C (n=4). All means are ± (standard deviation), mean values for each treatment with different letters (a, b for column) are significantly different $\alpha \geq 0.05$, NS not statistically significant, * <0.05, ** <0.01, *** <0.001

Protein in beef burgers is denatured during heating and releases the moisture and lipid; the myofibrils shorten as a result of heating, become more compact and squeeze the juice out of the matrix. This process leads to rubbery and tough texture of low-fat beef burgers used as the control. The burgers with added pulse flour retain the moisture and oil through the microfibrils of insoluble fiber and maintain the softer texture and juicy mouth feel as explained in section 4.2.4.

Shear force value for the control burger was $10.43 \pm 1.94\text{N}$ which was significantly ($P < 0.05$) higher than beef burgers with lentil and chickpea flour as reported in Table 4-10. However burgers with added pulse flour had shear force values between $6.22 \pm 0.77\text{N}$ and $7.63 \pm 3.06\text{N}$, with no significant difference shown between lentil and chickpea and micronization temperatures. In agreement with the results of our study, Der (2010) demonstrated the same results in shear force of low-fat beef-burger with added micronized (130 to 135°C) and non-micronized green and red lentil at 6%. All burgers in the Der study showed lower shear force values compared to the control without pulse flour. The micronization process increases water absorption capacity, starch gelation characteristics, starch retro-gradation, protein degradation (Khattab & Arntfield, 2009; Mwangwela, 2006). These characteristics could be important in the shear force of the cooked burgers. However, the results of the present study showed no significant difference in texture of burgers containing micronized ($130/150^\circ\text{C}$) and non-micronized pulse flour, therefore, inferring that micronization at 130 and 150°C does not cause any detrimental effect on functionality of lentil and chickpea flours.

As previously mentioned burgers with added pulse flour showed lower drip loss and cook loss and shrinkage during the cooking process. This shows that the moisture content is related to the level of tenderness and softness of cooked burgers.

To our knowledge, this is the first study evaluating the effect of micronization on lentil and chickpea without tempering and utilizes micronized chickpea and lentil flour in a minced meat matrix. Based on the this study, it can be concluded that micronization pretreatment at moderate temperature such as 130 or 150°C would be appropriate to enhance protein and starch functionality of pulse flour in a minced meat food matrix and as a result contribute to tenderness of final meat products extended with pulse flour.

4.2.6. Consumer acceptability testing

Consumer acceptability testing results for burgers containing 6% lentil and chickpea flours are given in Table 4-11 and Table 4-12. This test was conducted to evaluate the effect of addition of pulse flour and micronization treatment on acceptability of low-fat beef burgers for aroma, flavor, texture, appearance, and overall acceptance, as well as how often the consumers would eat the particular burger. The consumer test indicated that the effects of pulse flour and micronization on the acceptability of sensory parameters were significant ($P < 0.05$). The mean values for samples containing micronized lentil or chickpea flours for acceptability of all attributes, overall acceptability and frequency of eating (FACT) were over 6 on the 9-point hedonic scale.

Table 4-11 Consumer acceptability results four way ANOVA for source of variation

Attribute	Source of Variation (F-value)					
	G	A	GxA	T	μ	Txμ
Aroma ¹	0.24 NS ³	2.91 NS	0.83 NS	0.31 NS	6.63 **	7.46 **
Flavor ¹	4.49 *	1.02 NS	0.76 NS	3.76 *	6.85 **	4.55 *
Texture ¹	0.05 NS	0.21 NS	0.91 NS	8.49 ***	2.18 NS	1.43 NS
Appearance ¹	1.46 NS	1.32 NS	0.34 NS	4.02 *	0.79 NS	3.34 *
Overall Acceptability ¹	3.77 NS	0.47 NS	0.60 NS	5.88 **	4.31 *	3.97 *
Frequency of Eating ²	9.82 **	0.43 NS	3.65 *	6.45 **	3.80 *	2.90 NS

G=Gender (n=2), A=Age Group (n=3), T=Type (n=3), μ=micronization (n=3)
³Not Significant $p \geq 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4-12 Consumer acceptability test four-way ANOVA

Attribute	Gender		Age Group			Mean Value for Type			Mean Value for μ Level		
	Female (n=71)	Male (n=30)	18 to 24 years (n=45)	25 to 34 years (n=27)	over 34 years (n=29)	No Pulse (n=101)	Lentil (n=303)	Chickpea (n=303)	No μ (n=303)	130°C (n=202)	150°C (n=202)
Aroma ¹	6.3 ^a (0.07)	6.3 ^a (0.11)	6.5 ^a (0.10)	6.4 ^a (0.12)	6.1 ^a (0.12)	6.2 ^a (0.16)	6.4 ^a (0.09)	6.3 ^a (0.09)	6.1 ^b (0.09)	6.4 ^a (0.11)	6.6 ^a (0.11)
Flavor ¹	6.0 ^b (0.08)	6.4 ^a (0.13)	6.3 ^a (0.11)	6.3 ^a (0.14)	6.0 ^a (0.14)	5.6 ^b (0.18)	6.5 ^a (0.11)	6.2 ^a (0.11)	5.8 ^b (0.11)	6.3 ^a (0.13)	6.6 ^a (0.13)
Texture ¹	6.2 ^a (0.08)	6.2 ^a (0.13)	6.1 ^a (0.11)	6.2 ^a (0.14)	6.2 ^a (0.14)	5.3 ^b (0.18)	6.4 ^a (0.11)	6.3 ^a (0.11)	5.9 ^a (0.11)	6.3 ^a (0.13)	6.5 ^a (0.13)
Appearance ¹	6.1 ^a (0.08)	6.3 ^a (0.12)	6.1 ^a (0.10)	6.3 ^a (0.13)	6.3 ^a (0.13)	5.7 ^b (0.17)	6.3 ^a (0.10)	6.4 ^a (0.10)	6.1 ^a (0.10)	6.3 ^a (0.12)	6.4 ^a (0.12)
Overall Acceptability ¹	6.0 ^a (0.08)	6.3 ^a (0.12)	6.2 ^a (0.11)	6.2 ^a (0.13)	6.0 ^a (0.14)	5.4 ^b (0.18)	6.4 ^a (0.11)	6.1 ^a (0.11)	5.8 ^b (0.11)	6.2 ^a (0.13)	6.5 ^a (0.13)
Frequency of Eating ²	5.0 ^b (0.08)	5.5 ^a (0.12)	5.2 ^a (0.11)	5.3 ^a (0.13)	5.2 ^a (0.13)	4.7 ^b (0.17)	5.5 ^a (0.10)	5.1 ^a (0.10)	5.0 ^b (0.10)	5.3 ^{ab} (0.12)	5.6 ^a (0.12)

¹1=dislike extremely; 2=dislike very much; 3=dislike moderately; 4=dislike slightly; 5=neither like nor dislike; 6=like slightly; 7=like moderately; 8=like very much; 9=like extremely, ²1=I would eat this only if forced; 2=I would eat this if there were no other food choices; 3=I would hardly ever eat this; 4=I don't like this but would eat it on an occasion; 5=I would eat this if available but would not go out of my way; 6=I like this and would eat it now and then; 7=I would frequently eat this; 8=I would eat this very often, 9=I would eat this every opportunity I had, Not Significant $p \geq 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, a, b for Mean values (followed in brackets by the standard error of the mean) within the same variable "Gender", "Age Group", "Type" and μ Level" with the same letter within the same row (attribute) are not significantly different $p < 0.05$

4.2.6.1. Aroma acceptability

A significant presence of pulse type and micronization temperature interaction was found for aroma acceptability. The results indicate that the micronization effect on acceptability of aroma of beef burgers containing pulse flour is not the same for lentil and chickpea. Gramatina, Zagorska, Straumite and Sarvi (2012) evaluated the aroma characteristic of sausages containing 20% lentil flour using 30 trained panelists. Cooked sausages with 20% lentil flour were reported to impart an intense legume type aroma but it was not statistically significant. However the results of the present study showed there was a significant difference in acceptability of aroma of burgers containing two different pulse flours with two levels of micronization treatments

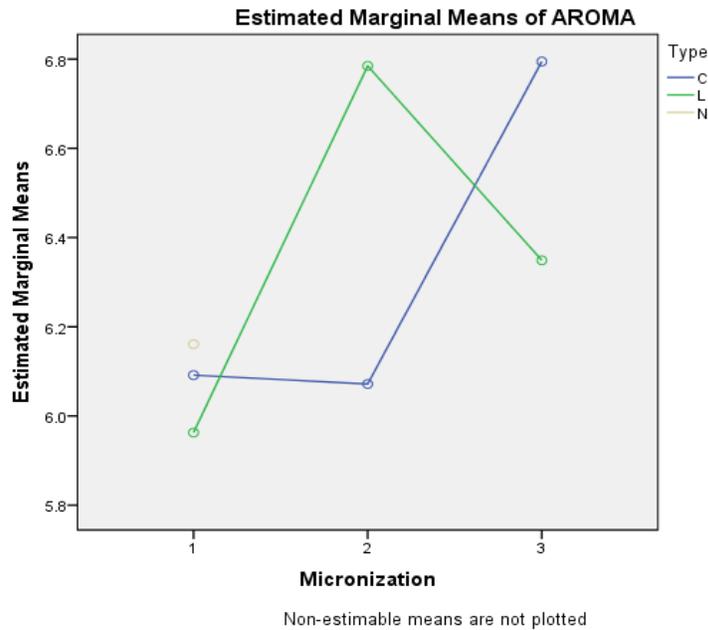


Figure 4-2 Type of pulses by micronization interaction for aroma of low-fat beef burger containing micronized and non-micronized lentil and chickpea flours

Micronization (1: no micronization, 2: micronization at 130°C, 3: micronization at 150°C) Type (C: chickpea, L: lentil, N: no pulse)

In contrast to our results Der (2010) also reported no significant difference in aroma score among burgers containing 6% non-micronized and micronized lentil flour with low-fat burgers with no-binder and 6% toasted wheat crumbs. Although based on Figure 4-2 burgers with micronized lentil at 150°C had lower aroma acceptability compared to those micronized to 130°C, the volatile analysis showed a significant decrease in compounds contributing to off-aroma in micronized lentil at 150°C compared to micronization at 130°C, while chickpea acceptability of burgers with micronized chickpea was different than lentil at two level of micronization. Overall, assessors' acceptability of aroma of the burgers with 6% micronized chickpea at 150°C was higher compared to non-micronized. Although the aroma scores for burgers with chickpea flour was the same as control in the present study, a decrease in beef aroma intensity was reported for meat loaves containing 30% chickpea meal (Shaner & Baldwin, 1979). In addition, the meat loaves with chickpea flour (30%) had significantly higher 'beany' aroma scores compared to the control containing no legume (Shaner & Baldwin, 1979).

There was no age and gender interaction observed for aroma acceptability score. Data analysis indicated that both male and female participants after age of 35 scored lower for acceptability of aroma.

4.2.6.2. Flavor acceptability

A significant presence of type of pulses and micronization treatments interaction was found for flavour acceptability of low-fat beef burgers with micronized and non-micronized lentil and chickpea flours (Table 4-11). The mean values for acceptance of aroma of beef burger with pulse flour was higher for micronized lentil compared to non-

micronized lentil, while there was no significant difference was observed between micronized lentil at 130°C and 150°C . Low-fat burgers containing micronized chickpea at 130°C and 150°C had the highest flavor acceptability compared to other burger treatments. The beef burger contain non-micronized lentil was scored as neither like or dislike category while burgers containing micronized lentil scored as like slightly. Although burgers containing micronized chickpea at 150°C had higher acceptability scores compared to those containing micronized chickpea at 130°C their acceptability score was lower than those containing micronized lentil. These results are in agreement with the results of flavour analysis which showed lower concentration of compounds contributing to beany aroma and flavour in micronized lentil compare to micronized chickpea.

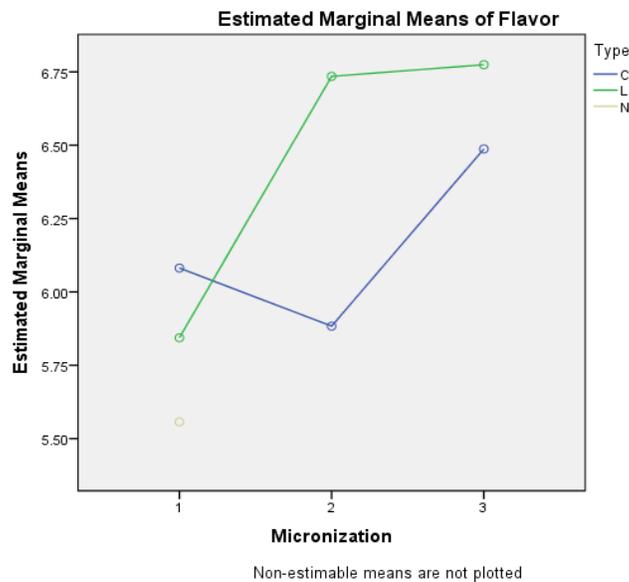


Figure 4-3 Type of pulses by micronization interaction for flavor of low fat burger containing 6% micronized and non-micronized lentil and chickpea flours

Micronization (1: no micronization, 2: micronization at 130°C, 3: micronization at 150°) Type (C: chickpea, L: lentil, N: no pulse)

Moreover, scores for flavor attributes were higher for burgers with 6% lentil and chickpea compared to the control. Burgers with micronized lentil and chickpea scored higher for flavor acceptability compared to non-micronized lentil and chickpea. There was no significant difference noted between flavor acceptability score of burgers containing micronized lentil at 130°C and 150°C. In agreement with the results of our study, aroma and flavor of beef burgers containing 5% moist-heated peanut, soy bean, and field pea scored higher than aroma and flavor of those with 5% unheated seeds (McWatters & Heaton, 1979). McWatter & Heaton (1979) reported, beef burgers with unheated seeds had more unpleasant 'beany' and spicy aroma. Although, the beany attribute of burgers containing pulse flour was not assessed directly using trained panelist in our study; the burgers containing non-micronized pulses scored lower than those with micronized. In contrast to our findings, Sanjeeva, Wanasundara, Pietrasik, & Shand (2010) reported no off-flavor or beany aroma in low-fat bologna with 2.5% or 5% Desi or Kabuli type chickpea flours. In agreement to results of the present study, Der (2010) reported beef burgers containing 6% micronized lentil at 135°C had more acceptable flavor compared to burgers with 6% untreated lentil flour and control burgers with no binder. Serdaroglu et al (2010) also reported the same flavor scores for meatballs with 10% lentil, and 10% chickpea flour compared to burgers with rusk. It should be noted that in the aforementioned study, chickpea and lentil seeds were soaked and cooked prior to utilization in the formulation of meatballs, which has an effect on the off-aroma and flavor ratings. Although there are some studies reported a lower flavor acceptability scores for beef burgers or meat loaves with added lentil flour, (Kurt & Kilinceker, 2012;

Shaner & Baldwin, 1979), contradictory results were reported in low fat beef products with 6% lentil and meatballs with 10% lentil (Der, 2010; Serdaroglu et al., 2005).

4.2.6.3. *Texture acceptability*

The type of pulses and micronization treatment interaction showed no significant differences. The texture acceptability scores were significantly higher for burgers with lentil and chickpea flours compared to those with no binder as shown in Table 4-12. In addition, micronization treatment had no effect on consumer acceptability of the texture of the burgers. Panelists liked the texture of burgers containing lentil and chickpea flour more than control burger regardless of micronization treatment or temperature of micronization. In this respect, Der (2010) reported that the addition of 6% micronized and non-micronized lentil significantly increased the acceptability of the texture of low-fat beef burgers compared to the control. Moreover, meatballs extended with 10% chickpea had the same perceived toughness as those with 10% lentil flour which support the results of the present study (Serdaroglu et al., 2005), while a firmer texture has been reported for low-fat bologna containing 5% chickpea flour compared to those with what or pea flour (Sanjeeva et al., 2010). Modi, Mahendrakar, Rao, and Sachindra (2003) also reported no detrimental effect on texture acceptability by addition of 8% pulse flours into buffalo burgers.

Since one of criteria for texture acceptability of beef burgers is juiciness and tenderness and both related to water holding capacity; the result of WHC showed no difference between the samples. However, cooking loss and drip loss results showed higher

moisture and oil release from burger without pulse flour compared to burgers containing pulse flour regardless of micronization treatment which is in agreement with the result of consumer acceptability of low-fat beef burgers with lentil and chickpea flour.

4.2.6.4. *Appearance acceptability*

The mean score for appearance was significantly higher for burgers with lentil and chickpea compared to control. Moreover, micronization treatment is shown to have no effect on acceptability of appearance of burgers. However, the interaction of type of pulses and micronization was significant for appearance of the burgers which indicated that micronization effect on appearance of burgers depended on the type of pulses. Burgers containing micronized chickpea at 130°C had higher acceptability scores compared to 150°C counterpart while burgers containing micronized lentil to 150°C was more acceptable than those with micronized lentil to 130°C as shown in Figure 4-6. The results are not in agreement with those reported by Serdaroglu and colleagues (2005) which indicated no significant difference in appearance of beef burgers with 10% lentil, chickpea, black bean flour and rusk. In contrast, Kurt et al. (2012) observed the lowest scores for color of beef burgers with 5% lentil flour compared to chickpea, rye, rice, corn, wheat, barley, and oat. In addition, Verma et al. (1984) found that replacing 30-40% of a sausage formulation with chickpea flour decreased the desirability of the color. Color of chickpea flour is pale yellow and replacing part of meat with chickpea flour results in less red pigmentation. In addition, cooking method might have a significant effect on appearance of the burger and pigment formation.

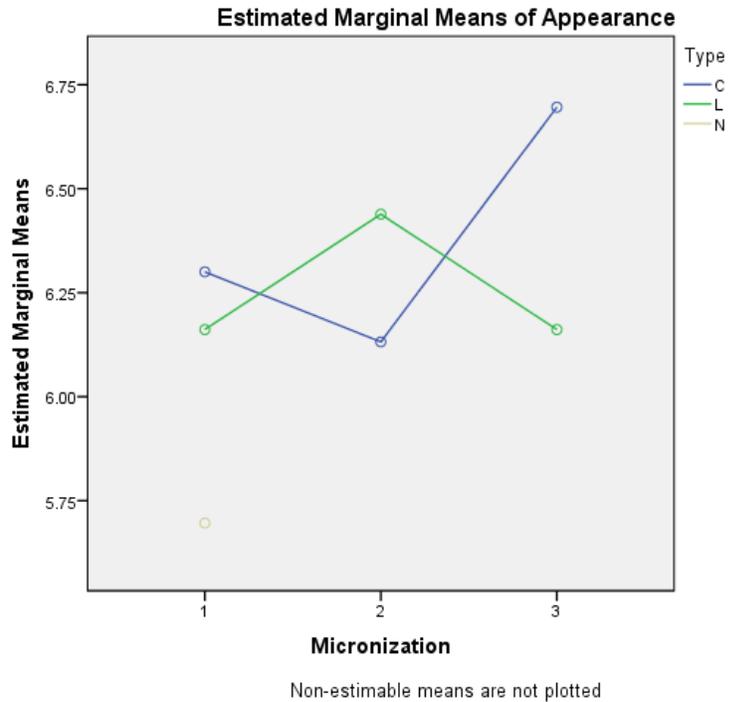


Figure 4-4 Type of pulses and micronization treatments interaction for acceptability of appearance of low fat beef burgers containing micronized and non-micronized lentil and chickpea flours

Micronization (1: no micronization, 2: micronization at 130°C, 3: micronization at 150°C) Type (C: chickpea, L: lentil, N: no pulse)

4.2.6.5. Overall acceptability

Pulse type and micronization treatment interaction showed a significant difference for mean score of overall acceptability. Figure 4-5 revealed that burgers containing 6% lentil or chickpea had significantly higher overall acceptability scores than those with no pulse flour. In addition, mean score for overall acceptability of burgers with micronized pulse flour is not the same for burger containing lentil and chickpea flours for both level of micronization. Burgers containing micronized lentil had a high overall acceptability score

regardless of micronization level. However, burgers containing micronized chickpea at 150°C were more acceptable compared to those containing micronized chickpea at 130°C. The mean score for acceptability of texture was the same for burgers with micronized and non-micronized pulse flours; and consumers rated appearance of low-fat burgers with pulse flour more acceptable than burgers with no pulse added. These results indicated that the aroma and flavor of burgers have a considerable effect on overall acceptability of burgers containing pulse flour.

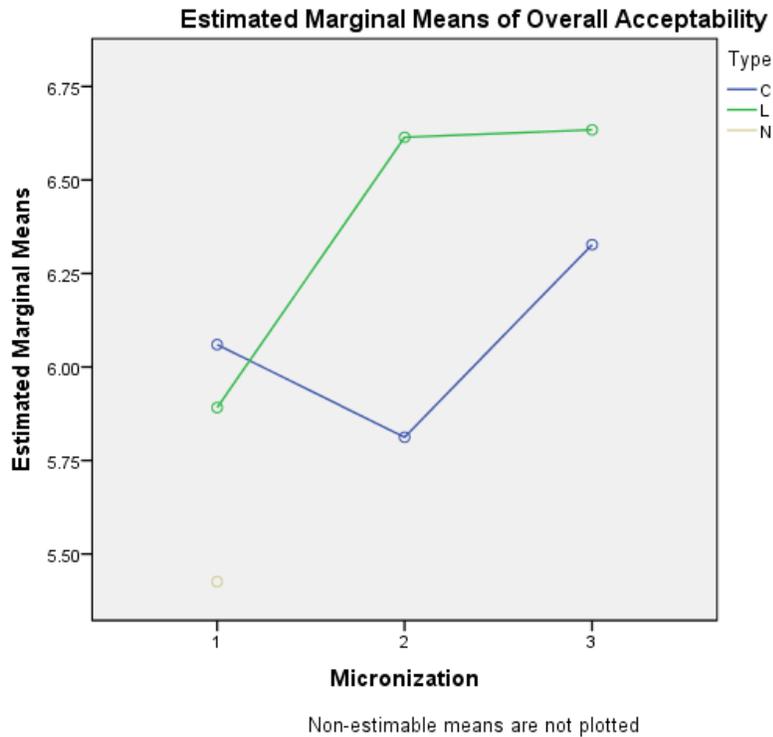


Figure 4-5 Type of pulses by micronization treatment interaction for overall acceptability of low-fat beef burgers containing micronized and non-micronized lentil and chickpea flours

Micronization (1: no micronization, 2: micronization at 130°C, 3: micronization at 150°C Type (C: chickpea, L: lentil, N: no pulse)

4.2.6.6. *Frequency of eating of burgers with pulse flour*

There was a higher scores for frequency of eating the low-fat burgers with 6% lentil and chickpea compared to low-fat burgers with no binder, although, the scores for FACT were lower compared to scores for overall acceptability and acceptability of sensorial characteristics. In addition, frequency of eating score was higher for burgers with micronized lentil or chickpea compared to those with non-micronized counterparts. Moreover, there was a significant ($P < 0.01$) sample effect as well as significant ($P < 0.05$) micronization treatment effect on the frequency of eating scores. In general, burgers containing micronized lentil (130°C), micronized lentil (150°C), micronized chickpea (130°C) and micronized chickpea (150°C) had the same acceptability score for flavor, aroma, texture, appearance and overall acceptability with lower eating frequency. as shown in Table 4-12. Although the results of chemical and instrumental color analysis show that lentil and chickpea behave differently when treated by different micronization temperatures (130°C and 150°C), these differences may not be large enough to make a difference in their acceptability when incorporated in beef burgers as a binder. In addition, there are other factors that should be taken into account when assessing sensorial properties of meat products with micronized pulse flours such as tempering of seeds prior to micronization (Arntfield et al., 1997) and type of pulses (Der, 2010). These aforementioned factors determine the micronization treatment parameters necessary to achieve the characteristics needed for acceptance as a meat binder. In addition other factors such as cooking process, type and concentration of amino acid and sugar in the final products, as well as pH and temperature are also important in development of aroma

and flavor in new formulated minced meat products (Martins, Leussink, Rosing, Desclaux, & Boucon, 2010). Therefore, the discrepancies among the results reported by other studies may be related to one or a few of these factors.

The type of pulses and micronization treatments interaction showed no significant differences for the frequency of eating the low-fat beef burgers with micronized pulses as it is shown in Table 4-11. However, the same trend was detected as overall acceptability.

4.2.6.7. *Effect of age and gender on consumer acceptability testing*

Consumer food choice could be influenced by demographic variables such as gender and age (Ares & Gambaro, 2007). Based on the data reported in Table 4-11, an age and gender interaction for frequency of eating was found to be significant. This can be concluded that mean value for frequency of eating was significantly higher for male participants than females; however, the difference is not consistent for all age groups as shown in Figure 4-6. Male participants age 25 and older were more willing to try the low-fat beef burgers with pulse flours than female in the same age groups while, younger male participants tend to willing to try the burgers the same as female participants. Females showed significantly less willingness to eat the new formulated burgers.

There was no gender and age interaction found for aroma, flavor, texture, appearance and overall acceptability of the burgers (Table 4-11).

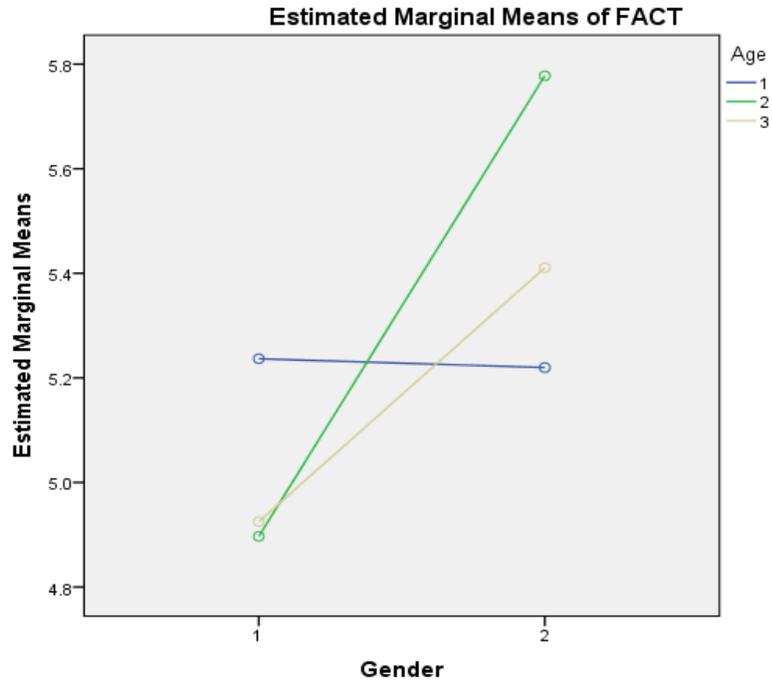


Figure 4-6 Age and gender interaction for frequency of eating burgers containing micronized and non-micronized lentil and chickpea flours

Micronization (1: no micronization, 2: micronization at 130°C, 3: micronization at 150°C) Type (C: chickpea, L: lentil, N: no pulse)

In the present study, gender and age had an influence in determination of new formulated food acceptability. Women gave significantly lower scores to acceptability of flavor of all burgers compared to men. This suggests women were less acceptant toward the burgers compared to men. Although, there was no significant gender effect found in acceptance of aroma, texture, appearance and overall acceptability; women tended to be more reluctant to eat the burger than men. The frequency of eating for female was significantly lower than for men. This was due to the fact that female ages 35 and over were more reluctant to try burgers in general while male in same age group showed more interest to try the burgers. Ares & Gambaro (2007) reported that men had more positive attitudes toward functional foods with different types of carriers and were more willing to try them

than women. Although, women were more acceptant toward healthier enriched products, they were significantly less willing to try them. In contrast, Der (2010) reported that males found the burgers with micronized lentil more acceptable than burgers with non-micronized while females found no difference in acceptability of both treatments, suggesting less sensitivity of females to change in sensorial properties of burgers with micronization treatment.

Although ageing causes less sensitivity to food flavor and aroma, and younger people are more sensitive to off-aroma, there was no significant age effect on acceptability of sensorial characteristics of burgers with different pulse flour and pulse flour treatment. In contrast to our results, Twigg, Kotula and Young (1977) reported older people were more acceptant of aroma than younger people while, children under the age of 19 were more acceptant of juiciness of burgers than older adults. Although, this segment of population (8-19 year-old) was not included in the present study, they have a high priority for meat producers. Meat and meat products provide important nutrients for growth and development; moreover, burgers and patties are considered a popular food among children and young adults. Another factor that should be taken to consideration is that the age and gender effects were decreased in laboratory environment testing while in real life played a major role in food choice and preference (King, Meiselman, Hottenstein, Work, & Cronk, 2007).

4.3. Correlation of chemical analysis with consumer acceptability testing

Partial Least Square regression (PLS) technique was used to predict the acceptability of low-fat beef burgers with 6% micronized and non-micronized pulse flour by using a large set of measured explanatory variables. PLS is a technique used to explain consumer liking scores by a set of laboratory measured independent variables (Krishnamurthy, Srivastava, Paton, Bell, & Levy, 2007). Figure 4-7 represents the overall correlation between specific volatile compounds contributing to beaniness, pH, and fatty acids with mean scores for consumer acceptability of burgers with pulse flour as binder including the control. This technique groups variables based on some measure of similarity taken from decomposition of X and Y variables and clearly visualizes both sensory and instrumental data based on their similarities and relationships. The map provides a visual representation of the relationship between burgers and consumer acceptance, and the level of volatile compounds contributing to 'beany' aroma and flavor of pulse flour after and before treating with micronization. The map clearly shows that the acceptance of the burgers is influenced by the intensity of aroma compounds contributing to beaniness. Burgers with non-micronized chickpea/lentil flours, lentil micronized at 130°C and control are present in clusters containing compounds with the 'beany' aroma attribute such as undecane, hexanol, 2-hexenal, furan-2-pentyl, heptanal, hexanal, octanal, and nonanal. This cluster is located at the opposite side of consumer acceptability of aroma and appearance. Based on the PLS map, burgers containing micronized chickpea at 130°C and micronized lentils at 150°C appear in the same quadrant as aroma and appearance and are in the opposite quadrant to the 'beany' aroma compounds. This

indicates that these micronized samples are associated positively with aroma and appearance acceptability and negatively with 'beany' aroma compounds. Moreover, the linoleic acid and pH in a cluster with burgers containing micronized chickpea at 130 and 150°C could be related flavor and texture acceptability found in the same area. It is not surprising to see pH in a cluster with acceptability for texture since pH has a direct relationship to WHC of burgers and as a result leads to better texture after cooking (Adam & Abugroun, 2010; Harrell et al., 1978). In addition, a higher level of linoleic acid means less decomposition of this fatty acid and less contribution to 'beany' aroma compounds as well as a possible direct effect of linoleic acid in flavor (Brewer, 2006, , 2012).

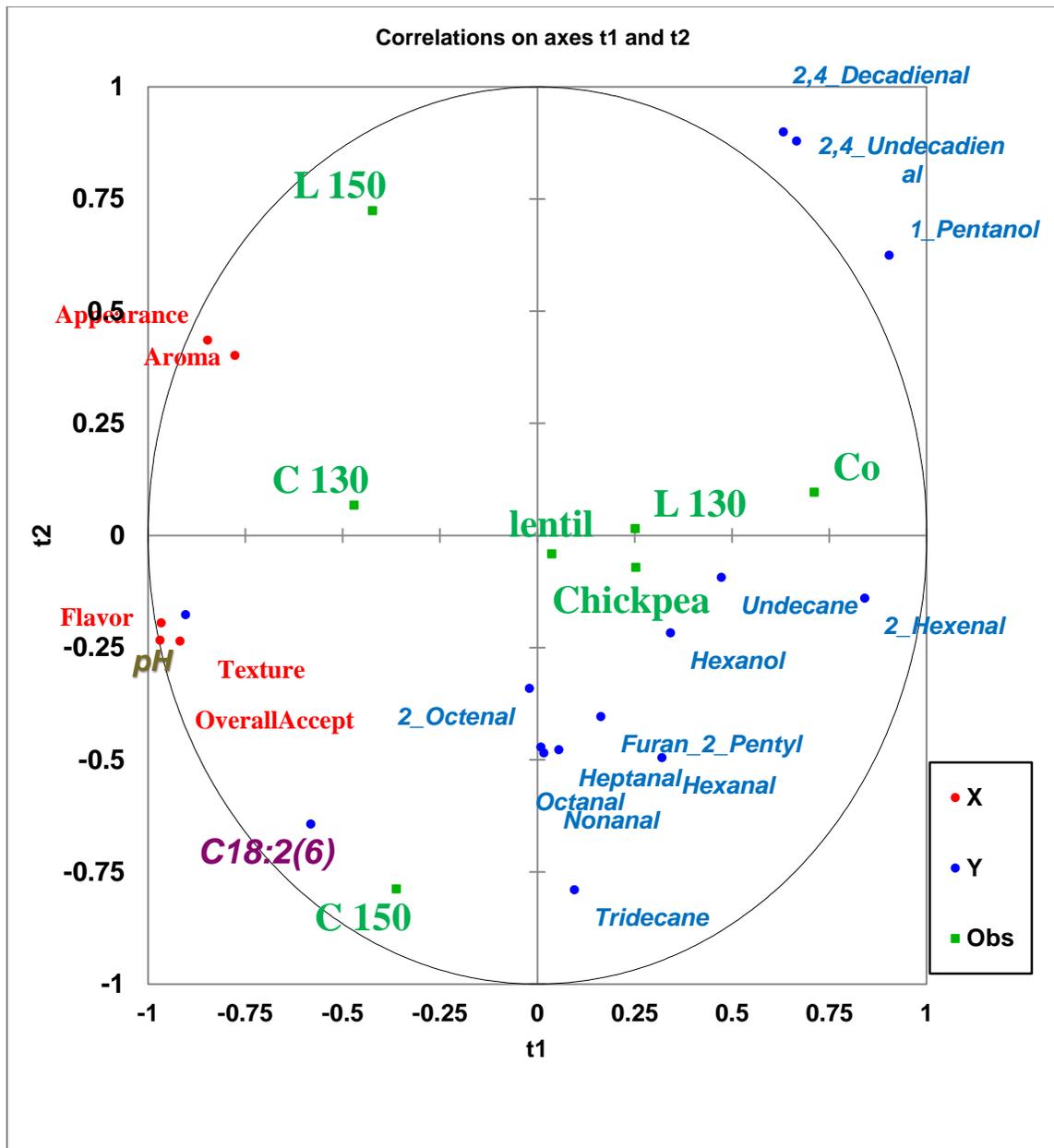


Figure 4-7 An overview of the correlation loadings from partial least squares (PLS) analysis

($p < 0.05$) with volatile compounds from pulse flours, fatty acid and pH as Y-variables and sensory attributes of cooked burgers as X-variables for seven burger treatments

Burger Treatments: Co – Control Burger, Lentil, Co + Green Lentil, L130: Co + MGL130 °C
 L150: Co + MGL150 °C, Chickpea: Co + Chickpea, C130: Co + MCP 130 °C, C150: Co + MCP150 °C

Fatty Acid: C18:2(6) – linoleic acid

Interestingly, burgers containing micronized lentil at 130°C also falls in the cluster with the selected volatile compounds compared to the lentil sample micronized at 150°C an indication that the higher temperature was more effective in decreasing some volatile compounds compared to micronization at 130°C. In addition, there might be other factors that contribute to acceptability scores of sensory attributes of burgers with treated pulse flours, such as water soluble compounds which contribute to taste. Drivers of overall acceptability of burgers with micronized chickpea are the low concentration of ‘beany’ aroma and flavor as well as increased WHC, which results in juiciness and softer texture. On the other hand, the acceptability of burgers with micronized lentil is driven by appearance and aroma attributes.

In addition, 2-hexenal, hexanol, undecane, and furan-2 pentyl have a closer location to control burgers as well as burgers with non-micronized chickpea and lentil flours, indicating these volatiles have a higher contribution to ‘beany’ aroma and flavor in abovementioned burgers.

Finally, presence of linoleic acid in the opposite area to aroma compounds and near burgers with micronized chickpea at 150°C and flavor appears to be related to higher acceptability score of flavor as well as overall acceptability scores. Moreover, the factors, in the model affecting the acceptability score of appearance of the burgers are micronization and type of pulses. Therefore, we can conclude that micronization is one of the important factors in acceptability of meat products containing pulse flour.

4.4. Conclusion

The present study showed that utilization of pulse flour at 6% in formulation of low-fat beef burgers improved the quality of the beef burgers to some extent regardless of micronization treatment. Utilization of chickpea and lentil flours in formulation of low-fat beef burgers improved the texture, decreased cooking loss and shrinkage. However, micronization of lentil and chickpea flours prior to use as extender improved the aroma and flavor of low-fat beef burgers.

The instrumental color analysis of raw beef burgers with lentil and chickpea flours showed that burgers with chickpea flour were lighter, more yellow and red in color, while micronization significantly increased the yellowness and redness further. In addition, utilization of micronized chickpea (150°C) in low-fat beef burger caused an increase in the redness and yellowness of burgers. In regard to lentil, utilization of lentil in burger formulation had no effect on lightness of the raw burgers, while increased both redness and yellowness significantly. Micronization increased the yellowness and redness of burgers with lentil flour, however there was no difference between the two micronization levels. It suggests that micronization can improve the color quality of raw burgers containing lentil and chickpea flour.

When cooked burgers were subjected to color analysis, it was found that burgers with lentil and chickpea flours had reduced lightness and yellowness while burgers with lentil flour had more redness. Micronization had no significant effect on color of cooked burgers compared to burgers with non-micronized lentil and chickpea flour.

Micronization of lentil and chickpea flours was the major factor contributing to increase consumer acceptability of aroma and flavor of the low-fat beef burgers with pulse flour with micronization being a major contributor to consumer acceptability rating.

5. General discussion:

Aroma of food which is sensed by olfaction plays an important role in the food flavor sensation (Lawless, 1991). Aroma is perceived through the interaction of chemoreceptors and a large number of volatile compounds that produce distinct smell (Lawless, 1991). Many times what is referred to as flavor and taste is basically perceived by sense of smell. Food aroma, flavor, and taste determine the enjoyment of food which is an important factor driving consumers to choose a specific type of food over another, especially in a society with large supply of nutritious foods (Clark, 1998). Furthermore, unpleasant beany' flavor as described in number of studies can result in decreasing consumption of these nutritious foods.

The objectionable aroma of pulses has been reported to be partly related to the volatile compounds which are formed by linoleic and linolenic acid enzymatic oxidation through LOX activities in the presence of oxygen (Siedow, 1991). Micronization technology among different types of heat treatments has the advantage of short exposure to infrared radiation which may protect nutrient content while decreasing enzymatic activities and decreasing formation of volatile compounds such as aldehydes and ketones in grains and legumes. The goal of this study was to develop a laboratory method procedure to test the effect of micronization treatment of lentils and chickpeas at 130°C and 150°C

temperatures on selected volatile compounds that had been reported contributing to 'beany' aroma and flavor using GC-MS. 'Beany' and off-flavor in relation to the LOX activity has been studied vastly in soy bean and soy products (Baysal & Demirdoven, 2007; Boatright & Lei, 1999; Lv et al., 2011) while the number of studies on volatiles contributing to beaniness in pulses such as lentil and chickpea is close to none to our knowledge. GC-MS appears to be highly beneficial to study, determine and quantify these volatile compounds.

Volatile analysis revealed that the effective micronization temperature to reduce selected volatile compounds is different for green lentil and Kabuli chickpea seeds. It was demonstrated that micronization influenced the level of selected volatile compounds contributing to beany aroma and flavor. Volatile analysis showed that micronization of chickpeas to surface temperature of 130°C significantly decreased the level of some volatiles such as hexanal, 2-hexenal, heptanal, furan-2-pentyl, undecane. Although not statistically significant compared to non-micronized chickpea, micronization of chickpea at 130°C also decreased the level of 2-hexenal, 2,4-decadienal, and 2,4-undecadienal. However, micronization to 150°C significantly decreased 1-pentanol, hexanal, 2-hexenal, 1-hexanol, Heptanal, furan-2-pentyl, 2-octenal, 2,4-decadienal, and 2,4-undecadienal. Micronization of green lentil to surface temperature of 130°C had no effect on the level of the selected volatile compounds while micronization to the surface temperature of 150°C significantly decreased 2-hexenal and 1-hexanol. Although there was no significant difference in the level of other volatile compounds contributing to beaniness in green lentil a decreasing trend was detected after micronization. The results of volatile analysis indicated that micronization to the surface temperature of 130°C and 150°C is

sufficient to produce Kabuli chickpea and green lentil flour with reduced objectionable aroma. To evaluate the effect of micronization on selected volatile compounds, a comparative analysis before and after micronization by GC-MS was used to detect and quantify the selected volatile compounds. However, using the human nose at the GC-MS to verify the compounds with the suggested aroma is a more valuable method (Michishita et al., 2010). In the present study GC-MS was used in combination with aroma attributes of selected volatile compounds based on literature. In addition, more information was obtained through literature review about the specific compounds behavior when combined with other aroma compounds (Arai et al., 1967; Blagden & Gilliland, 2006; Bott & Chambers-IV, 2006; Vara-Ubol et al., 2004). However, measuring the sensory aspects and naming the 'beany' attributes of the selected compounds remains the main challenge for study of volatiles contributing to beaniness. There is a need for a specific naming system and sensory description of these compounds which can be communicated through different studies such as studies done by Vara-Ubol (2004) and Bott and Chambers IV (2006). Using such a specific wording can eliminate confusion among different studies. In addition as Bott and Chamber IV (2006) suggests there is not a single compound in food that causes 'beany' aroma. The beany aroma and flavor of food could be caused by the combination of a few compounds. There is a need for more experimental design to evaluate the effect of different combinations of volatile compounds in pulses to draw a more accurate conclusion about beany aroma and flavor of cooked pulses and provide a better definition for 'beany' characteristic.

The effect of micronization on enzyme inactivation in large and small seed green and red lentil has been demonstrated by Der (2010). A 100-fold reduction in LOX activities in

green and red lentil has been reported after micronization to 135°C (Der, 2010). In the present study, although LOX activities have not been measured directly, a large reduction in volatile compounds such as hexanal and 2-hexenal in green lentil was observed after micronization to 150°C and in chickpea Kabuli type at 130°C and 150°C. This result suggests lentil seeds require a higher temperature to inactivate LOX enzymes due to its harder and denser structure in the dry state. The more porous and weaker structure of tempered lentil seeds (Arntfield et al., 1997) is shown to be effective in inactivation of LOX enzymes at lower temperature such as 130°C (Der, 2010).

Micronization to 130°C and 150°C in chickpeas and lentils had no effect on the level of linoleic acid and total ω -6 fatty acid while increased the linolenic and total ω -3 fatty acid in chickpea at 150°C. The higher concentration of linolenic acid may be related to decrease degradation of this fatty acid by activity of LOX isozymes.

In our study it was demonstrated that utilization of chickpea in low-fat beef burger formulation had a positive effect on the level of linoleic, linolenic, total ω -6 and ω -3 fatty acid and this increase observed in micronized chickpea at 130 and 150°C and micronized lentil at 150°C. Addition of chickpea had no impact on the level of linolenic acid and total omega-3 fatty acid, while, micronization of chickpea had a significant positive effect on the level of linoleic acid and total omega-3 fatty acid. In contrast to chickpea, utilization of lentil in burger formulation had no impact on the linoleic and total omega-6 fatty acid except for a positive shift in total omega-6 with micronization at 150°C which suggests the shift in level of linoleic and linolenic acid may be as a result of micronization treatment. Furthermore, micronization of lentil at 150°C had also a positive effect on the level of linolenic and total omega-3 fatty acid in low-fat beef burgers. The

shift in concentration of polyunsaturated fatty acid in burgers containing micronized chickpea and lentil could be either due to inactivation of LOX enzymes or antioxidant content of lentil and chickpea, or increase in the pH of the meat matrix by micronization. The second part of the study was conducted to examine the physical and sensorial properties of burgers and consumer acceptability of low-fat beef burgers formulated with micronized Kabuli chickpea and green lentil flour at two different micronization temperatures (130° and 150°C). The results were compared to those of burgers with non-micronized lentil and chickpea flour and with no added pulse flour. In general, adding 6% lentil and chickpea flour to the low-fat beef burger formulation decreased cooking loss and shear force compared to those without extender with no further effects by micronization processing of the pulses.

Instrumental color analysis revealed that lentil and chickpea have a different effect on the color of raw and cooked burgers. Overall, cooked burgers containing micronized chickpea and lentil flour received higher scores for appearance compared to those with no extender or non-micronized lentil and chickpea flour. Instrumental color analysis of cooked burgers containing chickpea revealed that burgers with chickpea are darker, redder and less yellow in color with micronization increasing the redness and yellowness further compared to control. However, cooked burgers containing lentil are darker less red and yellow in color with no significant difference by micronization. Liking or disliking of food greatly related to the attractiveness of the food (Molnar, 2009).

However, by experience some change in color will be acceptable if it is learned that the color is not related to food spoilage and is the natural color of the ingredients. Although, change of the color in meat products may have an effect on the consumer acceptance

score; size and shape of the food also greatly influences the acceptance of appearance of meat products (Molnar, 1995a; Molnar, 2009). Burgers with chickpea and lentil had less cook loss and shrinkage compared to the burgers with no pulse flour, therefore, as a result they are larger in size and contain more moisture compared to the control burgers. The higher score for the appearance of the burgers with pulse flour could be due to the fact that these burgers look juicier and larger compared to the control which has the most shrinkage and weight loss. In general, the acceptability of the appearance of the burgers is the first thing that influences the decision of one to choose the burger in the first place. Burgers with lentil and chickpea flour had higher scores for texture compared to no pulse burgers. In addition, participants gave higher scores for aroma and flavor of the burgers with micronized pulses with no difference between temperatures of micronization. A PLS model was used in the present study to link consumer acceptability of the seven low-fat beef burger formulations to the results of volatile analysis and linoleic acid content of the burgers. The PLS loading revealed that utilization of micronized lentil (150°C) and chickpea (130°, 150°C) in burger formulation is closely related to acceptability of appearance and aroma of the low-fat beef burgers. As explained earlier it could be related to increase in redness and yellowness of the burgers containing micronized pulse and the fact that the objectionable volatiles which form as a results of oxidation of linoleic acid decreases. Burger samples containing non-micronized lentil and chickpea flour and control are closely related to the objectionable volatile compounds and far from the consumer acceptability scores. Moreover, the graphical displays showed the burger samples formulated with micronized chickpea (150°C), high level of linolenic acid and high pH value with consumer acceptability of flavor, texture and overall acceptability

on the same plane. This is not surprising to have texture acceptability with pH closely related since the pH is a factor in water holding capacity of meat products which results in more juiciness and tenderness.

It would be highly advantageous to carry out instrumental volatile analysis using a diverse method of volatile extraction and isolation in parallel with human sensorial evaluation. It is also important to use many more genotypes and diverse growing environments to draw a firm conclusion about the effect of micronization on 'beany' volatile compounds. Such studies are important to increase domestic pulse consumption in the long term and contribute to health of the general population.

APPENDIX A Volatile compounds contributing to beaniness in legume foods reported in literature

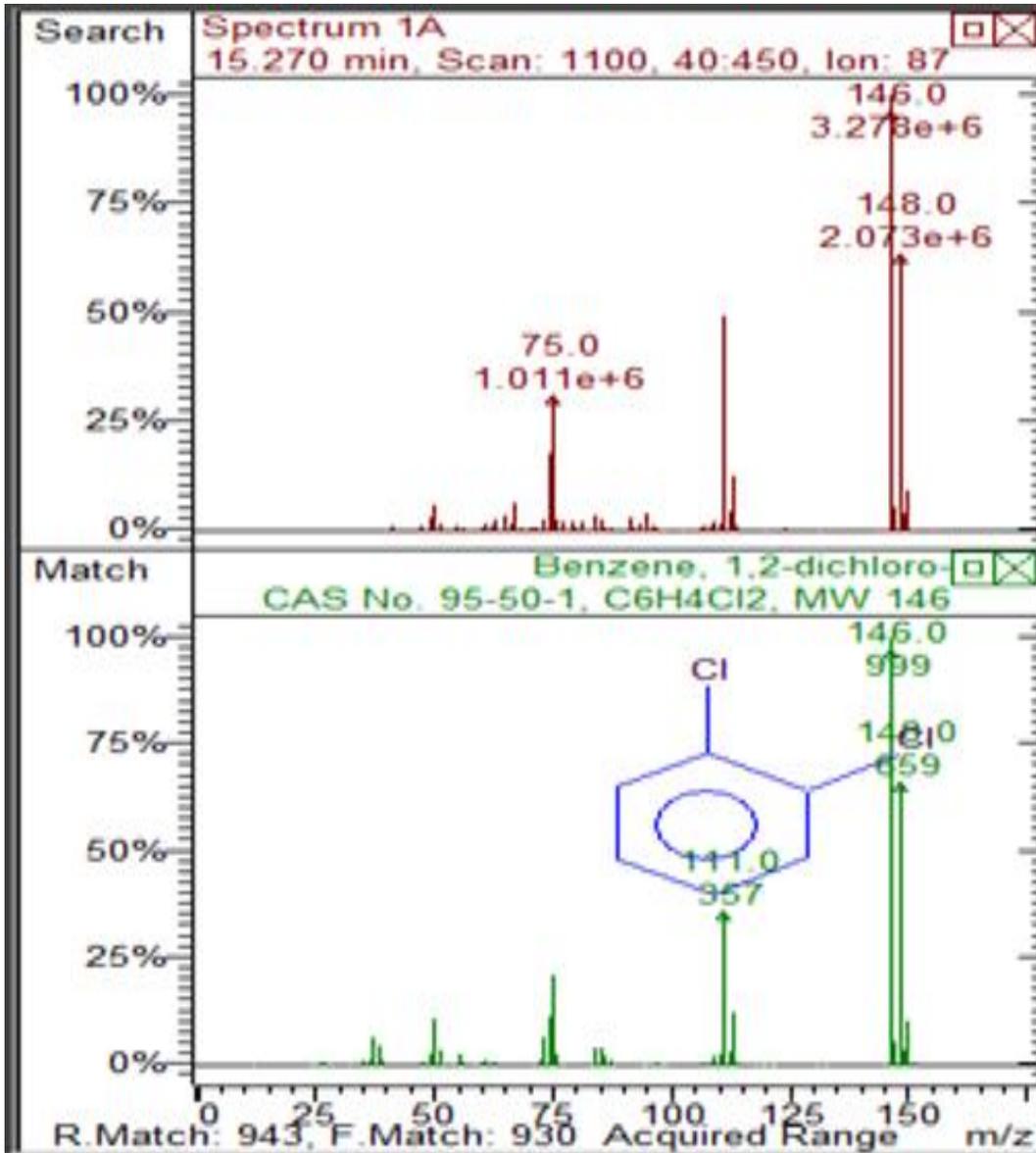
Chemical compounds potentially associated with 'beany' aroma and flavor in different types of food legumes reported in literature, (Vera-Ubol, Chambers and Chambers, 2004)

Reference	Food source	Volatile compounds	Sensory attribute
(Arai et al., 1967)	Raw soy bean	Isopentanol Hexanol heptanol	Green beanlike
(Wilkins & Lin, 1970)	Full fat soy milk	Hexanal Hexanol 2-hexanal 1-octen-3-ol Ethyl vinyl ketone 2-pentyl furan	Green 'beany' flavor
(Takahashi, Sasaki, & Chiba, 1979)	Soy bean	Hexanal pentanal	Green 'beany'
(Hsieh, Huang, & Chang, 1982)	Defatted soy flour	Ethyl vinyl ketone 2-pentyl furan Pentanol Hexanol 1-octen-3-ol Hexanal	'beany' grassy and green odor
(Mattick & Hand, 1969)	Soaked soy bean	Ethyl vinyl ketone	Green 'beany' odor
(Chang, Smouse, Krishnamurthy, Mookherjee, & Reddy, 1966)	Oxidized soy bean oil	2-pentyl furan	'beany' grassy flavor
(Hoffmann, 1962)	Oxidized soy bean oil	Hexanal Cis-3-hexenal Trans-3-hexenal 2 heptenal and 2-octenal have	Green 'beany' flavor Brown 'beany'
(Togari, Kobayashi, & Aishima, 1995)	Solvent extract of soy milk	Trans,trans-2,4-nonadienal Trans-2,4 decadienal Hexanal 2-pentyl furan 1-octene-3-one Trans-2-nonenal Trans,cis-2,4-nonadienal	'beany' odor
(Lao, 1972)	Soy milk	2,4-heptadienal	Painty oxidized flavor
(Oh, Lee, Lee, Lee, & Oh, 1988)	Soy milk prepared by soaking or not-soaking	propanal	Found
(Boatright & Lei, 1999)	Soy protein isolate	Dimethyl trisulfide Trans,trans-2,4-decadienal	'beany' flavor

Reference	Food source	Volatile compounds	Sensory attribute
		2-pentyl pyridine Trans,trans-2,4-nonadienal Hexanal Acetophenone 1-octen-3-one	
(Chiba, Takahashi, & Sasaki, 1979) and (Wang, Dou, Macura, Durance, & Nakai, 1998)	Soy protein isolate Defatted soy flour Soy milk	Hexanol Hexanal	Green 'beany' flavor
(Wang et al., 1998)	Soy milk	Concentration of above compounds linearly associate with lipoxygenase activity	
(Brown, Senn, Dollear, & Goldblatt, 1973)	Raw peanut	Hexanal Octanal Nonanal 2-octenal 2-nonenal	Green 'beany' flavor
(Hinterholzer, Lemos, & Schieberle, 1998)	Raw and cooked French bean	3-isobutyl-2-methoxypyrazine	Earthy 'beany'
(Tokimoto & Kobayashi, 1988)	Cooked Japanese adzuki beans	Hexanol 3-methyl-1-butanol	'beany'
(Mtebe & Gordon, 1987)	Winged beans	Hexanal 2-heptanone 2-pentyl furan Undecane Tridecane All formed by lipoxygenase activity	'beany'
(Brown et al., 1973)	Peanuts (roasted Spanish and Runner)	2-octenal	Described as tallow and fatty while other called it 'beany'
Badenhop and Wilkens 1969 Boatright and Lei 1999		1-octen-3-one 1-octen-3-ol (considered 'beany')	Described as musty, earthy, mushroom-like They may have a 'beany' characteristic in a specific range of concentration
(Apriyantono, Nurjanah, & Satiawihardja, 2001)	tempe	2,4-undecadienal	'beany' aroma

APPENDIX B Mass spectrum of selected volatile compounds contributing to beany aroma and flavor

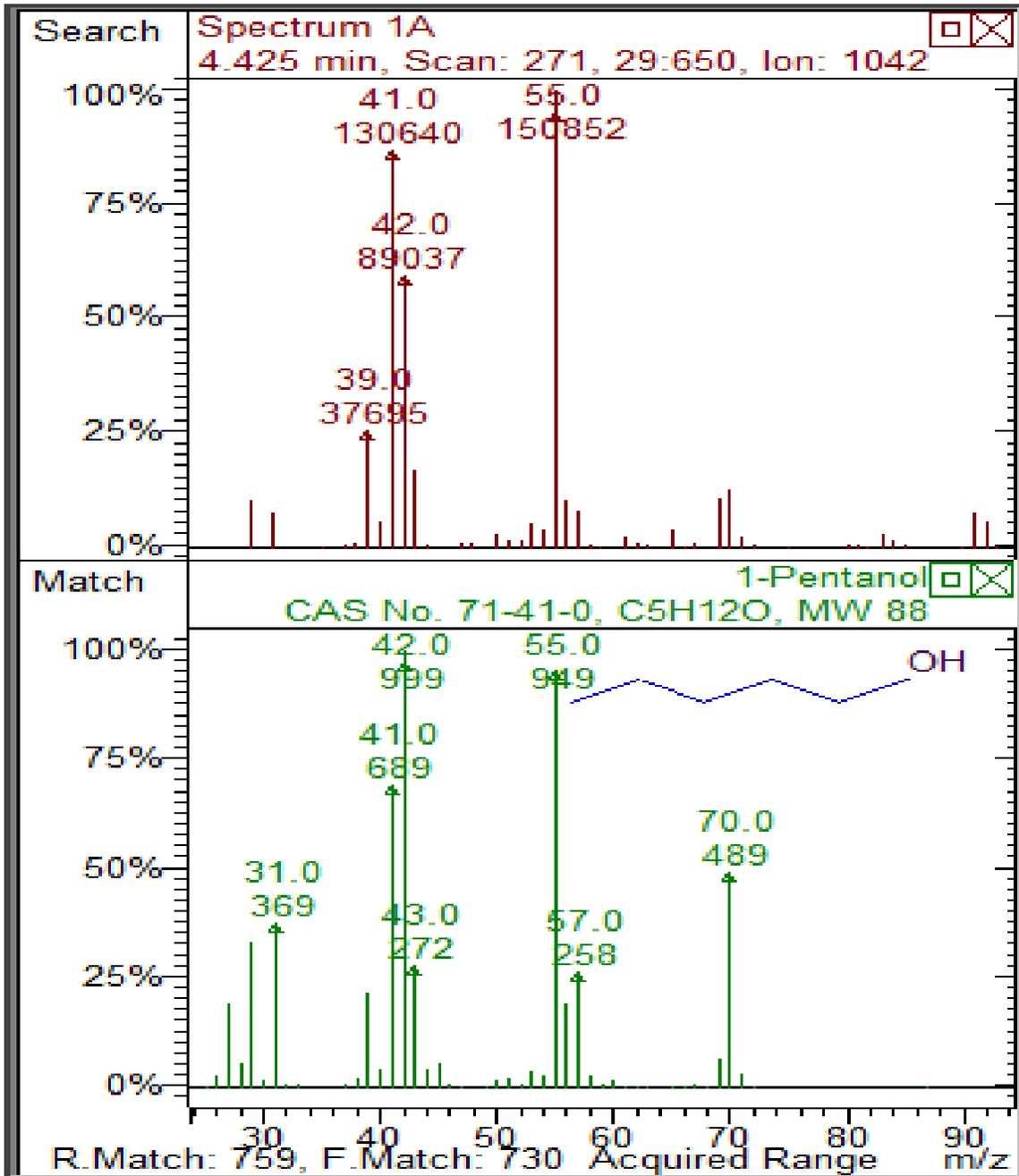
1,2-dichlorobenzene (Internal standard)



Spectrum 1A: Mass spectrum is obtained at 15.270 from scan 1100 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 1,2 dichlorobenzene from NIST library

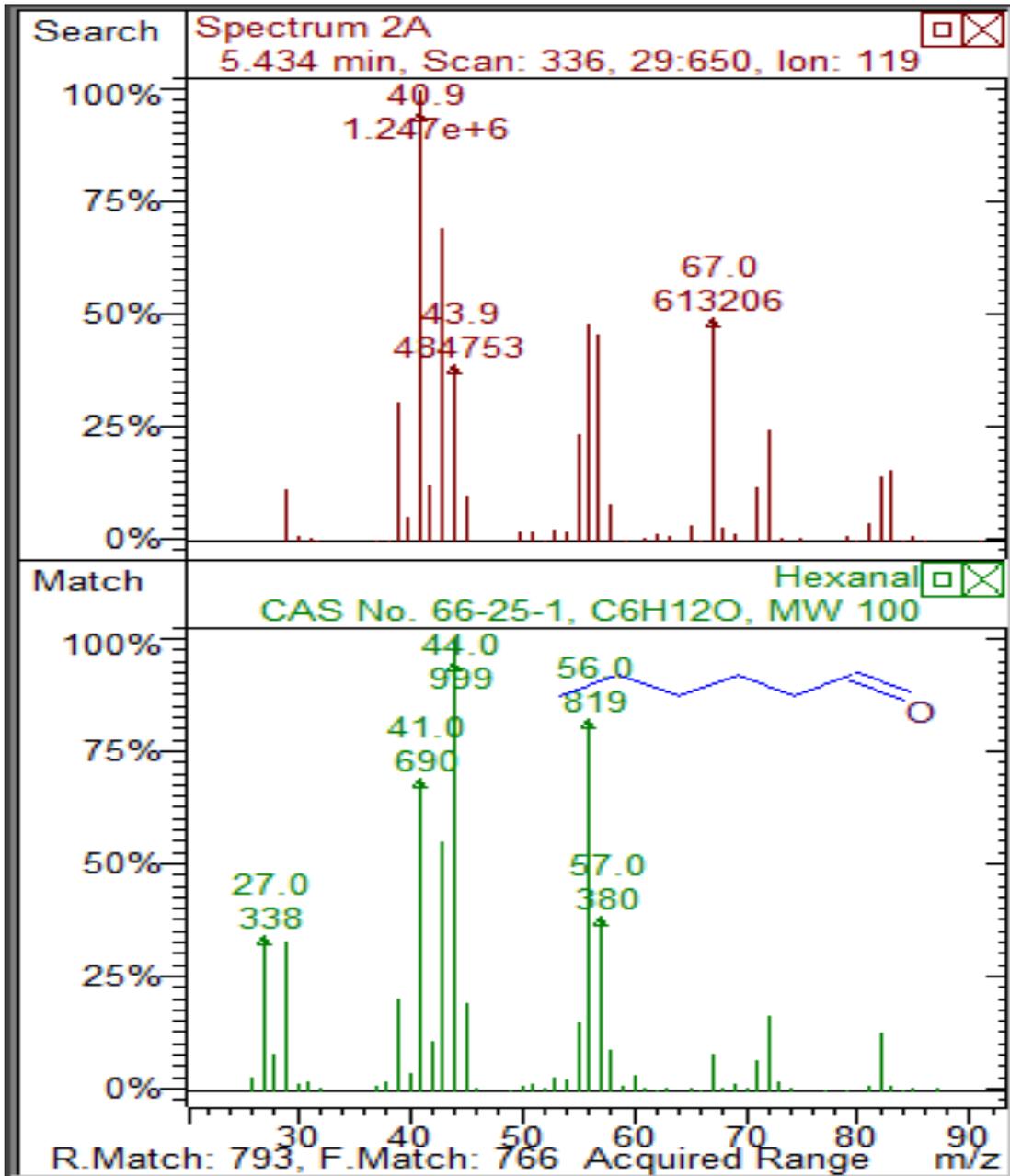
1-Pentanol



Spectrum 1A: Mass spectrum is obtained at 4.425 min from scan 271 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 1-Pentanol from NIST library

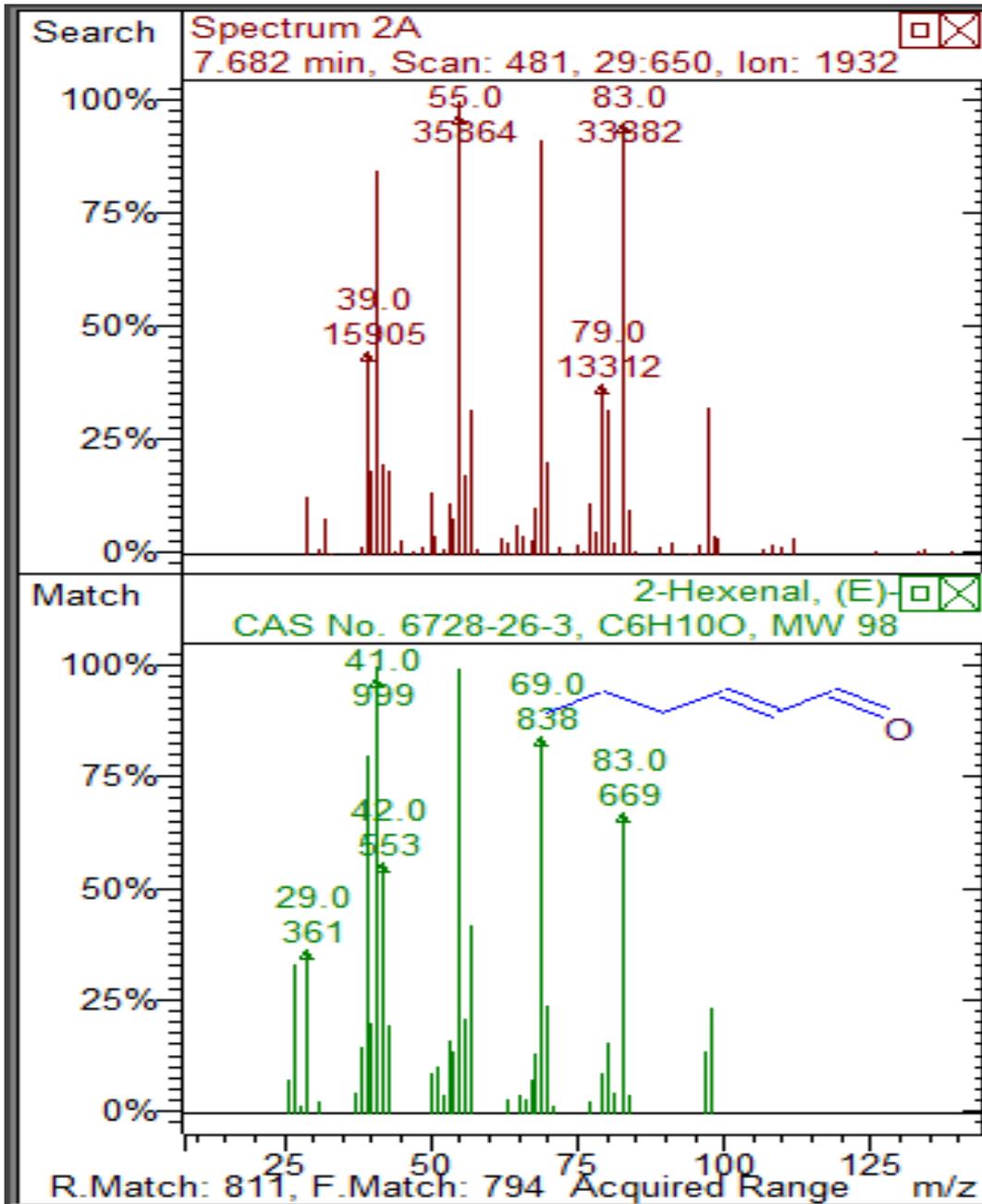
Hexanal



Spectrum 1A: Mass spectrum is obtained at 5.434 min from scan 336 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Hexanal from NIST library

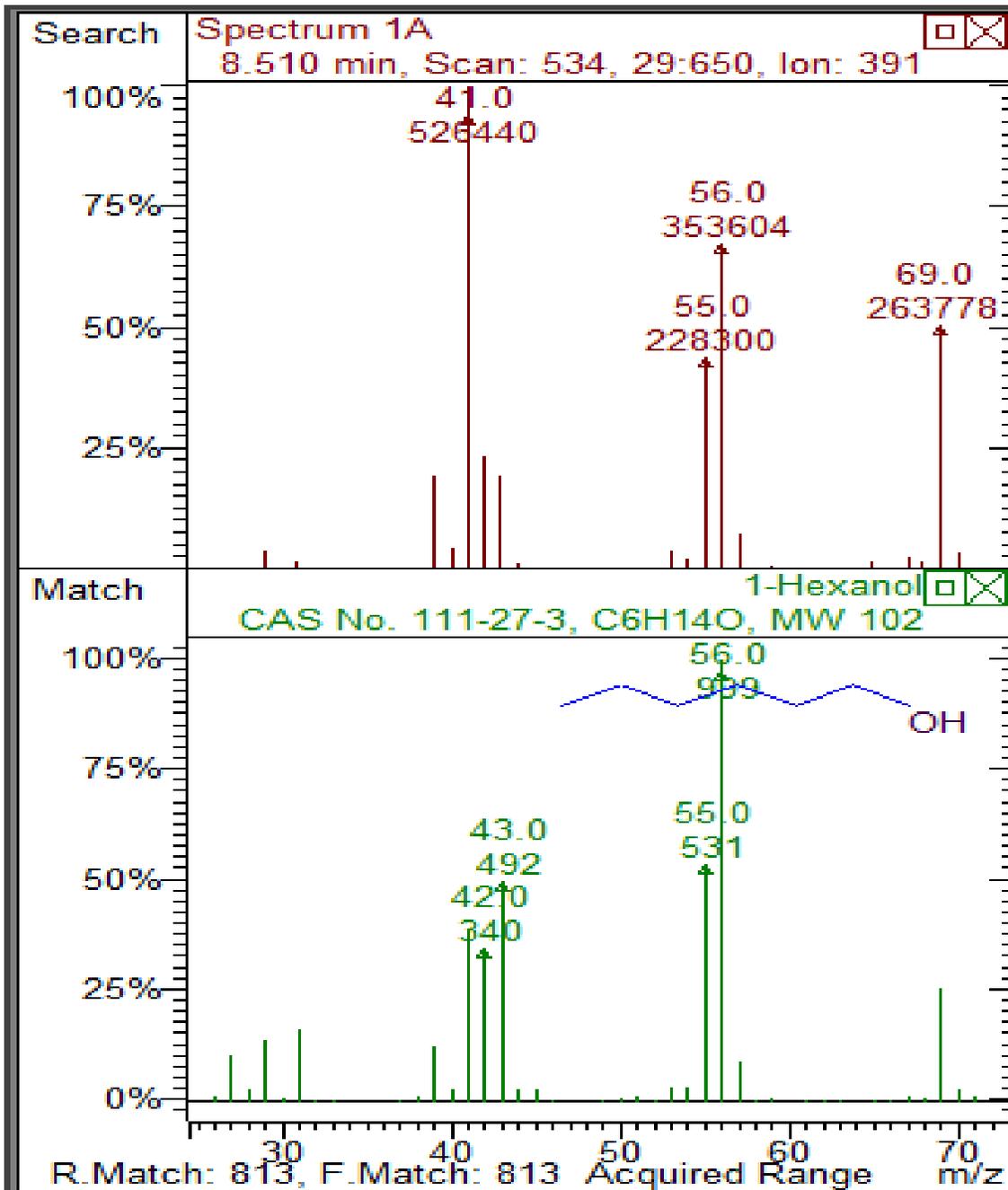
2-Hexenal



Spectrum 1A: Mass spectrum is obtained at 7.682 min from scan 481 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 2-Hexenal from NIST library

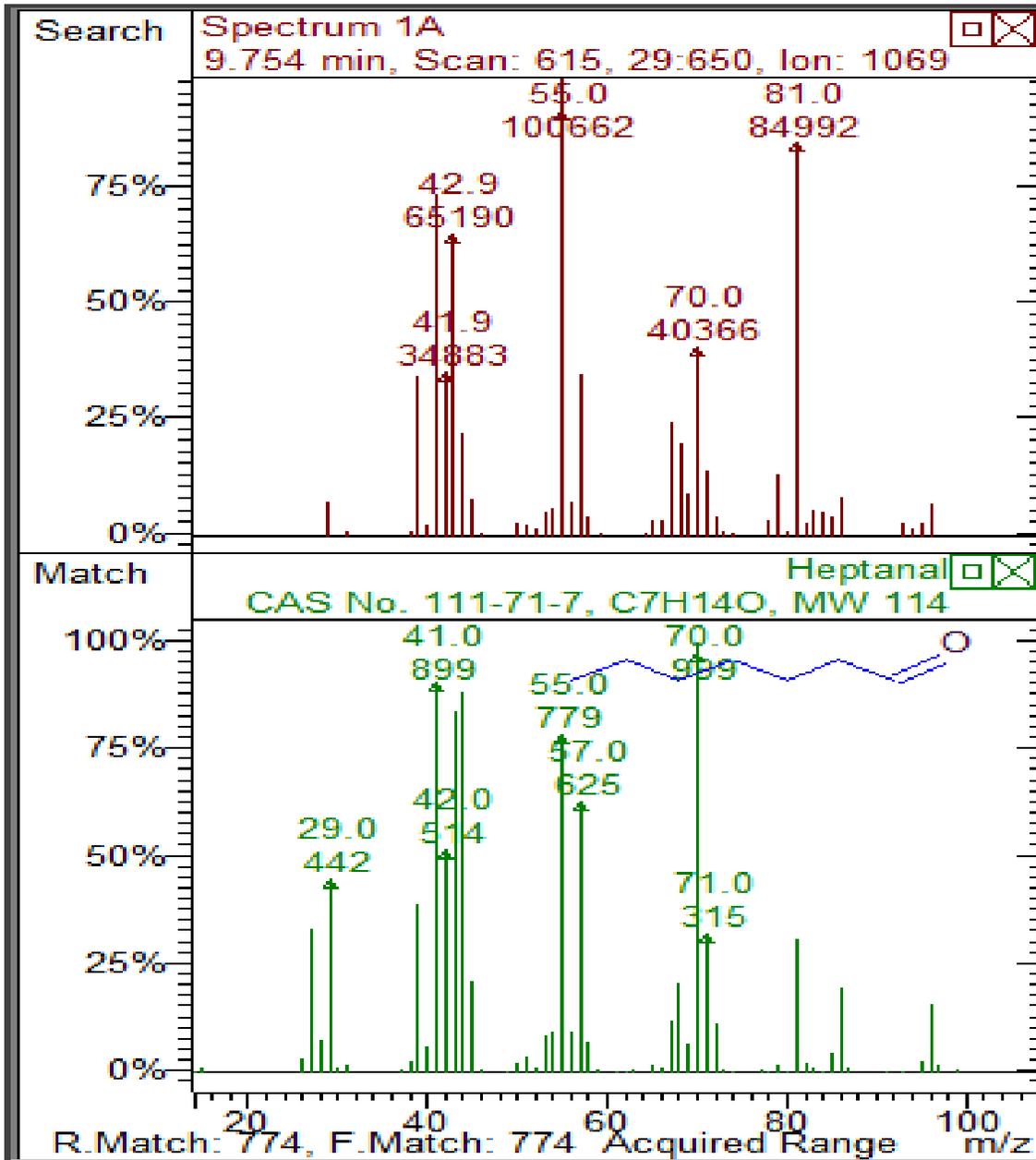
Hexanol



Spectrum 1A: Mass spectrum is obtained at 8.510 min from scan 534 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 1-Hexanol from NIST library

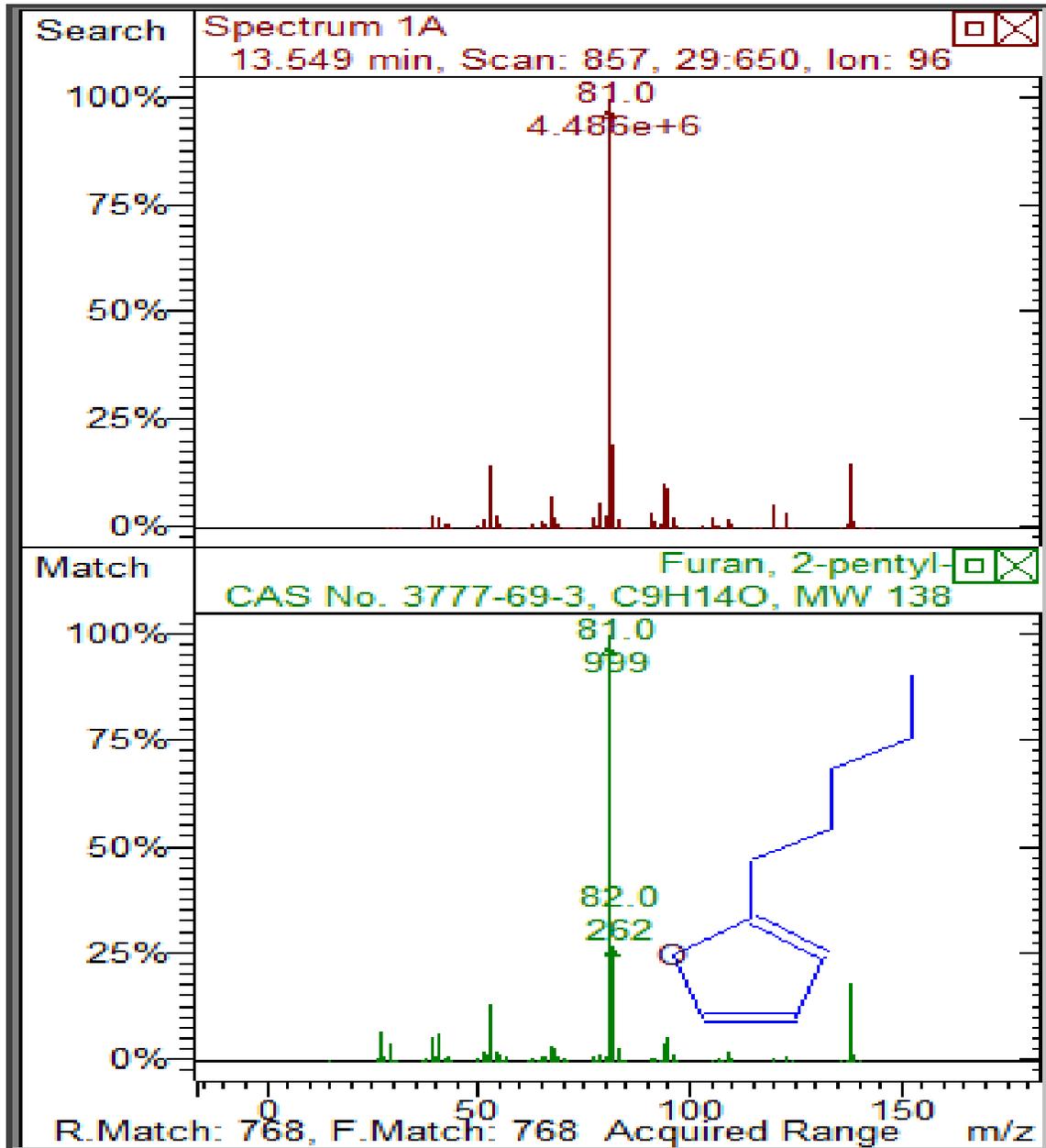
Heptanal



Spectrum 1A: Mass spectrum is obtained at 9.754 min from scan 615 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Heptanal from NIST library

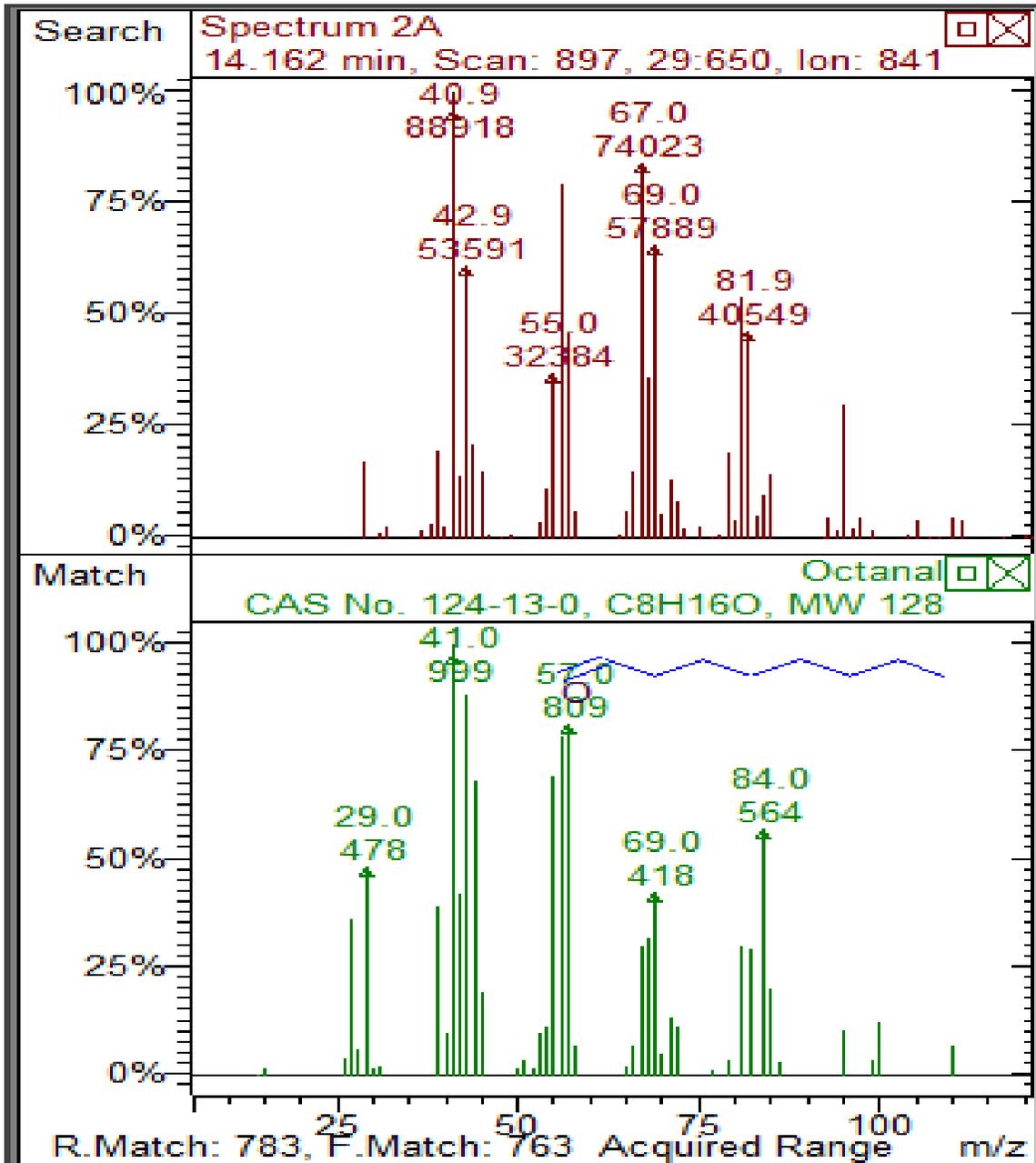
Furan 2-Pentyl



Spectrum 1A: Mass spectrum is obtained at 13.549 min from scan 857 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Furan 2-pentyl from NIST library

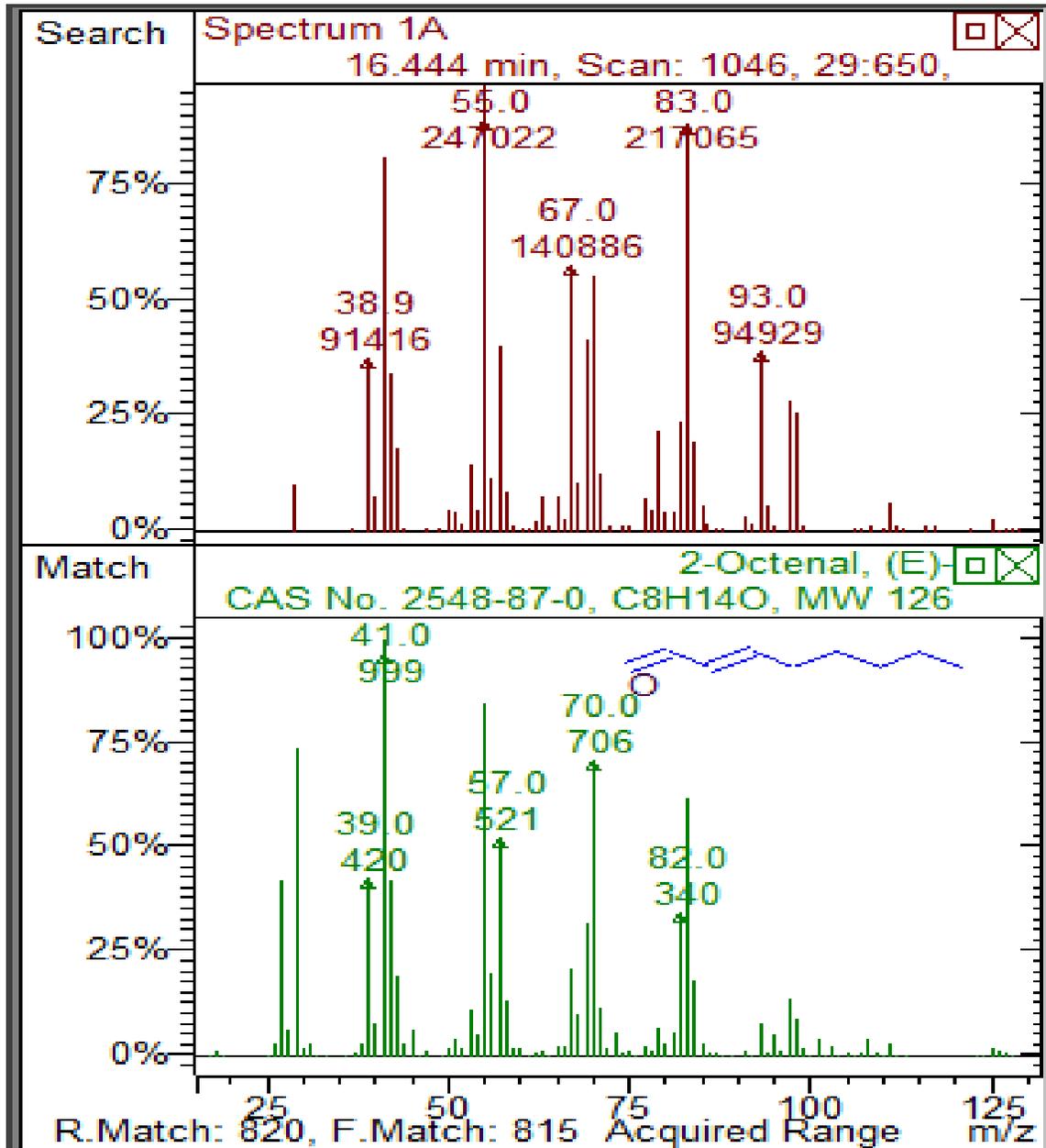
Octanal



Spectrum 1A: Mass spectrum is obtained at 14.162 min from scan 897 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Octanal from NIST library

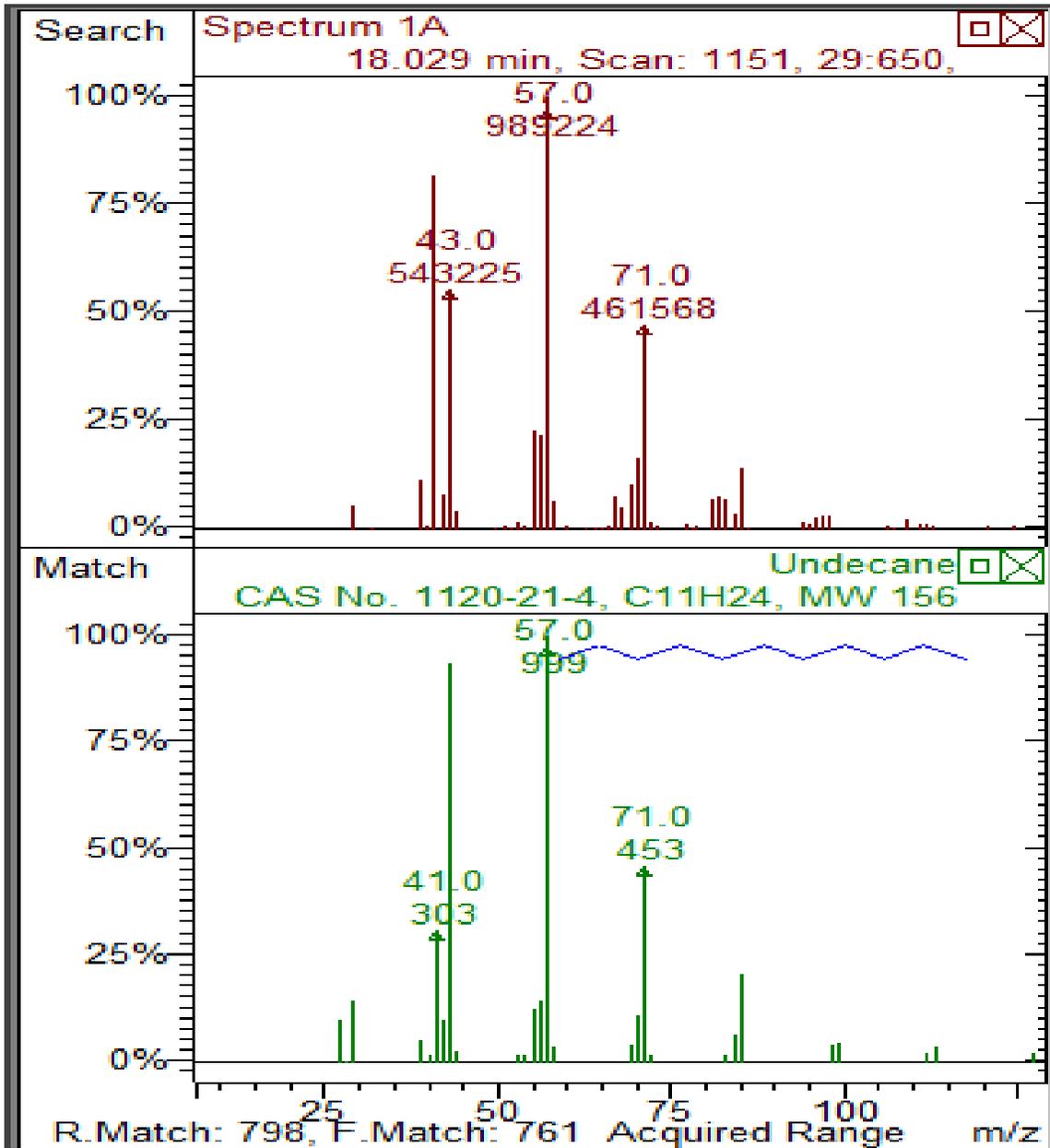
2-Octenal



Spectrum 1A: Mass spectrum is obtained at 16.444 min from scan 1046 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 2-Octenal from NIST library

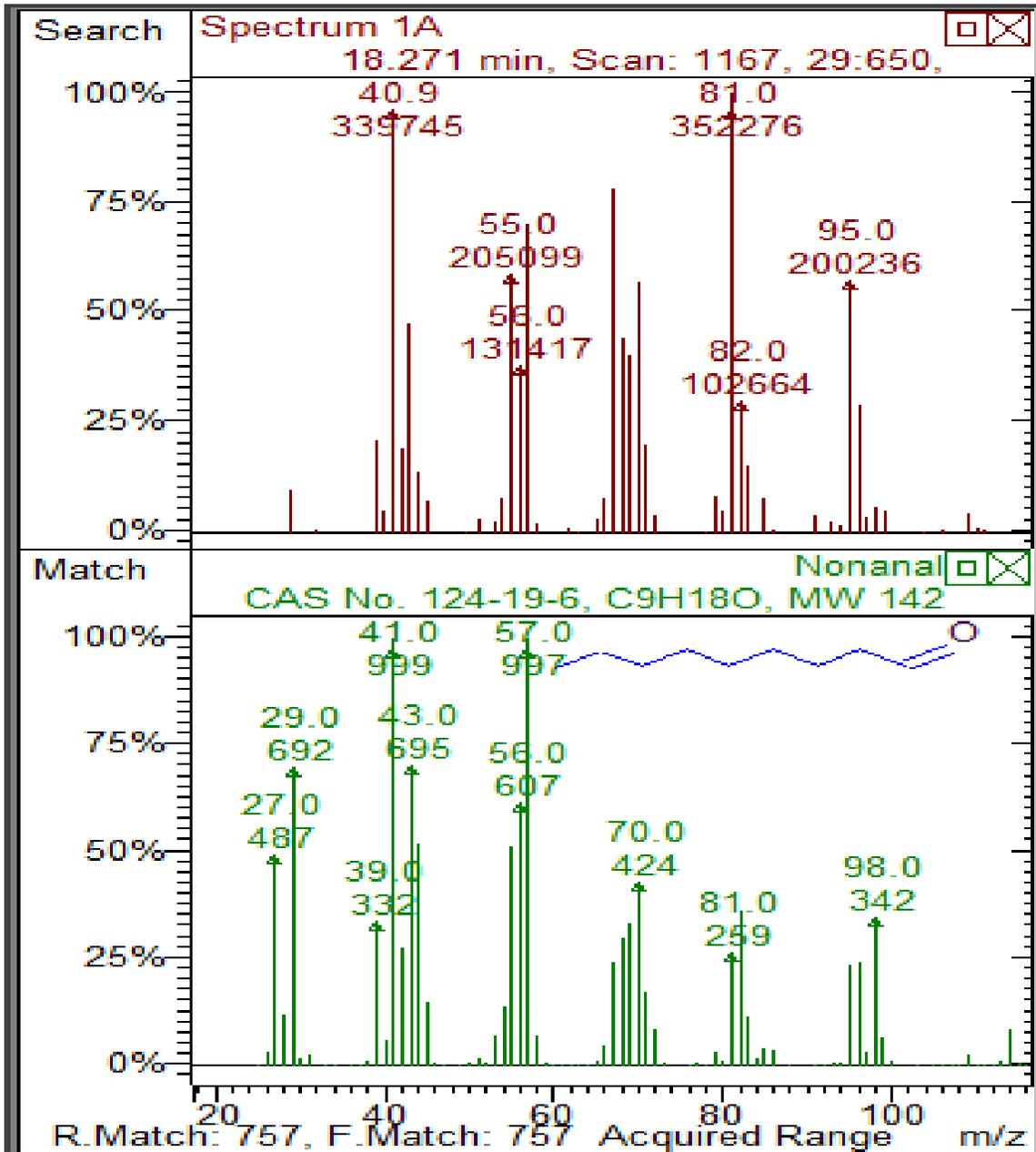
Undecane



Spectrum 1A: Mass spectrum is obtained at 18.029 min from scan 1151 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Undecane from NIST library

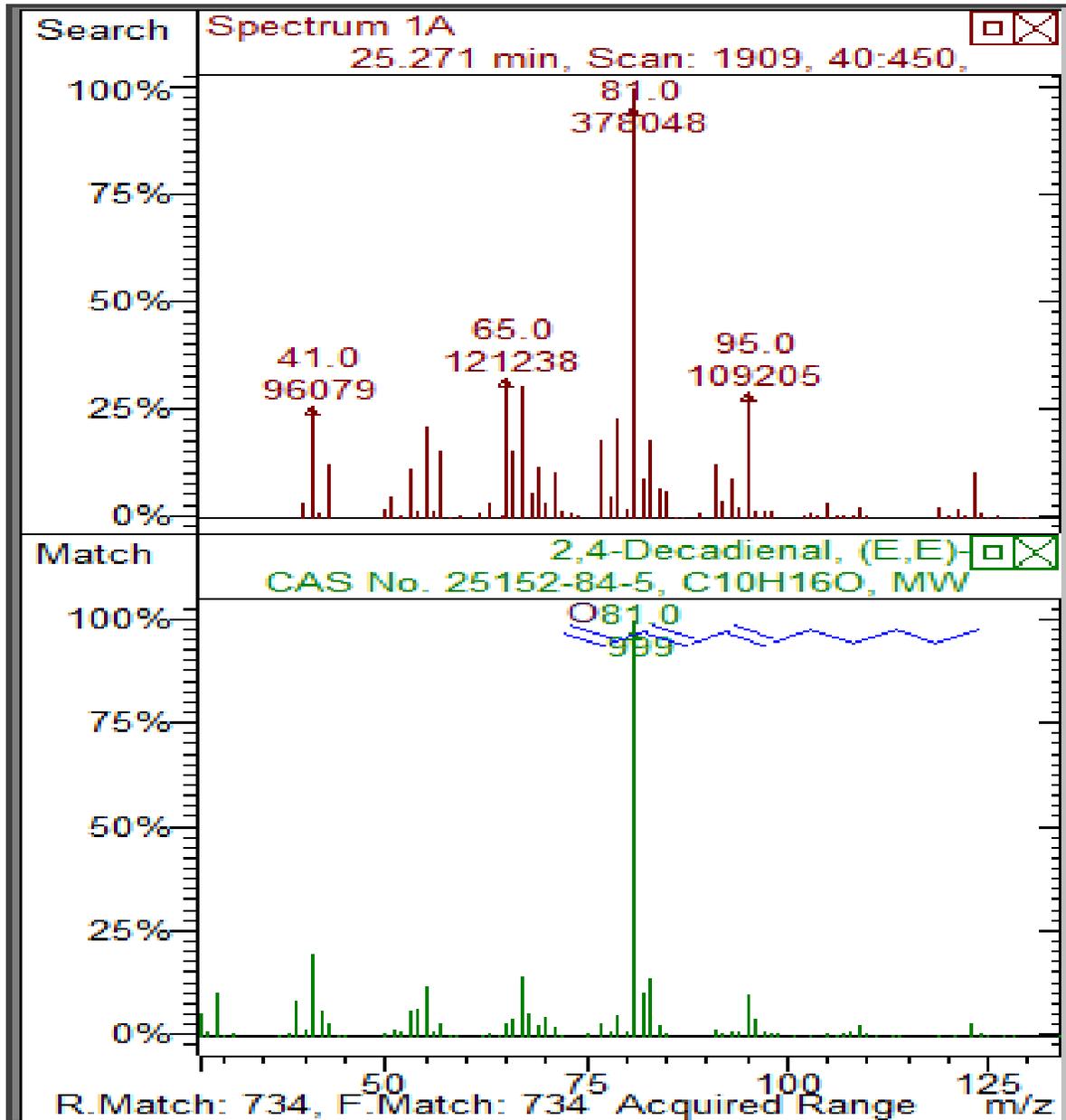
Nonanal



Spectrum 1A: Mass spectrum is obtained at 18.271 min from scan 1167 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Nonanal from NIST library

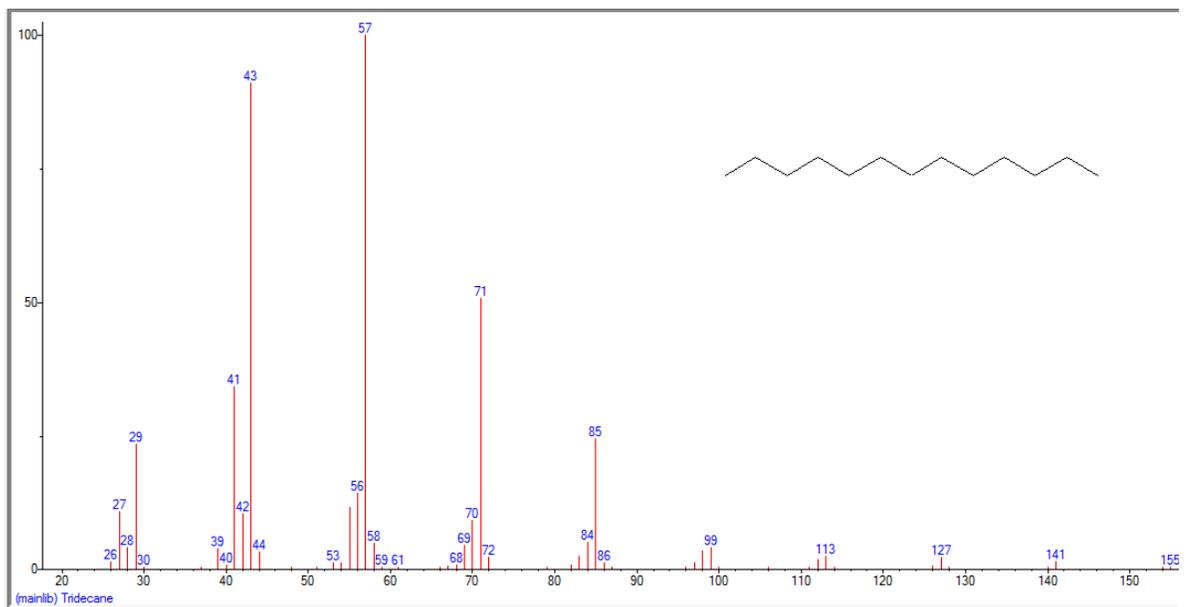
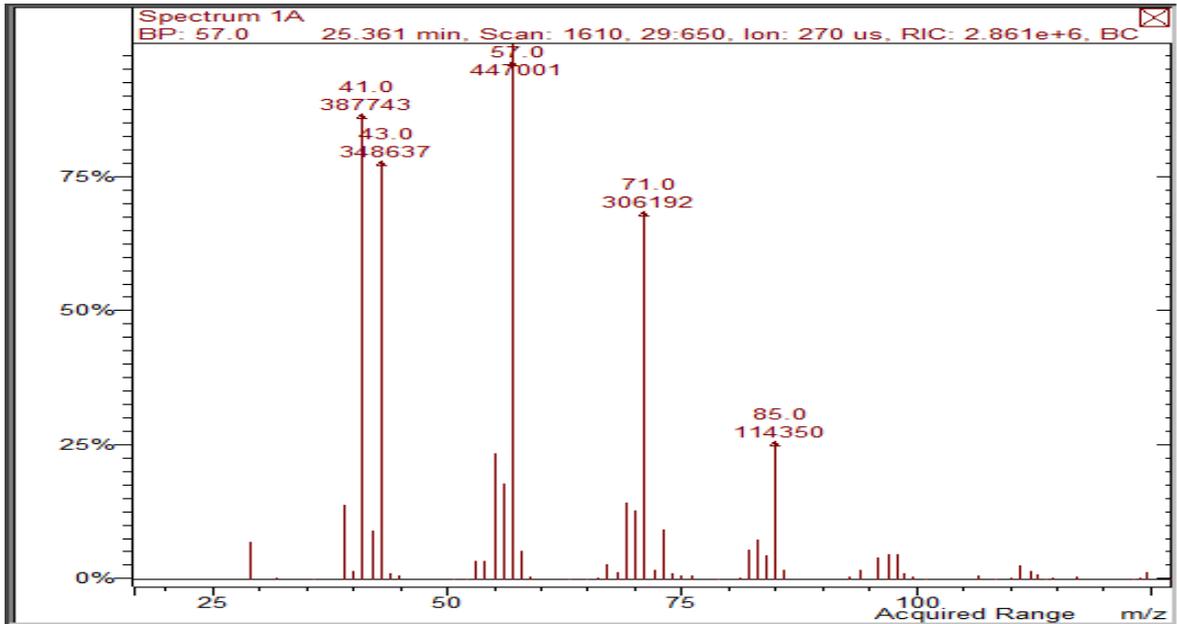
2,4-decadienal



Spectrum 1A: Mass spectrum is obtained at 25.271 min from scan 1909 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 2,4-Decadienal from NIST library

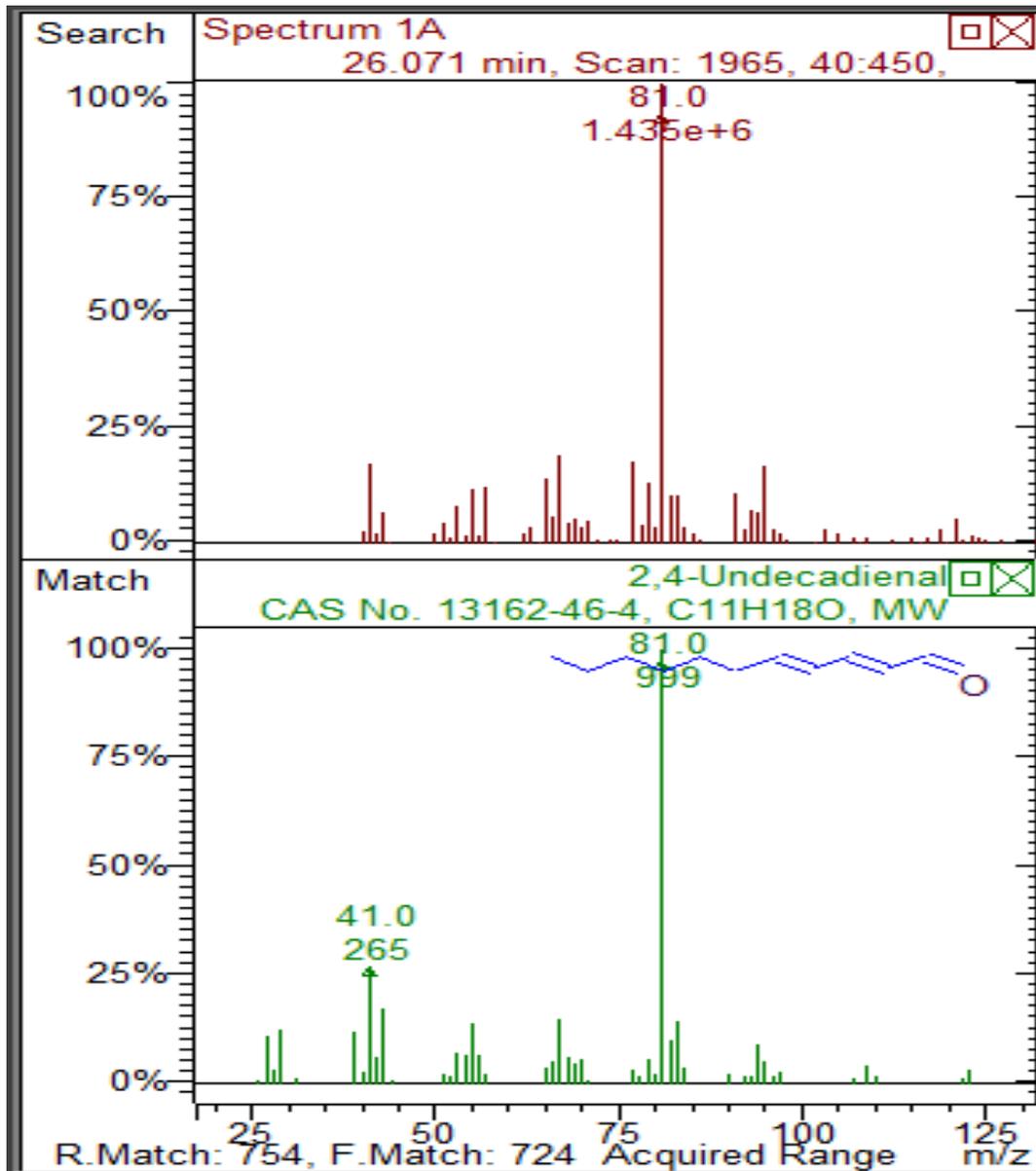
Tridecane



Spectrum 1A: Mass spectrum is obtained at 25.361 min from scan 1610 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of Tridecane from NIST library

2,4-Undecadienal



Spectrum 1A: Mass spectrum is obtained at 26.071 min from scan 1965 in non-micronized chickpea flour

Bottom: molecular structure and mass spectra of 2,4-Undecadienal from NIST library

APPENDIX C Research ethics and compliance approval certificate



UNIVERSITY
OF MANITOBA

Office of the Vice-President
(Research and International)
Research Ethics and Compliance

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

APPROVAL CERTIFICATE

January 23, 2012

Pulse Canada &
Canadian Agri-Science Cluster Initiative

TO: **Michel Aliani**
Principal Investigator

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

Re: **Protocol #J2011:155**
"Consumer Acceptability of Beef Burgers with Pulse Flours"

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.

umanitoba.ca/research/orec

APPENDIX D Consumer acceptability consent form and questionnaire

Faculty of Human Ecology

Department of Human Nutritional Sciences

CONSENT FORM

Research Project Title: Consumer Acceptability of Beef Burgers with Added Pulse Flours

Sponsored by: Pulse Canada and Canadian Agri-Science Cluster Initiative

Researchers: Dr. Michel Aliani, Shiva Shariati-Ievvari and Donna Ryland, Department of Human Nutritional Sciences

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** that can be scheduled during the weeks of February 6 and 13. Sign up times are - 11:30, 12:30, 1:30 and 2:30 on Mondays February 6 and 13 and Wednesdays February 8 and 15; 11:30 and 1:00 on Tuesday, February 7 and 11:30, 1:00 and 2:30 on Thursday, February 16. An honorarium of a \$10 gift card from the University of Manitoba Bookstore will be offered to those completing the session. Participants may request the citation of the study once it has been published 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 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, Assistant Professor, Department of Human Nutritional Sciences, telephone – 474-8070, e-mail – aliani@cc.umanitoba.ca.

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 at 474-7122.

Participant's Name (Please Print)

Participant's Signature

Date

Telephone

Number

E-mail

Address

Researcher and/or Delegate's Signature

Date

Delegate's contact information:

Donna Ryland, Sensory Evaluation Specialist
Room 400 Human Ecology Building
Telephone - 474-8071
E-mail - ryland@cc.umanitoba.ca

QUESTIONNAIRE

This information will be kept strictly confidential.

Panelist # _____

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.

Beef Burgers with Pulse Flour 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.

PROCEDURE

Evaluate the samples in the following order:

Sample Order

For each sample evaluate the aroma, appearance, flavor, texture, overall acceptability and frequency of eating before continuing to the next sample.

Check the descriptor which corresponds to your response.

Note comments if desired.

Ensure that all responses are completed.

Please see the researcher to sign for and receive your honorarium.

1. AROMA

Sample No. _____

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

GROUP CHARACTERISTICS

Click on the circle next to the descriptor which corresponds to your response.

ONLY ONE response will be accepted.

All information will remain strictly confidential

1. Gender

- Female
- Male

2. Age

- 18 to 24 years
- 25 to 34 years
- 35 to 44 years
- 45 to 54 years
- 55 to 64 years
- over 64 years

3. Frequency of eating pulses such as chick pea, lentil and beans.

- more than three times a week
- two to three times a week
- at least once a week
- at least once a month
- occasionally
- never

4. Frequency of eating beef burgers

- more than three times a week
- two to three times a week
- at least once a week
- at least once a month
- occasionally
- never

Thank you very much for taking the time to participate in our study!!!!

Remember to please see the researcher to receive and sign for your honorarium.

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