The acute effect of a pizza meal with partial or complete replacement of all-purpose wheat flour with lentil flour of similar particle size on postprandial blood glucose, subjective appetite, and food intake in healthy young adults

By

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ABSTRACT

Lentil consumption has been associated with a lower glycemic response. Previous *in vitro* study has shown that the larger the particle size of lentil flour, the less glucose is produced in the process mimicking the digestion in the gastrointestinal tract (Kathirvel et al., 2019). With the limited information reported on particle size in studies comparing various food flours and powders, it was unknown whether the differences detected in metabolic responses were determined by a difference in particle size, or composition, or both. The objective of this study was to investigate the effect of a pizza meal formulated with either lentil flour or wheat flour of similar particle size or their combination on postprandial blood glucose, subjective appetite, physical comfort and food intake. We hypothesized that the partial or complete replacement of wheat flour with processed lentil flour of similar particle size in a formulated pizza would result in lower blood glucose and subjective appetite due to a higher content of resistant carbohydrates and protein in lentil flour compared to wheat flour.

Methods: Twelve male and twelve female participants aged between 19 and 35 years completed a randomized controlled crossover by attending four sessions, with each session a week apart. The treatments were formulated as 100% lentil flour pizza (L), 100% wheat flour pizza (W), a mixture of lentil (50%) and wheat flour (50%) pizza (M), and water as an energy-free control (C). Blood was collected, and appetite sensations and physical comfort were recorded at 0 min (baseline) and 15, 30, 45, 60, 90, 120, 150 and 180 min following the treatment. *Ad libitum* food intake (FI) at 180 min was measured using a macaroni and cheese meal. Serum blood glucose (BG) concentration and calculated subjective appetite scores were used to compute the incremental area under the curve (AUC) for BG, maximum BG concentration and the total AUC for appetite, respectively.

Results: The ingestion of L led to reduced BG at 30 min ($P=0.0004$), and BG $iAUC_{0\text{-}180min}$ ($P=0.0008$) compared to W. The ingestion of M resulted in a lower BG $iAUC_{0\text{-}180min}$ ($P=0.02$) compared to W. There was no difference between L and M on BG $iAUC_{0\text{-}180min}$ ($P=0.7$). The relative glycemic response reduction was 65% and 47 % after L and M, respectively, compared to the W. All caloric treatments suppressed subjective average appetite over 180 min compared to C ($P\leq0.05$). Although there was a trend for the effect of a treatment on *ad libitum* FI at 180 min ($P=0.07$), there was an effect of sex ($P=0.004$), resulting in no effect of a treatment on FI in males

(P=0.9), and a higher FI after C compared to caloric treatments (L, W and M) in females (P=0.004). The whole or partial replacement of wheat flour with lentil flour in pizza meals resulted in a similar level of physical comfort between the treatments (P>0.05).

In conclusion, the ingestion of a pizza meal formulated with lentil flour resulted in a lower glycemic response compared to a pizza meal formulated with wheat flour of similar particle size. Pizza meals formulated with lentil or wheat flour or their mixture resulted in a similar subjective appetite, food intake and physical comfort.

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AA	Average Appetite
AGEs	Advanced glycation end products
AgRP	Agouti-related protein
ACE	Angiotensin-converting enzyme
ARH	Arcuate nucleus of the hypothalamus
BMI	Body Mass Index
BBI	Bowman-Birk type trypsin–chymotrypsin inhibitor
C	Energy-free water control
CVD	Cardiovascular disease
CCK	Cholecystokinin
CHD	Congenital heart disease
DNA	Deoxyribonucleic acid
DTE	Desire to eat
DVC	Dorsal vagal complex
GLP-1	Glucagon like peptide-1
GI	Glycemic index
GL	Glycemic load
GADPH	Glyceraldehyde-3 phosphate dehydrogenase
HbA1C	Glycosylated hemoglobin
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model of insulin resistance
ICV	Intracerebroventricular
iAUC	Incremental area under the curve
IGF-1	Insulin growth factor-1
IV	Intravenous
IHD	Ischemic heart disease
L	100% Lentil pizza
LDL	Low-density lipoprotein
M	Pizza made with 50% lentil flour and 50% wheat flour
C_{max}	Maximum blood glucose concentration
MS	Metabolic syndrome

MSVU	Mount Saint Vincent University
NHNES	National health and nutrition examination survey
NA	Nicotinic acid
NCDs	Noncommunicable diseases
ORAC	Oxygen radical absorbance capacity
PYY	Peptide YY
PARP	Poly ADP-ribose polymerase
PFC	Prospective Food Consumption
PPG	Postprandial glycemia
POMC	Pro-opiomelanocortin
PKC	Protein Kinase C
RDS	Rapidly digestible starch
RCF	Relative centrifugal force
RM-ANOVA	Repeated-measures analysis of variance
SSTs	Serum separation tubes
SD	Standard deviation
tAUC	Total area under the curve
TRAP	Total-radical trapping antioxidant capacity
TEAC	Trolox equivalent antioxidant capacity
T2DM	Type 2 diabetes mellitus
TH	Tyrosine hydroxylase
UPC	Universal product code
UK	United Kingdom
USDA	United States Department of Agriculture
VTA	Ventral tegmental area
VAS	Visual analogue scale
W	100% wheat flour pizza
WHO	World Health Organization

Chapter 1: Introduction

Obesity, diabetes, cardiovascular diseases, and cancers are the most prevalent diet-related chronic health conditions among Canadians (Health Canada, 2020). Approximately 27% of the Canadian adult population was obese in 2018 and one in three Canadians is diabetic or pre-diabetic (Lytvyak, Straube, Modi, & Lee, 2022; Ottawa: Diabetes Canada, 2022). Cancer is still the leading cause of death, followed by heart disease amongst Canadians (Canadian Cancer Society, 2022; Health Canada, 2017). These chronic conditions have a substantial social and economic burden. For example, the predicted health care costs related to diabetes was nearly $15.35 billion between 2011 and 2021 (Bilandzic & Rosella, 2017). Poor diet combined with other lifestyle risk factors has resulted in an increase in the number of adults with chronic metabolic conditions. The Canadian government is making efforts to encourage the plant-based protein foods in an attempt to improve the Canadian diet by emphasizing them in the 2019 version of the Canadian Food Guide (Health Canada, 2019).

Pulses are low-cost plant-based protein sources. They are defined as "edible dried seeds of certain legume family plants" (Pulse Canada, 2022). Examples of Canadian grown pulses are beans, peas, chickpeas, and lentils (Pulse Canada, 2022). Several epidemiological, long- and short-term animal and human studies have shown that consumption of pulses has beneficial effects on blood glucose control and weight management (Mollard, Wong, Luhovyy, Cho, & Anderson, 2014). However, pulse consumption is low in Canada due to its longer cooking time, which is not suitable for the fast-paced lifestyle of Canadians, and its unfavorable taste and flavor (Ipsos, 2010). One possible way to increase pulse consumption among Canadians is by introducing pulse products which take less preparation time. Canada is a large pulse producing country, and, therefore, has a unique opportunity to develop novel food products with pulse ingredients for both domestic consumption and export (Pulse Canada, 2022). This will help to boost the economy of Canada by decreasing health care costs related to chronic diseases and allowing for new business opportunities.

Lentils have gained popularity among consumers as well as food industries due to their excellent nutritional composition and functional properties (Escobedo & Mojica, 2021). Lentil flour is the primary lentil product used by food industries for various food applicationsas it is readily available and economical (Escobedo & Mojica, 2021). Lentil flour is incorporated into baked products (e.g., bread, cake, and crackers), extruded products (e.g., pasta and snacks) and other products such as

dressings, soups, dairy and meat products, and plant-based beverages (Escobedo & Mojica, 2021). Lentils are subjected to various processing methods to prepare a flour with desirable nutritional, technological, and sensorial properties. Lentil flour is produced in various particle sizes using different milling techniques, different sizes of milling screens and sieving meshes depending on the requirement of food product developers (Bourré et al., 2019; Maskus, Bourré, Fraser, Sarkar, & Malcolmson, 2016). The particle size of the lentil flour and the extent of the milling process affect the nutritional composition of the lentil flour and thus, its functional properties, such as its glycemic reduction potential (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). Based upon this, the current study aimed to study the effect of partial or complete replacement of all-purpose wheat flour with lentil flour of similar particle size and examine its effect on postprandial blood glucose. The secondary objective was to study the subjective appetite, physical comfort, and food intake after consumption of pizza made with lentil and/or wheat flour.

Chapter 2: Literature review

2.1 Non-communicable diseases in Canada

The World Health Organization (WHO) defines non-communicable diseases or chronic diseases as long-duration diseases that are the outcome of a combination of genetic, physiological, behavioral, and environmental factors (World Health Organization, 2021). Health Canada lists hypertension, periodontal diseases, osteoarthritis, ischemic heart disease (IHD), diabetes, osteoporosis, cancer, chronic obstructive pulmonary disease, asthma, and mood and anxiety disorders as the most common chronic diseases among Canadians (Health Canada, 2020). Of those, obesity, cardiovascular diseases, diabetes, and cancer are the most preventable diet and lifestyle-related diseases among Canadians (Health Canada, 2020).

2.1.1 Cardiovascular diseases

Heart disease is the second leading cause of death in Canada and one in 12 Canadian adults live with diagnosed heart disease (Health Canada, 2017). The incidence of IHD decreased from 217,600 in 2000-2001 to 162,730 in 2017-2018 (Health Canada, 2017). The known risk factors for IHD are smoking, excessive alcohol consumption, high cholesterol, high blood pressure, diabetes, lack of regular physical activity, and abdominal obesity (Health Canada, 2017).

2.1.2 Diabetes

Diabetes is a chronic condition in which the body is unable to maintain the blood sugar level within the normal range (Diabetes Canada, 2022). There are three main types of diabetes: Type I, Type II, and gestational diabetes (Diabetes Canada, 2022). Type I diabetes is an autoimmune disease in which insulin-producing cells in the pancreas are destroyed by the immune system of the body itself (Diabetes Canada, 2022). Type II diabetes is a metabolic disorder in which the pancreas doesn't produce enough insulin and/or cells become resistant to insulin (Diabetes Canada, 2022). The third category is gestational diabetes which occurs during pregnancy. Gestational diabetes can increase the risk of developing Type II diabetes at a later age (Diabetes Canada, 2022).

Of all three types of diabetes, the most common diabetes is Type II, which accounts for nearly 90% of the diabetes prevalence in Canada, followed by Type I, which accounts for 9% (Diabetes Canada, 2022). In 2022, the prevalence of individuals who had diabetes (Type I and Type II) was 10%, and it is expected to increase up to 12% by 2032 (Lytvyak, Straube, Modi, & Lee, 2022;

Ottawa: Diabetes Canada, 2022). Many Canadians face difficulties in compliance with treatments as they pay more than $1,500 annually from their income for prescribed medications, devices, and supplies (Lytvyak, Straube, Modi, & Lee, 2022; Ottawa: Diabetes Canada, 2022).

There are modifiable and unmodifiable risk factors of type II diabetes (Diabetes Canada, 2022). The unmodifiable risk factors are age, family history, gender, and ethnicity (Diabetes Canada, 2022). The modifiable risk factors are overweight/obesity, pre-diabetes, physical inactivity, having hypertension and/or high cholesterol, and a history of gestational diabetes (Diabetes Canada, 2022).

2.1.3 Overweight/Obesity

Obesity is a chronic disease defined as excessive or dysfunctional body fat/adipose tissue (Statistics Canada, 2019). Clinically, overweight and obesity are identified by BMI between 25 and 29.9 kg/m^2 and BMI $\geq$ 30 kg/m, respectively (Statistics Canada, 2019). In 2018, the overweight and obesity prevalence was 36.3 and 26.8% among adults 18 years and older, respectively (Statistics Canada, 2019).

Obesity increases the risk for chronic diseases such as heart disease, cancer, stroke, diabetes, and non-alcoholic fatty liver disease (Statistics Canada, 2019). It also affects the quality of life of individuals and reduces life expectancy (Statistics Canada, 2019). For example, diabetes prevalence was higher among obese individuals (13.4%) than individuals with normal weight (2.9%) in 2018 (Statistics Canada, 2019). Obese individuals have a higher prevalence of high blood pressure (29.5%) and heart disease (6.0%) than normal weight individuals whose high blood pressure and heart disease prevalence are 9.5% and 2.7% respectively (Statistics Canada, 2019).

The well-known immediate risk factors associated with obesity are genetics, inadequate physical activity, and an unhealthy diet (Obesity Canada, 2020). Besides, socio-economic, behavioral, and cultural, physical environmental risk factors also contribute to the development of obesity (Obesity Canada, 2020)

2.1.4 Cancer

Cancer occurs when abnormal cells grow uncontrollably and spread to nearby body parts or invade other organs in the body (World Health Organization, 2022). Among the many types of cancer, the most common type of cancers are breast, lung, colon and rectum and prostate cancer (World

Health Organization, 2019). Cancer is still the leading cause of death in Canada, it is predicted that two in five Canadian will develop cancer during their lifetime and one in four Canadian will die from cancer (Canadian Cancer Statistics Advisory Committee in collaboration with the Canadian Cancer Society, Statistics Canada, & Public Health Agency of Canada, 2021). The known risk factors for cancer are increasing age, smoking, genetic mutation, overweight /obesity, unhealthy diet, lack of physical activity, alcohol consumption, a certain type of infection, and exposure to harmful chemicals (Canadian Cancer Society, 2022).

2.2 Glucose homeostasis

2.2.1 Glucose homeostasis

Glucose is the body's primary fuel and energy substrate of the brain (Szablewski, 2011). It is vital to tightly regulate the blood glucose level as both hyper and hypoglycemia can lead to serious clinical consequences (Szablewski, 2011). Hypoglycemia can result in seizures, loss of consciousness and death, while long-term hyperglycemia may result in blindness, renal failure, vascular disease, and neuropathy (Szablewski, 2011).

Glucose enters the blood from various sources such as intestinal absorption of glucose from the diet, endogenous glucose synthesis (gluconeogenesis), and glycogenolysis, while glucose exits from the blood through glucose uptake by different tissues and organs such as the brain, liver, kidney, adipose tissue, and skeletal muscle for various functions including oxidation, glucogenesis and lipogenesis (Nirmalan & Nirmalan, 2017). The net effect of in and outflow of glucose leaves the arterial blood glucose level between 3.5 mmol/L (after exercise) to 9 mmol/L (following a meal), whereas post-prandial levels are limited within a narrow range of 4-5.5 mmol/L (Nirmalan & Nirmalan, 2017). Traditionally, glucose regulation was explained through the islet-centered insulin- glucagon mechanism (Schwartz et al., 2013). However, recent studies explain glucose regulation through an interactive model which represents the interaction among brain, liver, gut, pancreas, adipose tissue, and muscle in a complex network via hormones, neurotransmitters, and cytokines (Schwartz et al., 2013). Although glucose homeostasis is a multi-organ process, the brain and pancreas are the primary control organs (Schwartz et al., 2013).

Islet-centered glucose homeostasis is primarily driven by the level of the blood glucose level (Nirmalan & Nirmalan, 2017). An increased blood glucose level resulting from meal ingestion triggers the β-cells in the pancreatic islets of Langerhans to secrete insulin ((Szablewski, 2011;

Nirmalan & Nirmalan, 2017). Insulin secretion occurs in two phases: the first phase, an initial rapid release of preformed insulin, followed by increased insulin synthesis and release in response to blood glucose; the second phase (long term release of insulin), occurs when blood glucose remains high (Szablewski, 2011). The first phase of insulin release, also known as the cephalic phase, begins while eating a meal or anticipating a meal. This is a rapid and transient release which lasts approximately 10 minutes (Osundiji & Evans, 2013). Studies suggest that the first release of insulin is important as it determines the efficiency of subsequent meal glucose disposal (Osundiji & Evans, 2013). However, how it affects postprandial glucose disposal is unknown (Osundiji & Evans, 2013). Insulin regulates the postprandial blood glucose rise by inducing glucose disposal from the blood and increasing the blood glucose uptake of peripheral tissues such as adipose, liver, and skeletal muscle tissues (Aronoff, Berkowitz, Shreiner, & Want, 2004). Within 30-60 minutes after the beginning of a meal, insulin suppresses endogenous(hepatic) glucose production by inhibiting gluconeogenesis and glycogenolysis (Dimitriadis, Maratou, Kountouri, Board, & Lambadiari, 2021). Additionally, activated glycolysis and glucogenesis pathways further facilitate glucose transportation from the blood circulation to hepatic cells (Dimitriadis, Maratou, Kountouri, Board, & Lambadiari, 2021).

The opposite applies to the fasting state or inter-prandial state. During the fasting state, a lower blood glucose level stimulates the glucagon secretion from α-cells of the pancreatic islets (Aronoff, Berkowitz, Shreiner, & Want, 2004). Glucagon promotes hepatic glucose production through glycogenolysis (Aronoff, Berkowitz, Shreiner, & Want, 2004). In addition to glycogenolysis, the other energy-producing metabolic pathways, such as gluconeogenesis and ketogenesis, are activated during long-term fasting (Aronoff, Berkowitz, Shreiner, & Want, 2004). Moreover, the simultaneous suppression of the intra-hepatic glycolysis and glucogenesis inhibits the further glucose uptake by hepatocytes (Aronoff, Berkowitz, Shreiner, & Want, 2004). Though islet-centered glucose homeostasis is primarily regulated by coordinated action between insulin and glucagon hormones, several other modulators take part in the glucose dynamics (Nirmalan & Nirmalan, 2017). In the gut, blood glucose stimulates the L and K entero-endocrine cells to release the glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) respectively which then increases the glucose-induced insulin secretion by binding to the specific G-protein coupled receptors in pancreatic beta cell (Nirmalan & Nirmalan, 2017). GLP-1 and GIP may be responsible for around 50% of the insulin release by pancreatic beta-cells. GLP-1 is

released within a few minutes of a meal (Nirmalan & Nirmalan, 2017). The initial release of GLP-1 is followed by a sharp decline in secretion to prevent progression to hypoglycemia (Nirmalan & Nirmalan, 2017). The other role of the GLP-1 is to inhibit glucagon secretion by pancreatic alpha cells (Nirmalan & Nirmalan, 2017). Insulin growth factor-1 (IGF-1) also known as somatomedin-c is primarily produced in the liver and a limited amount in skeletal and adipose tissue (Nirmalan & Nirmalan, 2017). It has the similar structure to insulin and mimics the activity of the insulin but is less capable than insulin (Nirmalan & Nirmalan, 2017). It aids insulin in the glucose disposal from the blood thus both having an additive effect (Nirmalan & Nirmalan, 2017).

The brain has the ability to sense and directly control nutrients such as glucose, amino acids and fatty acids as well as hormones including insulin, GLP-1 and leptin (Fujikawa, 2021). During blood circulation, when glucose levels go beyond the defined level after meal ingestion, it is sensed by the brain through afferent input to both central (e.g., arcuate nucleus–median eminence, nucleus tractus solitarius) and peripheral sensing mechanisms (Fujikawa, 2021). The central glucose-sensing mechanism occurs between the gut and brain (Fujikawa, 2021). A high level of blood glucose resulting from meal ingestion triggers the sympathetic and parasympathetic nerves present in the gut, which in turn projects the impulse to the hypothalamic region and hindbrain. Peripheral glucose sensors are in the pancreas, carotid body, portal vein and myenteric plexus (Fujikawa, 2021). Pancreatic glucose sensors directly regulate blood glucose through insulin and glucagon hormones. Other peripheral glucose sensors are connected with the brain via sympathetic afferents (Fujikawa, 2021).

In addition to the peripheral action, insulin and GLP-1 act centrally to narrow down the blood glucose within the physiological range (Fujikawa, 2021). Insulin acts centrally in the arcuate nucleus of the hypothalamus (ARH), ventral tegmental area (VTA) and dorsal vagal complex (DVC) regions of the brain (Fujikawa, 2021). Insulin action on ARH regulates the lipid metabolism (suppress lipolysis and lipogenesis in white adipose tissues and promote lipid secretion in the liver) and hepatic glucose production via POMC neurons and hepatic glucose production via AgRP and neurons in the DVC whereas insulin action in the VTA and DVC control food intake via tyrosine hydroxylase (TH) neuron and neurons in the DVC (Fujikawa, 2021). Collectively, all the actions of insulin in the hypothalamus and the hindbrain area are directly or indirectly involve in glucose regulation (Fujikawa, 2021).

The brain's ability to control blood glucose in the absence of insulin was witnessed when studies infused leptin directly into brain ventricles (Schwartz et al., 2013). Leptin reduced the increased blood glucose levels in rats with insulin-deficient diabetes (Schwartz et al., 2013). The similar action of leptin was observed with systemic administration of leptin in rodent models with insulin-deficient diabetes (Schwartz et al., 2013). Though mechanisms underpinning this effect are still under investigation, it is suggested that inhibition of hepatic glucose production, along with increased glucose uptake in tissues such as skeletal muscle, heart, and brown adipose tissue may play a role (Schwartz et al., 2013). A recent study has found that other than leptin, the gastrointestinal hormone FGF19 also has the centrally mediated glucose-lowering effect (Schwartz et al., 2013). In genetically obese, leptin-deficient ob/ob mice, administration of a single, intracerebroventricular (ICV) injection of FGF19 (gastro intestinal hormone) improved the glucose tolerance without a change in the insulin secretion or sensitivity (Schwartz et al., 2013).

2.2.2 Postprandial glycemia

Postprandial glycemia (PPG) refers to the plasma blood glucose concentrations after eating a major meal (American Diabetes Association, 2001). It is usually measured over 2 hours of eating except for some specific conditions like gestational diabetes, where PPG is measured over a one-hour duration after a meal (American Diabetes Association, 2001). Blood glucose begins to rise 10 minutes after ingestion of a meal when the body starts to absorb carbohydrates, peaks at 60 min with the maximum level of 7.8 mmol/L, and again comes back to pre-prandial level after 2-3 hours of a meal in individuals without diabetes regardless of the persistent carbohydrate absorption which lasts up to 5-6 hours after a meal (American Diabetes Association, 2001). However, several factors such as carbohydrate quality, nutrient composition of the meal, energy density, acid levels, food form, processing and cooking methods influence the glycemic response of a meal (Shafaeizadeh, Muhardi, Henry, van de Heijning, & van der Beek, 2018). One of the conditions that result from defects in glucose homeostasis is postprandial hyperglycemia, which is defined as plasma glucose level>7.8 mmol/L1-2 hours after meal intake (Hiyoshi, Fujiwara, & Yam, 2017).

2.2.3 Significance of postprandial hyperglycemia

Postprandial glucose excursions tend to be higher and longer lasting with greater variability in patients with diabetes compared to healthy individuals ((Hiyoshi, Fujiwara, & Yam, 2017; (Monnier & Colette, 2009). Persistent hyperglycemia is an important contributor to diabetes

complications, including both macro and microvascular complications (Brownlee, 2005). Epidemiological evidence suggests that postprandial glycemia alone can significantly increase the risk of cardiovascular diseases (Gerich, 2003). For example, The Framingham offspring study discovered that for each 2.1 mmol increase in 2 hours PPG level, CVD risk increased by 12-42% independent of increased nonglycemic risk factors, fasting, or average hyperglycemia (Meigs, Nathan, D'Agostino, & Wilson, 2002).

Though hyperglycemia can occur in almost all the cells in the body, some cells, such as endothelial cells and mesangial cells, are more susceptible to hyperglycemia-induced tissue damage as they are unable to control glucose transportation (Brownlee, 2005). Hyperglycemic damage to the capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, and neurons and Schwann cells in peripheral nerves causes microvascular complications such as retinopathy, nephropathy, and neuropathy, respectively (Brownlee, 2005). Also, damage to the endothelial cells in the blood vessels leads to macrovascular disorders such as myocardial infarction, cerebral infarction, and lower extremity arteriosclerosis (Brownlee, 2005). In healthy cells, oxygen-free radicals are degraded by superoxide dismutase to hydrogen peroxide (Brownlee, 2005). In hyperglycemic cells, the body's natural antioxidant system is unable to degrade all the free radicals; thus, it damages the DNA, which activates the poly ADP-ribose polymerase (PARP), a DNA repairing enzyme (Brownlee, 2005). Activated PARP breaks the NAD^+ into Nicotinic acid (NA) and ADP ribose (Brownlee, 2005). ADP ribose modifies the glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GADPH), thus reduces its activity (Brownlee, 2005). Eventually, modified GADPH leads to the activation of the polyol pathway, increased formation of advanced glycation end products (AGEs), activation of Protein Kinase C (PKC) isomers, and activation of the hexosamine pathway through increased glycolytic intermediates (Brownlee, 2005).

The polyol pathway increases the intracellular oxidative stress by reducing the amount of reduced glutathione which is an intracellular antioxidant (Brownlee, 2005). AGE precursors damage the cell by modifying intracellular proteins which are involved in the regulation of the gene transcription, modifying extracellular molecules that affect the signaling between the cell and cellular matrix causing cellular dysfunction and modifying circulating protein in the blood such as albumin (Brownlee, 2005). Modified circulating proteins can bind to AGE receptors and activate

them. Then it produces inflammatory cytokines and growth factors which in turn cause vascular pathology (Brownlee, 2005).

The PKC activation pathway results in abnormal blood flow, vascular permeability angiogenesis, capillary occlusion, vascular occlusion, pro-inflammatory gene expression. N-Acetyl glucosamine is a product from the hexosamine pathway that modifies the transcription factors (Brownlee, 2005). Modified transcription factors result in pathological changes in gene expression such as increased expression of transforming growth factor-1 and plasminogen activator inhibitor-1 which are destructive for blood vessels (Brownlee, 2005).

2.2.4 Management of postprandial hyperglycemia

Management of postprandial glycemia helps to reduce the risk of NCDs and related complications (Brownlee, 2005). One of the ways in which this can be done is by introducing low glycemic index foods (GI). The term "Glycemic index" was originally introduced by Jenkins in his paper titled "Glycemic index of foods: a physiological basis for carbohydrate exchange" (Jenkins et al., 1981). In this study, Jenkins and colleagues studied the postprandial glycemic responses of 62 different carbohydrate sources (Jenkins et al., 1981). They calculated the glycemic index as "the area under the 2-hour blood glucose response curve for each food" and expressed it as a percentage of the area after ingesting an equivalent amount of glucose (Jenkins et al., 1981). GI is measured indirectly, as it measures the glycemic effect of the food and it is attributed to available carbohydrate in that food (Monro & Shaw, 2008). The amount of food used to measure the glycemic response doesn't need to be a usual intake or fixed amount (Monro & Shaw, 2008). Nevertheless, the amount of available carbohydrate present in the test food should be equal to the amount of the reference glucose (Monro & Shaw, 2008). Generally, 50g of carbohydrate is considered appropriate to use in the studies (Monro & Shaw, 2008).

Many studies have assessed the effect of low GI food compared to high GI food on postprandial glycemic response. Lobos and colleagues carried out a randomized controlled, crossover, single-blind clinical trial to assess the acute effect of low and high GI breakfast on the glycemic response and satiety in 10 obese subjects with T2DM under intensive insulin therapy (Lobos, Vicuña, Novik, & Vega, 2017). Their results suggested that a breakfast with a low GI produces a significantly lower glycemic response compared to a breakfast with a high GI (Lobos, Vicuña, Novik, & Vega, 2017). A recent systematic study also suggested the same (Toh, Koh, & Kim,

2019). A systematic review and meta-analysis of the study of 11 randomized control trials that compared the postprandial glycemic and insulinemic responses of dietary patterns with either a high or low GI breakfast in adults revealed that a low GI and low glycemic load (GL) is beneficial in reducing postprandial glycemic and insulinemic responses compared to high GI and High GL respectively (Toh, Koh, & Kim, 2019). Further, they added that the effect was more prominent in metabolically impaired patients than healthy individuals (Toh, Koh, & Kim, 2019).

2.3 Pulses in the regulation of appetite, blood glucose, and body weight

2.3.1 Pulses

Pulses are edible dry seeds of the legume family (Pulse Canada, 2022). They include dry peas, dry beans, chickpeas, and lentils (Pulse Canada, 2022). Pulses are known for their higher nutritional value as well as their low glycemic property (Pulse Canada, 2022). Many studies, including epidemiological, acute, and long-term studies, have evaluated the beneficial effects of pulse consumption on blood glucose and body weight management.

2.3.2 Epidemiological studies

Nutritional epidemiological studies often reveal whether there is any relationship that exists between diet and health. A systematic review and meta-analysis by Kastorini, *et al.* (2011) examined the epidemiological studies and randomized control clinical trials (n=534,906) which assessed the effect of a Mediterranean diet (comprised of fruits, vegetables, grains nuts, legumes, olive oil, fish) on metabolic syndrome (MS) and its components suggested that following Mediterranean diet helps to reduce the risk of developing MS as well as its components, including blood glucose (Kastorini et al., 2011). Similarly, another meta-analysis of 17 studies, including one clinical trial, nine prospective, and seven cross-sectional with a sample size of 136,846 participants, reported that higher compliance to the Mediterranean diet is associated with a 23% reduced risk of developing type 2 diabetes (Koloverou, Esposito, Giugliano, & Panagiotakos, 2014). The ATTICA (Greece) prospective cohort study carried out between 2001-2002 and 2011-2012 with 1,875 participants studied the effect of the Mediterranean diet on diabetes and CVD risk in subjects with impaired fasting glucose (Filippatos et al., 2016). This study observed a significant lower trend in diabetes and CVD incidence among participants with a medium and high adherence to the Mediterranean diet compared with low adherence (Filippatos et al., 2016).

Other than the studies that examined the relationship between legume consumption and appetite, blood glucose, and body weight as a part of Mediterranean diet studies, there are very few studies that examined the association specifically between pulse consumption, body weight, and blood glucose. Papanikolaou & Fulgoni (2008) examined the association between bean consumption and several physiological parameters and nutrient intake in adults using the American National Health and Nutrition Examination Survey data between1999 and 2002. They reported that compared with non-bean consumers, young adults who consumed a variety of beans and/or baked beans had lower body weight, a lower BMI, and a reduced waist circumference (Papanikolaou & Fulgoni, 2008). They further added that young adult bean consumers possess a 23% lower risk of having a larger waist size and 22% lower risk of being obese (Papanikolaou & Fulgoni, 2008). Interestingly, when they analyzed the baked beans separately, there was no significant difference between baked bean consumers and non-bean consumers in body weight, BMI, or waist circumference (Papanikolaou & Fulgoni, 2008). Also, there was no significant difference in risk of being obese or risk for increased waist size between the two groups (Papanikolaou & Fulgoni, 2008).

Frankenfeld and Wellace (2020) compared the diet and biomarkers of the health of hummus and chickpea consumers versus non-consumers using data between 2005 and 2016 from the National Health and Nutrition Examination cross-sectional study (Frankenfeld & Wallace, 2020). The dietary information was collected two times using a 24-hr recall during the study period (Frankenfeld & Wallace, 2020). The results showed that hummus and chickpea consumers have a lower incidence of MS, a decreased BMI, and a decreased waist circumference (Frankenfeld & Wallace, 2020). As supported by the other observations, hummus and chickpea consumption is also likely a marker of a healthier diet and lifestyle habits (Frankenfeld & Wallace, 2020).

2.3.3 Long term studies

The following studies evaluated the chronic consumption of pulses on blood glucose response.

A randomized controlled trial with 18 months follow-up with more than 100 healthy male and female volunteers revealed that incorporation of 4 servings of whole grains and 2 servings of pulses daily into a weight loss diet led to a greater reduction of waist circumference compared to the control group though there was no difference in body weight (Venn et al., 2010).

In another study, pulse consumption (five cups per week over eight weeks) was effective as an energy restriction diet in terms of lowering the metabolic syndrome risk factors such as waist

circumference, energy intake, glycosylated hemoglobin (HbA1c), glucose AUC and homeostasis model of insulin resistance (HOMA-IR) in overweight (n=19) and obese adults (n=21) (Mollard et al., 2012). Also, pulse consumption led to 5.4% of HbA1c reduction, whereas the energy restriction diet only reduced the HbA1c by 0.9% (Mollard et al., 2012). A similar effect was observed in type II diabetic patients (n=121) in a randomized parallel study (Jenkins et al., 2012). In that study, the consumption of legumes, including beans, chickpeas, and lentils as a part of a low GI diet (2 servings/ 190g per day) per day for 12 weeks, led to a 0.2 % greater reduction in HbA1c compared to a high wheat fibre diet (Jenkins et al., 2012).

Better glycemic response and increased satiation was also reported in some studies with navy beans and chickpeas alone. In 2015, Luhovyy et al., reported that consuming five cups of canned navy beans per week for four weeks reduced the metabolic risk factors related to obesity in overweight and obese adults (n=14) (Luhovyy, et al., 2015). Males had a 2.1cm reduction in waist circumference, while in females it was reduced by 2.5cm. They also observed a trend of decreased blood glucose AUC (Luhovyy et al., 2015). In another study, increased perceived satiation and reduced consumption of unhealthy high energy low-fibre snack foods were reported when following chickpea supplemented diet (104g/day) for 12 weeks in an ordered crossover design consisting of males (n=13) and females (n=29) (Murty, Pittaway, & Ball, 2010).

However, results are equivocal as Cryne, et al. (2012) carried out a randomized crossover study to investigate the effect of chickpeas, lentils, and peas on markers of CVD risk and glycemic control in healthy males (n=21) and found no significant results (Cryne et al., 2012). Participants had one of four treatments, including 100 g of spray-dried chickpeas, lentils, and 100 g of spray-dried chickpeas, lentils, peas, and 50 g of dehydrated potato flakes (control) for 28 days each, separated by 28-day washout periods. Consumption of chickpea, lentil, or pea for 28 days did not significantly affect serum lipids, homocysteine, or glycemic parameters (Cryne et al., 2012). There were several factors which were assumed to lead to the controversial results. This study was done on healthy individuals and each treatment was conducted for only 28 days (Cryne et al., 2012). The study duration may not have been long enough to improve/detect any changes in the glycemic response in healthy individuals. Also, the baseline data shows that the participants had normal fasting blood glucose levels at baseline (4.80 ± 0.24 mmol/l) and that might have made it hard to observe the improvement in fasting blood glucose (Cryne et al., 2012). Further, this study used the

pulse flour manufactured using a novel technique "spray drying" (Cryne et al., 2012). Spray drying might have changed the functional properties of the pulse flour relative to other pulse processing methods such as canning, heating, blending, or baking used in previous studies. Therefore, further research is required in this area to see if a larger dose, a different form, different processing method, different study group (individual risk for diabetes or study participant with diabetes), or longer duration would be beneficial for glycemic measures in healthy individuals or/and in individuals with diabetes

2.3.4 Acute studies

Many studies have investigated the acute effect of consumption of whole pulses either alone or as a meal on blood glucose, appetite, and food intake in healthy individuals as well as in people with disease conditions such as diabetes.

Jenkins, et al. (1982) conducted the first study on pulses (lentils) and glycemic status at the University of Toronto in 1982 (Jenkins et al., 1982). In this study, healthy volunteers (n=7) had either one of the four breakfasts including boiled whole lentils (226g), whole meal bread (280g), 1/4th whole meal bread (70g), and a slow breakfast (280g whole meal bread eaten slowly over 4 hours) (Jenkins et al., 1982). All meals were served with cherry tomatoes, cottage cheese or butter, and tea (Jenkins et al., 1982). A standard whole meal bread lunch was provided 2 hours after the treatment (Jenkins et al., 1982). The results of the study revealed that the glucose area under the curve (AUC) after breakfast was significantly lower with the lentils, 70g whole meal bread, and slow wholemeal bread breakfast compared to the wholemeal bread breakfast (Jenkins et al., 1982). Post-lunch blood glucose AUC was significantly lower for the lentil and 70g wholemeal bread breakfast, but not the slow whole meal bread breakfast compared to the wholemeal bread breakfast (Jenkins et al., 1982) The author concluded that the slow-release nature of lentils is responsible for this effect, not the carbohydrate malabsorption after conducting breath hydrogen studies (Jenkins et al., 1982).

Following that study, Wong, et al. (2009) performed three different randomized repeated measures experiments to examine the dependence of processing, recipe, and pulse variety on blood glucose response, satiety, and food intake in healthy young men (n=14) (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Experiment 1 compared canned navy beans, homemade navy beans and a 300mL glucose drink, each with 50g available carbohydrate. They found that food intake at a pizza

meal was lower for canned navy beans compared to glucose drink but not lower than homemade navy beans (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). However, there was no effect of treatment on cumulative food intake (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Homemade navy beans resulted in the lowest appetite scores over 2 hours, followed by the canned navy beans (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). However, there was no difference between canned navy beans on appetite scores (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). All bean treatments elicited a lower glycemic response at all time points except 90 min and 120 min than the glucose drink and net glucose AUC was lower for all treatment compared to the glucose drink (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009).

Experiment 2 assessed similar outcomes resulting from consumption of canned navy beans in tomato sauce, maple style, with pork and molasses, and homemade navy beans with pork and molasses and white bread, each containing 50 g of available carbohydrate (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). They reported that there was no effect of treatment on food intake or appetite net AUC (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Canned navy beans with pork and molasses and navy beans maple style resulted in higher blood glucose levels than white bread at 45 and 60 min (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Homemade beans resulted in the lowest blood glucose concentrations at 15, 30, and 45 min (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Higher blood glucose concentrations during the last hour were found after white bread compared with all bean treatments (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Navy beans in tomato sauce resulted in intermediate concentrations between homemade and the other treatments (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009).

Experiment 3 evaluated the effect of different types of equicaloric (300kcal) pulse foods that included chickpeas (341g), lentils (451g), navy beans (359g), and yellow peas (491g) compared to white bread (235g) and a water control on food intake on subjective appetite and glycemic response in healthy men (n= 15) (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009)They reported that there was no difference in food intake among pulses and water control (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). However, the treatment with chickpeas resulted in a slightly higher food intake at 2 hours compared to other pulses and the water control (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Regarding appetite, all treatments (chickpeas, lentils, navy beans, yellow peas, and white bread) lowered average appetite net AUC compared

with water (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Lentils and chickpeas resulted in the lowest blood glucose concentrations over the first hour compared with the other pulses (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009). Lentils and navy beans resulted in the lowest and highest AUC, respectively, while yellow peas and chickpeas were intermediate (Wong, Mollard, Zafar, Luhovyy, & Anderson, 2009).

Mollard, et al. (2011) and colleagues conducted another randomized repeated–measures study to further determine the effect of pulses that include chickpeas (222.8g), yellow peas (375.6g), navy beans (240.5), lentils (332.9) with nearly 50g of available carbohydrate on blood glucose (BG) and appetite following a fixed-size meal two hours later in healthy young men (n=15) (Mollard, Wong, Luhovyy, & Anderson, 2011). The study reported that consumption of chickpeas, lentils, and navy beans resulted in lower BG net AUC before the pizza meal compared with white bread (Mollard, Wong, Luhovyy, & Anderson, 2011). However, only lentils reduced the post pizza meal blood glucose AUC (Mollard, Wong, Luhovyy, & Anderson, 2011). Chickpeas, lentils, and navy beans resulted in lower BG net AUC (Mollard, Wong, Luhovyy, & Anderson, 2011). Regarding appetite, only lentils reduced the appetite AUC after the treatment compared to white bread control (Mollard, Wong, Luhovyy, & Anderson, 2011). However, this effect was not continued following the later meal (Mollard, Wong, Luhovyy, & Anderson, 2011).

Pulses are often consumed as a part of the meal. Therefore, the same group of researchers studied the effect of pulses when consuming as a meal on blood glucose, appetite, and food intake (Mollard, Zykus, et al., 2011). A within-subject, balanced, repeated-measures study was conducted in young healthy males (n= 15) to determine the effects of pulse meal on food intake, appetite, and blood glucose before and after test meal and on food intake at the test meal (Mollard, Zykus, et al., 2011). The results of the study revealed that by replacing 44% of the energy of high glycemic carbohydrate meals (Pasta with tomato sauce) with a different type of pulse (chickpeas, lentils, navy beans, and yellow peas) which reflects the regular diet, pulses lowered the blood glucose after ad libitum intake regardless of pulse types but the reduction of blood glucose after a second meal was dependent on the pulse type compared to the pulse-free meal (Mollard, Zykus, et al., 2011). Concerning food intake and satiety, all pulse types lowered the food intake at that meal but there was no effect on satiety following the first or second meal (Mollard, Zykus, et al., 2011).

Schafer, et al. (2003) also studied the effect of the pulse consumption in a mixed meal in type II diabetes patients (Schäfer, Schenk, Ritzel, Ramadori, & Leonhardt, 2003). They found that consumption of 36g of carbohydrate from dried peas in a mixed meal produced significantly lower glycemic and insulinemic responses compared to a similar amount of carbohydrate from potato in a mixed meal in type 2 diabetic patients (Schäfer, Schenk, Ritzel, Ramadori, & Leonhardt, 2003).

2.4 Lentils

2.4.1 Definition

Lentils (*Lens culinaris)* are edible, dried seeds that belong to the family *Leguminosae* (Cokkizgin, 2013). They are originally from southwestern Asia and the Mediterranean region (Cokkizgin, 2013). They have been in the human diet since prehistoric times and remains as a staple diet in Indian and Middle eastern diets (Siva et al., 2017). Now, it is commercially cultivated globally and is recognized as one of the popular cuisines around the world (Siva et al., 2017).

2.4.2 Chemical composition of lentils

Lentils are a nutritious food, rich in protein and dietary fiber, high in carbohydrate and low in fat.

Table 2.1 Nutrition composition of raw and cooked lentils

	Dry raw lentils(100g)		Cooked lentils (1 cup)	
Energy	352Kcal		243 Kcal	
Protein	24.6g		18.9g	
Total Fat	1.1g		0.8g	
Carbohydrate	63.4g		42.1g	
Dietary Fibre	10.7g		8.9g	
Total Sugar	2.0g		3.8g	
Iron	6.5mg	46%DV	7.0mg	50%DV
Magnesium	47mg	11.2%DV	75mg	18%DV
Zinc	3.3mg	30%DV	2.7mg	25%DV
Potassium	677mg	14%DV	772mg	16%DV
Folate	479µg	120%DV	379 µg	95%DV

All values are taken from the Canadian Nutrient file (Health Canada, 2012); %DV- Percent Daily Value

2.4.2.1 Proteins

Lentils are an important source of protein. They have a high nutritional value with good leucine/Isoleucine and leucine/lysine ratios (Jarpa-Parra, 2017). The downside of lentil protein is

less availability of sulfur-containing amino acids such as cysteine and methionine (Jarpa-Parra, 2017). That is why it is always emphasized to consume grains and legumes together to compensate for the missing amino acids from each other (Jarpa-Parra, 2017). Most of the protein in lentils is present as storage protein which is in the cotyledon part of the lentil seed (Jarpa-Parra, 2017). Lentils contain 16% albumins (enzymatic proteins, protease inhibitors, amylase inhibitors, and lectins), 70% globulins, and 3% prolamins (Jarpa-Parra, 2017; Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). Storage proteins are biologically active and generally considered anti-nutritional compounds (Jenkins et al., 1982). Anti-nutritional compounds such as trypsin and chymotrypsin inhibitors may affect protein digestibility, however, they are generally destroyed during processing (Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). Bowman-Birk type trypsin–chymotrypsin inhibitor (BBI) is present at higher concentrations compared to other plant families and tissue (Faris, Takruri, & Issa, 2012). The protein portion of the nitrogen is high in lentils which accounts for nearly 89% of total nitrogen present in lentils (Faris, Takruri, & Issa, 2012). Thus, lentils have the potential to be used as edible protein sources in food industries (Faris, Takruri, & Issa, 2012).

2.4.2.2 Carbohydrates

Most of the carbohydrate in the lentils is present as starch (47.1%) (Faris, Takruri, & Issa, 2012). The starch in lentils is mainly distributed in the cotyledons, dispersed in a protein matrix, and exists in granular form (Faris, Takruri, & Issa, 2012). Nutrients which are inaccessible for digestive enzymes are called “colonic nutrients” or “prebiotics” (Siva et al., 2017). Sugar alcohols and dietary fiber are examples of prebiotic carbohydrates (Siva et al., 2017). Lentils are rich in sugar alcohols. The average concentrations of sorbitol and mannitol present in lentils are 1126–1392 mg/100 g and 45–69 mg/100 g, respectively (Siva et al., 2017). Lentils are a valuable source of dietary fibre with insoluble dietary fibre occupying the most (93-99.7%) of the dietary fibre present in lentil (Faris, Takruri, & Issa, 2012)**.** Resistant starch and oligosaccharides are two prominent resistant carbohydrates dietary fibre present in lentils. Lentils have around 25g of resistant starch per 100g which makes up about 48-62% of total starch (Faris, Takruri, & Issa, 2012). Oligosaccharides present in lentils belong to the alpha-galactoside or raffinose family of oligosaccharides which represents around 53% of oligosaccharides and total sugars present in lentils (Faris, Takruri, & Issa, 2012). Among these oligosaccharides, stachyose is present in the highest amount, followed by ciceritol and raffinose (Faris, Takruri, & Issa, 2012).

2.4.2.3 Fat

Lentils are low in fat which makes them low-energy food (Faris, Takruri, & Issa, 2012). Fats in lentils are comprised of 16.7% of saturated fatty acids, 23.7% of monounsaturated fatty acids, and 58.8% of polyunsaturated fatty acids 58.8% (Faris, Takruri, & Issa, 2012).

2.4.2.4 Micronutrients

Lentils are a good source of some micronutrients. Consumption of one cup of cooked lentils provides half of the daily required amount of iron and almost satisfies the daily value of folate (Health Canada, 2012). Though lentils contain a considerable amount of iron, bioavailability could be affected by chelating agents present in the lentils (Faris, Takruri, & Issa, 2012). However, chelating agents can be minimized by cooking, germination, and fermentation of the lentils before consumption (Faris, Takruri, & Issa, 2012). Lentils also provides 18% and 25% daily value of magnesium and zinc, respectively (Health Canada, 2012). Lentils are also a good source of B vitamins, including thiamin and riboflavin (Faris, Takruri, & Issa, 2012).

2.4.2.5 Non-nutritional compounds

There are many anti-nutritional compounds present in lentils. Lentils are a natural source of phytosterols, lectins (biologically active or glycol proteins), saponins (25mg/100g), phytic acid, and its related phytates and polyphenolic compounds (Faris, Takruri, & Issa, 2012).

Higher antioxidant capacity (Ferric Reducing Antioxidant Power (FRAP) and Total-radical trapping Antioxidant Capacity (TRAP) of lentils has been proved in several studies (Faris, Takruri, & Issa, 2012). Lentil became the second-highest antioxidant potential when measuring as Trolox equivalent antioxidant capacity (TEAC). Based on USDA, lentils have a higher ORAC value than most common fruits and vegetables, including apples, plums, blackberries, cherries, figs, peaches, pears, oranges, garlic, cabbage, and almonds (Faris, Takruri, & Issa, 2012).

Compared to other pulses, lentils have the highest amounts of simple polyphenols, total phenolic compounds with the total TPC amount of 26mg gallic acid equivalent (GAE)/100g FM (Faris, Takruri, & Issa, 2012). The highest antioxidant capacity of the lentils could be coupled with a higher number of phenolic compounds present in the lentils. Seed coat and cotyledon differ in their phenolic compounds (Faris, Takruri, & Issa, 2012). The cotyledon has nonflavonoid phenolic compounds such as free and combined hydroxybenzoic and hydroxycinnamic acids while, the seed

coat has tannins, trans-resveratrol-3-glucoside, and proanthocyanins (Faris, Takruri, & Issa, 2012). Major phenolic acids present in the lentils are tannins and tannin-related compounds (Faris, Takruri, & Issa, 2012). 100g of lentil provides around 915mg of tannin (Faris, Takruri, & Issa, 2012). Catechin glucosides, procyanidin dimers, quercetin glycoside, and trans p coumaric acid-dominant compounds are present in green lentils, while quercetin diglycoside, catechin, gallate procyanidin, p dihydroxy benzoic acid are the dominant phenolics are present in red lentils (Faris, Takruri, & Issa, 2012).

2.4.3 Lentil production in Canada

Canada started its lentil production in the 1970s, and now it has become one of the major lentil producers and leading exporters in the world (Lentils.org, 2015). The lentil varieties produced in Canada are whole green lentils, whole red lentils, split red lentils, French green lentils, peppery, and braised beluga lentils (Lentils.org, 2015). The more common varieties of lentils are large green lentils and split red lentils and French green lentils, beluga lentils are lesser common varieties (Lentils.org, 2015). In 2020, the lentil production was about 2.2Kt and was expected to increase to 2.48 Kt in 2021 (Agriculture and Agri-Food, 2022). The province of Saskatchewan is the largest lentil producer, responsible for 90% of production, followed by Alberta and Manitoba (Agriculture and Agri-Food, 2022).

2.4.4 Lentil consumption in Canada

Pulse, specifically lentil, consumption is low among Canadians (Ipsos, 2010). The more frequent consumption of beans and less frequent consumption of lentils indicates that Canadians' pulse consumption depends on the variety (Ipsos, 2010). In a survey, 20 % of Canadians aged 18 years and older didn’t consume any type of pulses at home or restaurant for the past six months. Only 60% consumed one or more types of pulses, either one to three times per month or less than once per month for the past 6 months. Only 20% of adults reported weekly consumption of at least one type of pulses (Ipsos, 2010). Among the reported pulse consumers, the weekly cooked pulse consumption was one cup when all Canadians were considered (Ipsos, 2010). Consumption was dependent on the type of pulse with the highest consumption level found for beans and lowest for lentils (Ipsos, 2010).

A study by Mudry, et al., (2012) reported that on any given day, 13 % of Canadians consume pulses, with the highest consumption among the Asian population (Mudryj et al., 2012). People

who ate pulses had a higher intake of carbohydrates, fibre, and protein (Mudryj et al., 2012). Also, the micronutrient intake of pulse consumers improved as only a few individuals estimated the average requirement for thiamin, vitamin B_6, folate, Fe, Mg, P, and Zn were below the average (Mudryj et al., 2012).

In another survey conducted with caregivers about their lentil consumption, more than half of the respondents (58%) mentioned that they never or rarely consumed lentils (Phillips, Zello, Chilibeck, & Vandenberg, 2015). The barriers included the belief that the family wouldn't accept the lentils as well as lack of knowledge around the cooking of lentils (Phillips, Zello, Chilibeck, & Vandenberg, 2015).

2.4.5 Challenges in reformulation of food products with lentils

There are many lentil products available in Canada as well as around the world. Examples of those products are lentil chips, pasta, lentil soup, lentil soup mix, sprouted lentils, lentil crackers, ready-to-eat lentils, rice, and lentil cake mix, and roasted lentils. Some products targeted for babies include lentil and chickpea puff, lentil and chickpea rusks, lentil puree, lentil snacks, and lentil crisps. Also, the lentil bowl is available as a frozen food.

Food Scientists have been trying to develop baked goods from lentils which have similar sensory qualities to their wheat-containing traditional food products. Lentil based products are produced through various processing methods such as extrusion, baking, and deep frying (Bresciani & Marti, 2019). Each process affects the sensory acceptability, nutritional composition, functional properties, and biological potential in a positive or negative way (Bresciani & Marti, 2019).

The process of baking often involves mixing the dry ingredients, adding water to make a dough, letting the dough ferment, shaping it, baking it, and then cooling it (Bresciani & Marti, 2019). Snack bars, cookies, biscuits, bread, and chips are produced through baking. The major challenge reported in studies in making bread using pulses is lack of gluten present in the pulses as gluten plays a major role in structure of the bread (Bresciani & Marti, 2019). The other barrier is insufficient interactions between pulse and wheat flour protein produces bread with poor crumb structure, color and texture as the weaker interactions reduce the formation of viscoelastic dough and affect air incorporation and gas retention during leavening (Bresciani & Marti, 2019).

However, the above-mentioned barriers could be overcome by adding suitable ingredients such as vital gluten and hydrocolloids or emulsifiers to the recipe (Bresciani & Marti, 2019). Portman et

al., (2018) reported that with the inclusion of gluten, it is possible to obtain the best loaf and crumb quality when baking with wheat-lentil flour at minimum concentrations of up to 5% or up to 20% (Portman et al., 2018). In recent study, Marchini, et al. (2021) reported that regardless of lentil flour particle size, a 10% substitution level of wheat flour with lentil flour offers the best rheological qualities, although at higher substitution levels (15–30%), a coarse fraction can perform better than unfractionated flour and finer fractions (Marchini et al., 2021).

The other common baked good reformulated by researchers was cake. de la Hera, et al., (2012) investigated how the addition of lentil flour with two different particle sizes (fine and coarse) to layer cakes and sponge cakes affects the qualities of the batter and the finished product (de la Hera, Ruiz-París, Oliete, & Gómez, 2012). Wheat flour was substituted at 0%, 50% and 100% level by each type of lentil flour (de la Hera, Ruiz-París, Oliete, & Gómez, 2012). Inclusion of lentil flour resulted in the reduced density of layer-cake batter, lower layer-cake volume, symmetry index, cohesiveness and springiness and increased hardness (de la Hera, Ruiz-París, Oliete, & Gómez, 2012). There were no noticeable changes in volume or symmetry index, but the total replacement of wheat flour with lentil flour increased the hardness and reduced the cohesiveness in sponge cake (de la Hera, Ruiz-París, Oliete, & Gómez, 2012). Also, increased cake volume and reduced hardness was observed in sponge cakes made from lentil four with fine particle size (de la Hera, Ruiz-París, Oliete, & Gómez, 2012).

In contrast, developing cookies and biscuits by incorporating lentil flour was not as challenging as bread making as the cookie making does not require gluten formation or an elastic nature of the dough (Bresciani & Marti, 2019). Substitution of two types of lentil flour (coarse and fine) at 25%, 50%, 75% and 100% level with wheat flour in a traditional cookie recipe showed the possibility of partial or complete replacement of lentil flour with desirable qualities in cookies (Zucco, Borsuk, & Arntfield, 2011). Further, the authors reported that fine and coarse flour cookies affected the physical characteristics such as hardness, thickness and spread factor of the cookie in an opposite way (Zucco, Borsuk, & Arntfield, 2011). The observed challenge with inclusion of lentil flour resulted in sticky and less spread dough, but the addition of lentil flour gave desirable features to the cookies, such as increased hardness (Zucco, Borsuk, & Arntfield, 2011). Another food product that developers had success with was not only partial replacement but also the complete substitution in crackers (Malcolmson, Boux, Bellido, & Frohlich, 2013). Crackers made

from lentils were superior to the control in terms of color, texture, and taste (Malcolmson, Boux, Bellido, & Frohlich, 2013).

Product developers not only research on substituting lentil flour for wheat flour but also, been trying to study the possibility of creating novel food products with lentils. Jeske, et al., (2019) tried to produce plant-based milk using lentils with similar nutritional and sensory qualities to cow's milk (Jeske, Bez, Arendt, & Zannini, 2019). The lentil-based milk alternative formulated in this study had textural and organoleptic profiles similar to commercial plant-based milk substitutes (Jeske, Bez, Arendt, & Zannini, 2019). The authors further added that lentil protein isolates have high potential to be used formulating milk substitutes with a high-protein content, similar to cow's milk (Jeske, Bez, Arendt, & Zannini, 2019).

Besides the technological barriers, the sensory profile of lentils, especially the nutty flavor of the lentils is not suitable for the for all food products. During the reformulation of a food product the macronutrient composition should be adjusted carefully as inappropriate mixing could affect the sensory profile of the finished good negatively. For an instance, though higher dietary fibre content of a food is regarded as a good feature it may affect product attributes like texture, flavour, colour, and appearance negatively (Escobedo & Mojica, 2021). Despite the fact that the lack of dietary fat in lentils is a nutritionally desirable feature, a moderate fat concentration could enhance functional qualities and sensory acceptability of the product. Thus, it creates the need to add external fat such as animal fat which may increase the production cost and reduce health benefits (Escobedo & Mojica, 2021).

2.4.6 Potential health benefits of lentils

The health benefits of lentils are attached to nutritional (dietary fibre, protein, vitamins, and minerals) and antinutritional compounds (enzyme inhibitors and phenolic compounds) present in the lentils.

The high protein content of the lentils makes it as a potential source of protein for all stages of human life, especially for malnourished children and elderly people who have higher protein requirements (Alberta Pulse Growers, 2022; Hossain, Islam, Wahed, Khatun, & Kabir, 2009). Lentils are considered as an important source of protein for vegans who solely depend on plant protein sources and vegetarians who depend largely on plant protein sources (Alberta Pulse Growers, 2022). The gluten-free nature of the lentils makes it an option for the gluten-free diet

(Alberta Pulse Growers, 2022). Adding lentils to the gluten-free diet enhances the nutritional quality of the gluten-free diet (Alberta Pulse Growers, 2022).

Individuals with noncommunicable diseases (diabetes, obesity, cardiovascular disease, and cancer) are advised to add pulses, including lentils into their diet (Lentils.org, 2015). The dietary fibre present in the lentils lowers the rate and extent of starch digestibility by slowing down gastric emptying (Rebello, Greenway, & Finley, 2014). The higher amount of amylose than amylopectin is related to the higher amount of resistant starch which is inaccessible to digestive enzymes (Rebello, Greenway, & Finley, 2014). Biological peptides formed from protein digestion enhance the release of gut peptide hormones such as CCK, glucagon-like peptide (GLP 1), peptide YY (PYY), which delays gastric emptying by regulating the pyloric pressure and gastric motility (47) thus affecting the food intake and blood glucose control. Also, dietary proteins suppress the release of ghrelin which stimulates gut motility (Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). Postprandial elevation of amino acids increases both insulin and glucagon. Thus, it alters hepatic glucose metabolism by changing the insulin and glucagon ratio (Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). The presence of protease inhibitors also slows down gastric emptying and thus lowers the plasma glucose and insulin concentration in the blood (Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). Phytates may reduce the starch digestibility and lower the postprandial glucose response (Rebello, Greenway, & Finley, 2014). Amylase inhibitors present in the lentils also prevent starch digestibility. All the mentioned components present in lentils are working collectively to contributes to the benefits for acute and long-term blood glucose control. Association between lentil consumption and increased satiety and reduced food intake are also reported in many studies (Dastgiri, Mahdavi, TuTunchi, & Faramarzi, 2006; Siva et al., 2018).

A cross-sectional study performed with 300 men and women revealed that legume consumption, including lentils, reduces the risk of obesity (Dastgiri, Mahdavi, TuTunchi, & Faramarzi, 2006). In an animal study, rats fed with a lentil-based diet had significantly decreased body weight and reduced body fat percentage and pathogenic gut bacteria compared to the control corn diet (Siva et al., 2018). Though it's not clear yet which components available in lentils are responsible for weight loss, it has been suggested that high fibre and protein content of lentils may act as weight loss aids by promoting satiety, decreasing energy intake, and increasing energy expenditure (Rebello, Greenway, & Finley, 2014a). Indigestible carbohydrates, including fibre and resistant

starch, reduce energy intake by being inaccessible to digestive enzymes. Protein enhances energy expenditure through diet-induced thermogenesis (Rebello, Greenway, & Finley, 2014a). The slow gastric emptying effect of fibre enhances the nutrient and gastric wall interaction which stimulates the release of appetite-regulating hormones (Rebello, Greenway, & Finley, 2014a).

Several animal and human studies have explored the beneficial effects of lentil consumption on cardiovascular risk factors and the underlying mechanisms. A randomized cross-over clinical trial conducted with type II diabetic patients found that consuming 50g cooked lentil and 6g canola oil for 6 weeks significantly reduced the cholesterol level compared to control 30g bread and 20g cheese break first meal (Shams et al., 2008). In an animal study, cooked whole lentil significantly increased HDL compared to raw whole lentil and raw dehulled lentil but not compared with control (Barbana, Boucher, & Boye, 2011). There was no effect on other components of lipid profile such as LDL, total cholesterol, and triglycerides (Barbana, Boucher, & Boye, 2011). There was no difference between cooked whole lentil and cooked dehulled lentil in terms of HDL (Barbana, Boucher, & Boye, 2011). Consumption of legumes including beans, chickpeas, and lentils as a part of a low GI diet (2 servings/ 190g per day) per day for 12 weeks improved glycemic control and reduced the CHD risk in type II diabetic patients (Jenkins et al., 2012).

The protective effect of lentils against cardiovascular could be related to dietary fiber, protein, phytosterols, folate, and ACE inhibitors present in the lentils (Barbana, Boucher, & Boye, 2011). The lowering cholesterol effect of protein could be related to its bile acid-binding capacity and hydrophobicity (Jahan-Mihan, Luhovyy, El Khoury, & Anderson, 2011). Barbana, et al. (2011) reported the bile acid-binding capacity of red and green lentil varieties. Also, they further reported that there is no difference between red and green lentil varieties. Further, they also mentioned that the bile acid-binding capacity of lentils could be relatable to fiber and protein present in the lentil (Barbana, Boucher, & Boye, 2011). The same study group reported the ACE inhibitory activity of lentil (Barbana & Boye, 2011). In contrast, an animal study which compared the hypercholesterolemic effect of different types of pulses revealed that different types of pulses possess hypocholesterolemia effect in different degrees and that effect is not related to the concentration of fecal bile acids or neutral sterols (Dabai et al., 1996).

Folate rich diet is effective as folate capsules in reducing plasma homocysteine levels in male patients with hypercholesterolemia as daily consumption of folate-rich foods including 50 g of

lentils combined with other folate-rich foods at a level of 500 µg folate reduced homocysteine level by 8.6% (Pinto et al, 2005). Therefore, the cardiovascular disease risk lowering effect of lentils could be attributed to higher folate levels which help in reducing homocysteine levels in the body. However, recent studies concluded that there is no causal relationship between plasma homocysteine level and coronary heart disease or acute myocardial infarction (Miao et al., 2019).

Several epidemiological and case-control studies have shown the positive association between lentil consumption and different type of cancers. A prospective cohort study conducted between 1976 and 1983 observed that lentil consumption is protective against pancreatic cancer in 34,000 California Seventh-day Adventists (Mills, Beeson, Abbey, Fraser, & Phillips, 1988). The same research group reported the negative association between lentil consumption and prostate cancer from a cohort study with 78,000 Adventist men. (Mills rt al., 1989). A case-control study by Jain et al., (1999) confirmed the same (Jain, Hislop, Howe, & Ghadirian, 1999). Consumption of beans or lentils has shown an inverse association with breast cancer risk in a study conducted with 90,630 women compared to other flavanol-rich sources such as tea, onions, apples, string beans, broccoli, green pepper, and blueberries (Adebamowo et al., 2005). Lentils are considered as one of the cancer-preventive foods in Turkey (Akcicek, Otles, & Esiyok, 2005). In a case-control study, legume consumption including lentils was inversely associated with colorectal cancer risk in African- American men and women (Agurs-Collins, Smoot, Afful, Makambi, & Adams-Campbell, 2006). A multisite case-control study carried out between 1996 and 2004 reported that increased intake of lentils lowers the risk of developing several types of cancers related to the digestive tract such as oral cavity and pharynx, esophagus, larynx, stomach, colorectum, and kidney (Aune et al., 2009). There was no significant association between legume consumption and breast, lung, prostate, and bladder cancers (Aune et al., 2009). In a prospective cohort study, lentil consumption was associated with lower cancer risk mortality in adults aged between 55-80 years (Papandreou et al., 2019). An animal study conducted to compare the cancer-preventing ability of raw and cooked lentils revealed that lentils could be protective against colon cancer and hydrothermal treatment of the whole lentil improved the chemo preventive potential further (Faris, Takruri, Shomaf, & Bustanji, 2009).

The chemo preventive ability of the lentils could be attributed to several important components, including fibre, folic acid, defensin, phytates, a protease inhibitor (Bowman-Birk type trypsin–

chymotrypsin inhibitor), lectin, and phenolic compounds such as flavonoids (flavanones, flavan-3-nols, flavones, flavanols, anthocyanidins, tannins and proanthocyanins) (Faris, Takruri, Shomaf, & Bustanji, 2009; Ganesan & Xu, 2017)**.** Plausible mechanisms responsible for the chemo preventive ability of the lentils have been suggested by researchers. Polyphenols may act through involvement in uptake of carcinogens, detoxification, DNA repair, and binding to ribosomes, thus inhibit protein synthesis (Ganesan & Xu, 2017). Lectins could prevent tumor growth by binding to cancer cell membranes or receptors, thereby causing cytotoxicity, apoptosis, and autophagy (Ganesan & Xu, 2017). Kahlon et al., (2014) suggested that secondary bile acid is a carcinogen, and therefore, removing bile acid from the body is one way to reduce the risk of cancer development (Kahlon, Berrios, Chiu, & Pan, 2014). Studies have shown that lentils possess the bile acid-binding capacity (Kahlon, Berrios, Chiu, & Pan, 2014).

2.4.7 Pulse processing

The processing of pulses can be classified into three levels such as primary processing, secondary processing, and tertiary processing (Joshi, Timilsena, & Adhikari, 2017). Primary processing begins with the cleaning of pulses to remove unwanted organic and inorganic materials (Joshi, Timilsena, & Adhikari, 2017). This step is followed by sorting and grading to obtain pulses with desirable quality in terms of size, shape, density, and color by using series of mechanical separation (Joshi, Timilsena, & Adhikari, 2017). The second level of processing consists of decortication, splitting, sorting, and polishing of whole or split seeds (Joshi, Timilsena, & Adhikari, 2017). The next level of processing involves grinding/milling of whole or decorticated seeds and fractionation of protein and starch-rich components which can be incorporated into various food products (Joshi, Timilsena, & Adhikari, 2017).

2.4.7.1 Milling and milling techniques

Milling simply refers to "size reduction". In pulses, size reduction can be achieved through various processes such as dehulling/decortication, splitting, and flour milling (Wood & Malcolmson, 2011). Dehulling involves the removal of the seed coat (Wood & Malcolmson, 2011). Splitting cleaves the two cotyledons and produces dhal or splits (Wood & Malcolmson, 2011).Dehulling and splitting occur simultaneously in the same milling process for some pulses (Wood & Malcolmson, 2011). Flour milling/grinding produces pulse fours from whole seeds or cotyledons (Wood & Malcolmson, 2011).

Traditionally, milling was performed by hand pounding with stones or mortar and pestle and later using quern stones (Wood & Malcolmson, 2011). Milling was done at the household or community level. Eventually, saddle querns progressed to rotary and oscillatory querns (Wood & Malcolmson, 2011). These are built with a concave shaped top stone and a convex bottom stone that fit together (Wood & Malcolmson, 2011). There is a hole in the top stone through which pulse seeds are fed while the top stone is rotated by a wooden handle fixed on the top stone (Wood & Malcolmson, 2011). At the beginning of the process, it produces dehulled split pulses and can be converted to pulse flour by keep rotating it (Wood & Malcolmson, 2011). After milling, either edible oil or water is added to dehulled seeds to improve the appearance. On some occasions, a cone-type polisher or a buffing machine was used to polish the pulses (Wood & Malcolmson, 2011).

In the modern-day, attrition-type mills and carborundum roller mills are used for processing pulses (Wood & Malcolmson, 2011). Under runner disk shellers (URD Shellers) were developed using the same two stone concept of saddle quern (Wood & Malcolmson, 2011). However, carborundum coated artificial stones with various abrasive grades used as an alternative to real stones (Wood & Malcolmson, 2011). It has some additional features including using electric power instead of manual power and an adjustable gap between the stones according to the seed size (Wood & Malcolmson, 2011). Carborundum roller mills have a cylindrical carborundum stone which rotates inside a perforated metal casing (Wood & Malcolmson, 2011). The stone or casing can be moved to adjust the gap depending on the seed size. The quality of the final product depends on several factors including the abrasiveness of stones (grit), speed of stones/rollers, gap size, feed rates, and time seed is retained within the mill (Wood & Malcolmson, 2011).

2.4.7.1.1 Dry vs wet milling

Pre-treatment is done to pulses to facilitate the removal of seed coats from the cotyledon (Wood & Malcolmson, 2011). Based on the pre-treatment used, pulse milling is classified as wet vs dry milling. Wet milling differs from dry milling mainly by soaking seeds (Wood & Malcolmson, 2011). In the wet milling process, after cleaning and grading pulse seeds are soaked for 3-12 hours (Wood & Malcolmson, 2011). Soaking helps to loosen the bond between the seed coat and cotyledon (Wood & Malcolmson, 2011). Followed by soaking, seeds are dried under the sun. Alternate sun-drying and conditioning are continued for nearly 2–6 days (Wood & Malcolmson,

2011). Red earth is added after soaking to increase the rate of drying and red earth is removed through sieving before milling (Wood & Malcolmson, 2011).

In contrast, edible oil is added at a rate of ≈0.4% (w:w) before conditioning for 2–3 days followed by water at a rate of ≈10% (w: w) before tempering for 4–8h in dry milling process. The following steps such as drying, milling, and sieving are similar for both the wet and dry milling process. During milling, broken pieces and seed coats are removed by aspiration. And during sieving larger broken pieces are removed and larger seeds may put through the process again (Wood & Malcolmson, 2011). As sun drying is not feasible in western countries hot air is used using a batch bin drying technique for drying of pulses. However, inappropriate time duration and temperature of hot air heating may produce an unfavorable appearance, taste, and cooking quality (Wood & Malcolmson, 2011).

2.4.7.1.2 Industrial milling

Automotive mills are used for industrial pulse processing (Tiwari, Gowen, & McKenna, 2011). These mills are functioning based on the following milling techniques such as impact milling, attrition milling, knife/cutting milling, and direct pressure milling (Tiwari, Gowen, & McKenna, 2011). Physiochemical and functional properties of the pulse flour are affected by many factors including the type of mill used, milling conditions, and selection of screen and/ or sieves for separation of the ground material (Wood & Malcolmson, 2011; Tiwari, Gowen, & McKenna, 2011)).

Impact milling

Mills such as hammer mills, pin mills, cage mills, universal mills, and turbo mills use the impact technique to reduce the particle size of the pulses (Tiwari, Gowen, & McKenna, 2011). For instance, a Hammer mill is employed with a vertical or horizontal shaft fitted with hammer bars that spin inside of a steel drum as seeds are fed into the mill (Tiwari, Gowen, & McKenna, 2011). Seeds are reduced until it becomes the size which could be exit through the metal screener (Tiwari, Gowen, & McKenna, 2011). Another example of the impact mill is the roller mill (Wood & Malcolmson, 2011),78). In a roller mill, series of cylindrical rollers are built with an adjustable decreasing gap between subsequent rollers (Wood & Malcolmson, 2011; Tiwari, Gowen, & McKenna, 2011). The first roller does the job of removing the seed coat and crush the seed. The

following rollers allow particular size flours to pass through the screen (Wood & Malcolmson, 2011; Tiwari, Gowen, & McKenna, 2011).

Attrition milling

Attrition mills reduce the size of the pulse seeds by sheering and cutting (Tiwari, Gowen, & McKenna, 2011). Ball mill is an example of attrition milling (Tiwari, Gowen, & McKenna, 2011). In ball mills, a horizontal rotating cylinder/ vessel which is filled with balls converts the pulse seeds into small free-flowing spherical particles (Tiwari, Gowen, & McKenna, 2011). It can be used to produce fine particles as it can reduce the 1,000-micron (20-mesh) size to less than 1 micron (78).

Cutting/knife milling

This type of mills employs a rotating sharp blade which reduces large particles to predetermined small particles by applying a sheer force (Tiwari, Gowen, & McKenna, 2011). Examples of mills which use cutting techniques are dicing mills and guillotine mills (Tiwari, Gowen, & McKenna, 2011). It can reduce two-inch or larger chunks or slabs of material to 250 to 1,200 microns.

Direct pressure milling

Pressure milling equipment is built with two rotating bars or one rotating bar and a stationary plate that can crush the grinding material (Tiwari, Gowen, & McKenna, 2011). It can pulverize one inch or larger chunks to 800 to 1,000 microns**.** Roll mills, cracking mills, and oscillator mills are an example for pressure milling (Tiwari, Gowen, & McKenna, 2011).

Impact of processing on the characteristics of pulses

2.4.7.2 Impact of processing on pulse characteristics

Conventional pulse processing includes dehulling and milling, thermal treatments, germination, and fermentation. In addition to conventional pulse processing methods, there are some innovative methods such as extrusion, microwave heating, micronization, and irradiation are applied in industrial pulse processing (Patterson, Curran, & Der, 2017).

Soaking and dehulling

Soaking is an important step in pulse preparation. Soaking allows water to get into the legume kernels and soluble nutrients to dissolve (Patterson, Curran, & Der, 2017). Soaking temperature, time, nature of the soaking solution (water, acidic or basic), and pulse type may affect the anti-nutrient components present in the pulses (Patterson, Curran, & Der, 2017). However, in general, soaking results in lower phytate content due to its water solubility and the activation of endogenous phytase, and loss of phenolic compounds (Patterson, Curran, & Der, 2017).

Dehulling or decortication

Dehulling or decortication involves the removal of the seed coat (Patterson, Curran, & Der, 2017). As most of the phenolic compounds present in the seed coat, dehulling leads to the removal of phenolic compounds such as tannins (Patterson, Curran, & Der, 2017). Loss of antinutrient factors such as phytic acid and polyphenols which inhibit the activity of alpha-amylase may be responsible for the reduction of resistant starch after soaking and dehulling, and damage or removal of the seed coat makes starch more accessible to hydrolytic enzymes (Al-Tibi, Takruri, & Ahmad, 2010). Dehulling results in reduced cooking times, improved flour and protein quality, palatability, and digestibility (Al-Tibi, Takruri, & Ahmad, 2010). In contrast, it increases the other antinutrient concentration such as trypsin inhibitors, chymotrypsin inhibitors, and α-amylase inhibitors in the cotyledon fraction (Patterson, Curran, & Der, 2017).

Thermal treatments

Open pan cooking and pressure cooking are commonly used thermal treatments (Al-Tibi, Takruri, & Ahmad, 2010). Moisture and temperature together cause cell disruption in pressure cooking which makes starch more accessible to hydrolytic enzymes (Al-Tibi, Takruri, & Ahmad, 2010). Also, gelatinization and dispersion of starch molecules resulting from hydrothermal treatments make starch available for enzymatic digestion (Al-Tibi, Takruri, & Ahmad, 2010). Overall, thermal treatment (boiling or cooking under pressure) increases the digestibility and nutritional quality by inactivating or eliminating heat-labile anti-nutrients (Patterson, Curran, & Der, 2017; (Al-Tibi, Takruri, & Ahmad, 2010).

Extrusion

Extrusion is a complex multivariable process that is used to produce cereals, snacks, and other textured products (Patterson, Curran, & Der, 2017). It uses an appropriate level of moisture, pressure, temperature, and mechanical shear to produce a product with unique physical and chemical characteristics (Patterson, Curran, & Der, 2017). The advantages of extrusion cooking of pulses are reduced cooking time and improved textural, nutritional, and sensorial characteristics (Patterson, Curran, & Der, 2017). Extrusion type of cooking also results in starch gelatinization, breakages of amylopectin molecules into smaller digestible molecules, increased digestibility of protein, and loss of antinutritional factors which all increase the digestibility (Pasqualone, Costantini, Coldea, & Summo, 2020).

Micronization

Micronization or infrared heating is another novel technique used in pulse processing to improve their digestibility and palatability of foods and food ingredients such as precooked flours, infant foods, breakfast cereals, snacks, and modified starches (Patterson, Curran, & Der, 2017). Exposure to the 1.8-3.4μm wavelength IR waves for 2-3 min causes vibration of the pulse molecules at 60000-150000 MHz which produces rapid internal heat. It raises the temperature to 200^0c which is enough for gelatinizing the starch present in the pulses in 2-3 minutes. Then pulses are flaked and milled into powders (Anderson et al., 2014).

2.4.7.3 Studies on processed pulses

The effect of pulses on slow release of glucose was observed in healthy volunteers who had hachis paramentier (shepherd's pie) made of bean purée compared to hachis paramentier made of potato puree (Leathwood & Pollet, 1988). The second study by the same researchers revealed that in healthy volunteers who consumed six hachis parmentier (three with bean purée, three with potato purée, topped with spinach, ratatouille or tomatoes), the bean purée delayed the return of hunger and had decreased ratings for propensity to eat a tasty snack than potato puree (Leathwood & Pollet, 1988). In healthy volunteers, consumption of 50g carbohydrates either from red kidney beans or Bengal gram improved postprandial blood glucose level compared to dextrose and a similar amount of carbohydrate from wheat and rice (Dilawari, Kamath, Batta, Mukewar, & Raghavan, 1981). Hummus, a processed form of chickpeas showed four times lower glycemic

response than white bread with a similar available carbohydrate (50g) load in healthy individuals without compromising insulin levels (Augustin et al., 2015).

Eyaru and colleagues performed a study to investigate how different domestic processing techniques affect the starch digestibility of red kidney beans and peas and reported that the processing converts the resistant starch fractions to more digestible starch regardless of the type of processing technique (Eyaru, Shrestha, & Arcot, 2009). Further, they added that even after processing, legumes possess a higher amount of resistant starch compared to other starchy food sources. Thus, still legumes are beneficial in terms of lowering plasma glucose (Eyaru, Shrestha, & Arcot, 2009). Tovar, et al. (1992) studied the glycemic and insulinemic response of various red kidney bean products including boiled, autoclaved, boiled-freeze dried, milled-precooked flour, milled-steam cooked-freeze dried flour (Tovar, Granfeldt, & Bjoerck, 1992). All red kidney bean products lowered postprandial glycemic response than white bread (Tovar, Granfeldt, & Bjoerck, 1992). Among the variously processed red kidney bean products, boiled beans elicited the lowest metabolic response while milled-steam cooked freeze-dried flour showed the highest response and autoclaved and milled -pre-cooked flour showed the intermediate response (Tovar, Granfeldt, & Bjoerck, 1992).

A study comparing the physiological effects of various types of whole pulses and respective commercially produced pulse flour revealed that pulse powders possess the same physiological properties as whole pulses (Anderson et al., 2014). Further, it added that the cooking time of pulses positively correlates with the starch hydrolysis rate (Anderson et al., 2014).

Other than the type, nutritional composition, and food form, processing method also determines the starch digestibility andthus the glycemic response of the food (Singh, Manickavasagan, Shobana, & Mohan, 2020). For instance, a study by Ramdath et al., (2018) has shown that boiling and pureeing produce lower glycemic response whereas spray drying, freezing and roasting food prior to cooking lead to a higher glycemic response (Ramdath et al., 2018). It is proposed that the change in glycemic response resulting from processing could be attributable to particle size(milling) and temperature (roasting and freezing) (Ramdath et al., 2018). As a result of the possible implications of particle size in the product quality as well as in the functionality of the food product, granulometry has recently become an interesting topic which is worth exploring further (Marchini et al., 2021).

Despite being the gold standard and known for providing more accurate results, *in vivo* studies in animals and humans are expensive, invasive, time-consuming, and require special skills (Jeong, Han, Liu, & Chung, 2019). As a result, many useful alternative *in vitro* study methods have been developed and they have been extensively used to predict the glycemic response of a food (Jeong, Han, Liu, & Chung, 2019).

To add to the knowledge on how particle size affects starch digestibility, an *in vitro* study was conducted in our lab that assessed the carbohydrate digestion of raw and baked navy bean flour with various particle sizes (coarse- 1101.6 µm, regular-630.7 µm, fine-301.7 µm, very fine-144 µm, and super fine-28.6µm) (Luhovyy, Hamilton, Kathirvel, & Mustafaalsaafin, 2017). Decreasing carbohydrate digestion rate was observed with increasing particle size and baked navy bean flour also showed a similar pattern of digestion rate (Luhovyy, Hamilton, Kathirvel, & Mustafaalsaafin, 2017). However, the digestion rate difference between different particle sizes was smaller in baked navy bean flour compared to raw navy bean flours (Luhovyy, Hamilton, Kathirvel, & Mustafaalsaafin, 2017). This study shows the potential of incorporating navy bean flour into baked products without compromising the slow carbohydrate releasing effect of pulses (Luhovyy, Hamilton, Kathirvel, & Mustafaalsaafin, 2017).

As a next step to that study, our lab examined how particle size of whole laird lentil (*Lens culinaris*) flour affects the carbohydrate digestion rate (in vitro) using a modified Englyst method (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). For this study, raw, baked, and moist-heat cooked lentil flour samples were prepared using five different particle sizes of whole laird lentil flour (coarse - 1,101.6 µm, regular- 630.7 µm, fine - 301.7 µm, very fine-VF, 144 µm, and superfine- SF, 26.8 µm) (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). There was an effect of particle size of raw, baked and moist cooked lentil flour on glucose tAUC over 180 minutes (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). Total AUC was significantly higher for superfine of raw, baked and moist cooked lentil flour compared to other particle sizes (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). There was no significant difference between coarse and regular, fine and superfine raw lentil flour whereas, in baked lentil flour, there was no significant difference between coarse and regular, regular and fine, fine and very fine respectively (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). In moist cooked flour, the difference in glucose release between regular, fine and superfine wasn't significantly different (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). Further, the authors reported

the significant relationship between mean particle size and raw, baked and moist cooked lentil flours (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). The study concluded that larger particle size was associated with lesser glucose release and this feature was partially retained in baking and moist cooked samples (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019). The authors indicated that higher content of resistant starch and a lower content of RDS are present in the larger particle size flour (Kathirvel, Yamazaki, Zhu, & Luhovyy, 2019).

Similar results were reported by Mandalari et al with the wheat flour (Mandalari et al., 2016). They studied the bio accessibility of starch and protein of different wheat endosperm products varying in particle size (Mandalari et al., 2016). They observed the significant increase in glucose release with a decreasing particle size (Mandalari et al., 2016). For example, 92.16% of total glucose release was observed with a particle size of 0.11mm whereas the meal with the particle size of 1.95mm released only 47.39% of total glucose (Mandalari et al., 2016).

2.4.7.4 Use of pulse flours for substitution of wheat flour

Wheat flour is popular than any other cereal grain flour and it has been used massively in the production of a wide range of food products especially in baked goods due to its physicochemical properties (Figoni, 2004). The important feature of wheat flour is gluten formation when mixing with water (Figoni, 2004). The other preferable feature of wheat flour is its mild nutty flavour (Figoni, 2004). However, growing health concerns among consumers, and nutritional limitations such as lack of essential amino acids, low in dietary fibre, and high GI to food industries look for alternative flour for food production (Figoni, 2004). Besides, the gluten-free market is growing now due to the demand from consumers who are allergic to wheat, have celiac disease, and gluten sensitivity (Figoni, 2004). Pulses seem like a good alternative due to their rich nutrient profile and their promising health effects. Also, it's an inexpensive and sustainable ingredient. Making baked goods without gluten will cause some technical issues which in turn affects the sensory quality of the food product (Augustin et al., 2015).

Numerous studies have investigated the feasibility of substituting or incorporating pulse flour in bakery products which are usually made with wheat flour. However, only a few studies have studied the health effects resulting from the replacement of wheat flour with pulse flour in baked goods. Hall, et al. (2009) reported that substituting 10% of wheat flour with Australian sweet lupin flour in bread making lowered the glycemic index and increased the insulinemic index (50 g

available carbohydrate load), however, there was no effect on satiety or food intake at the meal after 3 hours at ad libitum in healthy individuals (Eyaru, Shrestha, & Arcot, 2009). In another study, when replacing 25% of the wheat flour with chickpea flour in pasta products produces a significantly lower glycemic response compared to regular wheat pasta products (Tovar, Granfeldt, & Bjoerck, 1992). However, when replacing 25% of whole wheat flour in bread with chickpea flour didn't show any significant difference in blood glucose response compared to whole wheat bread but with white bread (Zafar et al., 2013). Substituting 35% whole wheat flour with chickpea flour significantly reduced the blood glucose response compared to white bread and whole wheat bread (Zafar et al., 2013).

In a recent study, the substitution of wheat flour or conventional flour with mung bean flour (30%) or lentil flour (30%) or a mix of mung bean flour (15%/15%) and lentil flour in muffins with the load of 50g available carbohydrate lowered the postprandial blood glucose and food intake at later meal and improved the appetite in healthy females compared to control (Naseer et al., 2019). However, the mung bean substitution showed better results in terms of satiety, food intake, and glycemic control compared to other treatments (Naseer et al., 2019). Another recent randomized control in healthy adults assessed the effect of lentil (red and green) substitution (0.75 cups) in wheat flour muffins and rice flour chilies on satiety and food intake (Conte, 2018). It revealed that consumption of chilies made from green lentils increased satiety but there was no difference in food intake at later meal compared to control (Conte, 2018). Muffins made from either green or red lentils as a substitution for wheat didn't have any effect on neither satiety nor subsequent food intake. It shows that lentil variety and product type may influence satiety and food intake (Conte, 2018).

Conclusion:

Diet-related chronic conditions such as obesity, diabetes, cardiovascular diseases, and cancers are more prevalent among Canadians. These chronic conditions have a substantial social and economic burden. A healthy diet has the potential of preventing the development of those chronic conditions and management of chronic conditions and the relevant complications. Pulses are low-cost plant-based protein sources. Most national dietary guidelines including Health Canada recommends pulses as part of a healthy diet. Lentil (a type of pulse) consumption is associated with reduced blood glucose, increased satiety, and reduced food intake. Despite the health benefits, lentil

consumption is low among Canadians. One way of encouraging lentil consumption is increasing the availability of the ready-to-eat or ready-to-cook lentil flour food products in the market. However, milling is one of the many factors which could influence the glycemic response of the lentils. Change in glycemic response results from milling could be attributable to particle size. In vitro studies reported that glycemic response varies with particle size where the highest and lowest glycemic response were obtained for superfine and coarse flour respectively. But there is a scarcity of human studies to confirm the findings of the *in vitro* study. As such, there is a strong need for a human study to understand whether there is any effect of particle size on the glycemic response. Having a better knowledge of the implications of particle size on the functionality of the food product (glycemic response) will help to develop a high-quality functional lentil food product.

Chapter 3: Rationale, objectives, and hypothesis.

3.1 Rationale

Previous acute studies have demonstrated that consumption of lentils as a simple meal or as part of a complex meal lowers the postprandial blood glucose level compared to other high glycemic carbohydrate sources in healthy individuals as well as individuals with diabetes. In addition, processing methods such as canning, blending, and milling preserve the glycemic-lowering ability of lentils. Despite being a readily available, economical, and a potential food source for blood sugar control, lentil consumption is low among Canadians. Introducing lentil products using lentil flour with minimal cooking time is one way to increase the lentil consumption. Previous *in vitro* study reported that the extent of milling influences the glycemic response of lentils as larger particle produced lower glycemic response and smaller particle size led to higher glycemic response. With the limited information reported on particle size in studies comparing various food flours and powders, it was unknown whether the differences detected in metabolic responses were determined by a difference in particle size or composition, or both.

3.2 Objective

The objective of this study was to investigate the effect of a pizza meal formulated with either lentil flour or wheat flour of similar particle size or their combination on postprandial blood glucose, subjective appetite, physical comfort and food intake.

3.3 Hypothesis

We hypothesized that the partial or complete replacement of wheat flour with lentil flour of similar particle size in a formulated pizza would result in lower blood glucose and subjective appetite due to a higher content of resistant carbohydrates and protein in lentil flour compared to wheat flour.

Chapter 4: Methodology

4.1 Study design

The study was conducted at the Appetite lab at Mount Saint Vincent University (MSVU) and was approved by the MSVU University Research Ethics Board (Ethics file # 2018-201). Written informed consent was obtained from all the participants after explaining the procedures of the study. This study followed a within-subject, randomized, single-blind, and repeated-measure design, in which participants acteds as their own controls.

4.2 Study participant recruitment & selection

Healthy young adults 19-35 years of age with a body mass index (BMI) between 20 kg/m^2 - 24.9 kg/m^2 were recruited for the study from MSVU and surrounding communities using posters (Appendix 1), social media advertisements (Facebook and Instagram), and by word of mouth. Interested participants were invited for a telephone screening. The telephone screening was done by using a telephone screening questionnaire (Appendix 2 & 3). Individuals who had any kind of diseases, smoking, were taking medications known to influence blood glucose, did not regularly eat breakfasts, had emotional or learning problems that would affect their ability to participate in the study, had known food allergies, and/or were lactose intolerance were excluded from the study. In addition to this, females with irregular menses were also excluded from the study. Eligible participants based on inclusion criteria were given brief information about the study and were scheduled for the information session. On the information session day, the information sheet and consent form (Appendix 4) were given to participants and their height, weight and body composition were measured via bioelectrical impedance analysis using a Tanita Body Composition Analyzer TBF-300A and Stadiometer HR-200 (Tanita Corporation of America, Inc, Arlington Heights, IL). Participants who gave consent to participate in the study were scheduled for the sessions. Male participants were scheduled for four sessions with each session one week apart. Female participants were scheduled at the follicular phase of their menstrual cycle to avoid a potential effect of the menstrual phase on insulin sensitivity. Participants were asked to follow their usual diet and activity routine on the day before the study session and arrive to the lab the following day between 8-10 am with overnight fasting (12 hours).

4.3 Sample size

Twenty-four participants were recruited for the study. However, based on the data from the current study, only 14 participants are required to detect a 10% (0.49mmol/L) difference in peak blood glucose response rate with the SD of 0.6mmol/L, power of 0.8 at 95% confidence level. If the dropout rate of 20% is considered the participant requirement would be 17.

A sample size of 111 is required to find 10% difference in subjective appetite (5.08mm), with the power of 0.8 at 95% confidence level. Twenty-four participants are enough to detect only 24% difference in subjective appetite (12.7mm), with a power of 0.80 at 95% confidence level.

With respect to food intake, sample size of 24 is enough to detect a treatment effect of food intake of 154kcal with the SD=258.1, power of 0.8 at 95% confidence level.

4.4 Treatments

Treatments were formulated and prepared in the kitchen at the Center of Applied Research, Mount Saint Vincent University using fresh ingredients. Treatments included three types of pizzas which contained 100% wheat flour (W) or 100% lentil flour (L) or 50 %wheat and 50%lentil flour (M) and a energy free control water (C). The ingredients and amounts of ingredients used in the formulation of dietary treatments are shown in Table 4.1 and Table 4.2 respectively

Table 4.1 List of ingredients

Ingredients	Universal product code (UPC)
All purpose flour	067716003336
Lentil flour	515 (Lot No-1820)
Olive oil (light taste)	041790004403
Pizza cheese	055742553536
No salt added tomato sauce	027000390078
Pizza yeast	064217000079
Table salt	066010001055
Sugar	058891252220

Table 4.2 Pizza formulations (9-inch pizza)

Ingredients	100% wheat flour (W)	100% lentil flour (L)	50%wheat flour/50%lentil flour (M)
Wheat flour + dust, g	150 + 8	N/A	75+4
Lentil flour + dust, g	N/A	150+8	75+4
Olive oil (light taste), g	37.5	37.5	37.5
Pizza cheese, g	42	42	42
No salt added tomato sauce, g	35	35	35
Pizza yeast, g	6.5	6.5	6.5
Table salt, g	2.5	2.5	2.5
Sugar, g	2.8	2.8	2.8
Warm water, g	48	48	48

Cooking directions

Each pizza was prepared individually using fresh ingredients to achieve weight consistency. To make the pizza, all the dry ingredients (flour, yeast, salt, and sugar) were measured individually,added into a bowl andmixed well using a spatula. The warm water (50-55$^{\circ\circ 0}$C) and oil were then added to the bowl with dry ingredients andmixed by hand until all the ingredients were combined. The dough was placed on a lightly floured countertop using the flour for that treatment. The dough was gently kneaded and punched down till it became a firm ball. The dough was then rolled out to make the pizza crust. Finally, toppings (unsalted tomato sauce and pizza cheese) were added. After making pizza, it was baked for 2 minutes at 425$^{\circ 0}$F in the convection oven and was vacuum packaged, labelled, and stored in the freezer (-18$^{\circ 0}$C). On the study day, the pizza was baked for 16 minutes at 425$^{\circ 0}$F in the oven. Half of the baked pizza was served to the participant. The nutrient composition of the dietary treatments is shown in Table 4.3

Table 4.3 Nutrition composition of the treatments (1/2 of the 9-inch pizza)

	W	L	M
Serving size (1/2 of the 9"pizza), g	166	166	166
Energy, kcal	532.5	520.6	526.5
Total Fat, g	25.8	25.2	25.4
Saturated Fat, g	5.6	5.6	5.6
Trans Fat, mg	0	0	0
Cholesterol, mg	14	14	14
Sodium, mg	642	647.8	644.9
Total Carbohydrate, g	60.2	52	56
Available carbohydrate, g	54.3	46.8	50.4
Dietary fibre, g	2.5	12.7	7.6
Total sugar, g	3.0	4.8	3.9
Added sugar, g	1.5	1.5	1.5
Protein, g	14	22.9	18.4
Vitamin D, mcg	0	0	0
Calcium, mg	169.5	201.4	185.5
Iron, mg	3.5	7.5	5.5
Potassium, mg	152	797.7	474.1

4.5 Study protocol

Upon arrival, the participant completed a health and activity questionnaire (Appendix 5) which assesses the previous 24-hour period food intake, stress, and physical activity. Height, weight, and body composition were measured via bioelectrical impedance analysis using a Tanita Body Composition Analyzer TBF-300A and Stadiometer HR-200 (Tanita Corporation of America, Inc, Arlington Heights, IL). Compliance with the fasting was assessed by taking a baseline blood glucose measurement from a discard tube and was checked with a handheld glucometer - HemoCue® Glucose 201(HemoCue AB, Ängelholm, Sweden). Participants with baseline blood glucose under 4mmol/L or over 6mmol/L were rescheduled for another day.

Participants completed the visual analogue scale (VAS) questionnaires (Appendix 6) which assessed their physical comfort and subjective appetite before having an intravenous catheter inserted into their arm for blood collection by a Registered Nurse. Blood collection was performed by using a

system manufactured by Becton, Dickinson, and Company (Mississauga, ON) that includes a shielded intravenous catheter (BD Insyte Autoguard™) which was inserted into the antecubital vein and connected with Luer-Access split septum device (BD Q-Syte™) and Luer-Lok™ access device (BD Vacutainer®). The blood was collected into BD Vacutainer® Plus serum separation tubes. An initial baseline sample was taken for time 0 (t_0). Following this, participants consumed one of the four treatments. Average weight of the lentil (L), wheat (W), and mixed pizza (M) were 151.3±0.9g, 151.9±1.2g, and 151.41g respectively. Time 0 (t_0) was marked for blood glucose when participants took the first bite of the treatments or took the first sip of water. Participants were given 12 minutes to consume the complete treatment. A VAS and 9-point hedonic scale were given to participants to assess the treatment palatability (Appendix 7). Afterwards, blood samples were drawn at 15, 30, 45, 60, 90, 120, 150 and 180min time points. Immediately, after the blood collection, participants were asked to complete the questionnaires (Appendix 6) which evaluated physical comfort, subjective appetite, and gastrointestinal wellness. Participants completed the physical activity questionnaires (Friedenreich et al., 2006) and restraint scale and three factor eating questionnaires (Stunkard & Messick, 1985) only during their first session.

At 180 min post-treatment, participants were served either original Kraft dinner (Kraft Canada *Inc*) or vegetable added kraft dinner based on their preference to consume as an *ad libitum* meal with water and were instructed to eat until comfortably full. More water was given if requested. Kraft dinner was prepared on the same day at the kitchen according to the instructions provided by the manufacturer with slight alterations. For the original Kraft dinner, skim milk and margarine were replaced by 80mL of 2% milk (UPC- 067997042000) and 30 grams of unsalted butter (UPC-066096123054) respectively. Additionally, 30 grams of frozen pepper (15 grams of yellow pepper and 15 grams of red pepper) and 30 grams of frozen broccoli were blended with milk and were added to the Kraft dinner. Participants were not allowed to change their preference for different study sessions. Food intake was measured using a weight difference, with the weight of macaroni and cheese consumed used to determine caloric intake based on information available on the nutrition facts table from the manufacture. Water intake was calculated by deducting the final weight of the cup plus remaining water from the weight of the cup plus water before serving to the participant. Food and water intake values were recorded in the food intake sheet (Appendix 10)

Participants were rescheduled for each subsequent treatment after a washout period of at least 1 week. A 1-week washout period was selected based on established lab protocols, and similar published studies investigating the effects of lentils on short-term food intake, subjective appetite, and blood glucose.

4.6 Data

4.6.1 Blood glucose

Established lab procedures related to blood collection, handling, and storage, were followed. After consuming the treatment, and at each time point throughout the study session (0, 15, 30, 60, 90, 120, 150 and 180 min), the Registered Nurse flushed the IV catheter with saline solution, drew enough blood into a discard tube to remove any saline from the IV line, and then filled the 5 mL serum separation (SST) tubes. The SST tube was immediately inverted 5 times and was allowed to clot at room temperature for at least 30 minutes before the centrifugation at 4˚C at 1300 g (RCF) for 10 minutes. The serum was aliquoted into 2 mL microtubes in equal amounts (500µL), and immediately transferred to -18˚C freezer where it was stored until glucose analysis.

Blood samples of each time point were analyzed as triplets for serum concentrations of glucose against a glucose standard solution of known concentration usingYSI 2900 Biochemistry Analyzer (YSI Inc., Yellow Springs, OH, USA).

4.6.2 Appetite

Subjective appetite was recorded using VAS which were completed at 0, 15, 30, 45, 60, 90, 120, 150, and 180 min by participants in the blood collection area. VAS questionnaires were administered using Compusense software (Compusense Inc., Guelph, ON). They measured the subject's appetite on different aspects including desire to eat, hunger, feeling of fullness, and subjective food intake speculation. According to previously published guidelines, the subjective average appetite was calculated using the following formula from the VAS scales: appetite score = [desire to eat + hunger + (100 – fullness) + prospective consumption]/4 (Mollard, Zykus, et al., 2011).

4.6.3 Physical comfort

Subjective feelings of physical comfort include subjective feelings of nausea, stomach discomfort, wellness, flatulence, and diarrhea were measured using VAS questionnaires at 0, 15, 30, 45, 60. 90, 120, 150, and 180 min by using Compusense software (Compusense Inc., Guelph, ON).

4.6.4 Treatment palatability

Treatment palatability was measured using VAS and a 9-point hedonic scale. The questionnaire was completed immediately after having the treatment using Compusense software (Compusense Inc., Guelph, ON). The VAS scale was used to assess the pleasantness, bitterness, chewiness, lentil flavour and prospective purchasing of the treatments and 9-point hedonic scale was used to assess the taste, texture, flavour, and aftertaste of the treatments.

4.6.5 Food intake

At 180 min after consuming the treatment, participants were served a glass of water and an *ad libitum* macaroni and cheese (Kraft Canada *Inc*). The participant was instructed to eat until comfortably full. Food intake was calculated as weight difference, measured through subtracting the weight of the leftover macaroni and cheese meal plus plate after eating from the weight of the macaroni and cheese meal plus plate served to the participant. Caloric intake was calculated using the information provided from the manufacturer of each ingredient added. Water intake was calculated in the same way used for calculating the food intake.

4.7 Statistical analysis

All statistical analyses related to this study was performed using the SAS version 9.4 (Statistical Analysis Systems, SAS Institute Inc., Cary, North Carolina) software suite. All data from each outcome were checked for normality by using mean, kurtosis, skewness, and a histogram output. Skewness and kurtosis of less than ±2 was used as a reference to assess normality, as well as the general shape of the histograms.

The average concentrations of glucose from triplicates for each time was used to find the incremental area under the curve (AUC) for blood glucose. The two-way repeated-measures Analysis of Variance (RMANOVA) tests was used to test the effect of a treatment, time and their interactions on blood glucose. If there was a significant effect of a treatment, a one-way ANOVA and Tukey's posthoc analysis was performed to find the differences at individual time points.

The calculated appetite scores were used to calculate the total area under the curve for appetite. The three-way RMANOVA tests was used to test the treatment, time, session, and their interaction effects on appetite. If there were any significant effects found, it was followed by one-way ANOVA and Tukey's post hoc analysis to find the treatment differences at individual time points.

The macaroni and cheese weights were converted into calories using the weight consumed multiplied by the caloric information found on the Nutrition facts table provided by manufacturers. One-way ANOVA with Tukey's post hoc analysis was conducted to determine a significant difference between mean caloric intake of macaroni and cheese across all four treatment sessions.

Correlation analyses among outcome measures was performed using Pearson's Correlation Coefficient. All results were presented as mean ± standard deviation (SD). Statistical significance was concluded with a P-value of less than 0.05.

Chapter 5: Results

5.1 Participant flow and characteristics

Twenty-four participants including males and females participated in the study (Figure 5.1). The participants were 25.04 ±4.8 years old, with BMIs of 22.5± 2.2 kg/m2. (mean ± SD) (Table 5.1).

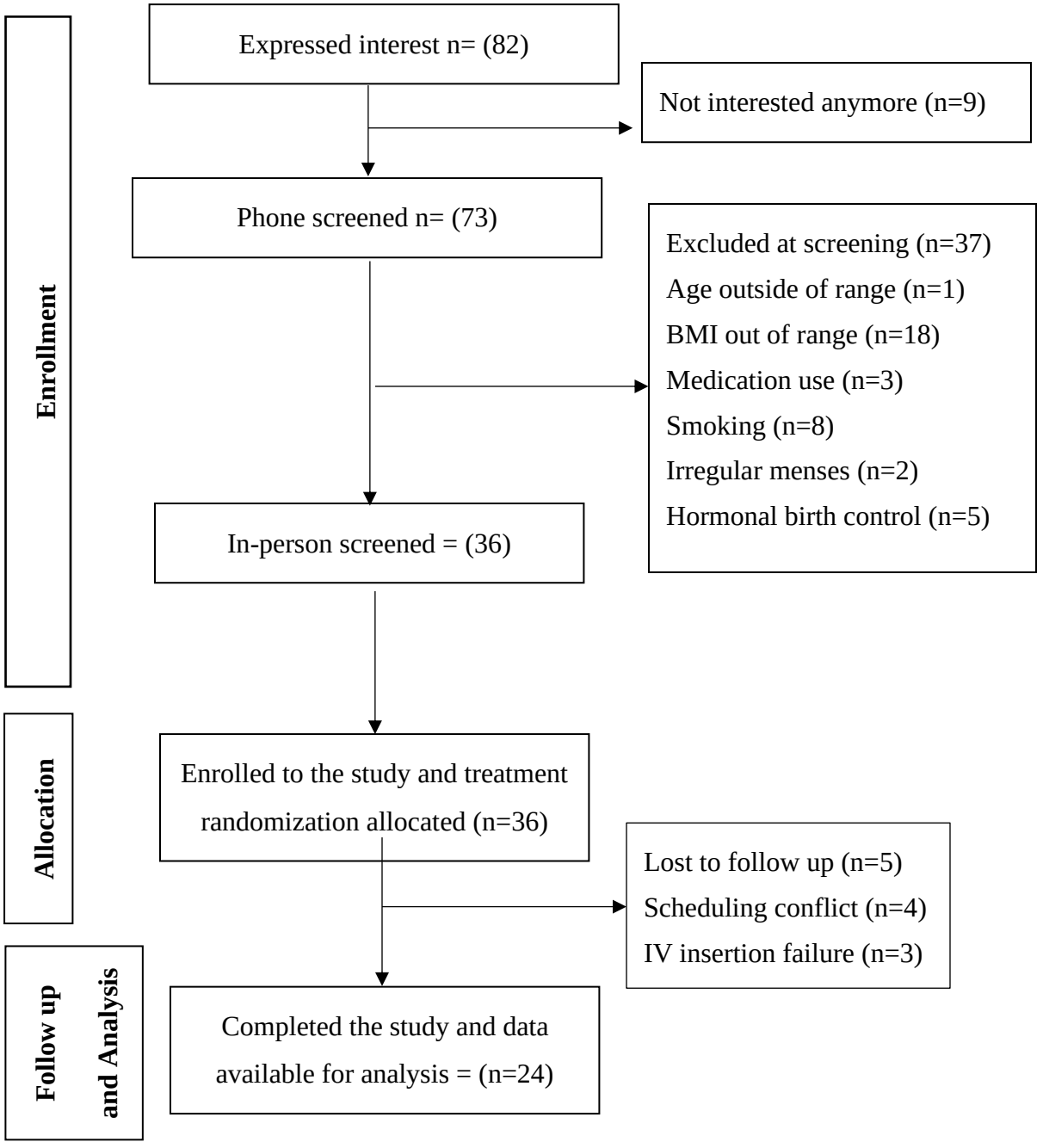

Figure 5.1 CONSORT participant flow diagram. BMI=body mass index.

Table 5.1 Participant baseline characteristics

	Male (n=12)	Female (n=12)	P value
Age (years)	24.9±5.2	25.2± 4.6	0.45
Body weight (kg)	68.5± 7.0[a]	54.4 ±4.1[b]	0.0001
Height (cm)	169.3± 5.8[a]	160.1± 5.6[b]	0.0054
BMI (kg/m^2)	23.9 ±1.8[a]	21.0± 1.6[b]	0.0007
Fat mass (kg)	12.5± 3.0	13.6± 2.9	0.21
Fat free mass (kg)	56.1± 5.2[a]	40.9± 1.9[b]	0.0000
Restraint scale	11.7±4.2	10.2. ±4.5	0.24
TFEQ Factor 1	12.3±3.0	12.3±2.7	0.47
TFEQ Factor II	10.1±2.7	11.1±2.8	0.18
TFEQ Factor III	22.6±5.3	21.3±5.7	0.31

Values are Means ±SD, BMI= body mass index, TFEQ-Three factor eating questionnaire, Unpaired t-test followed for male and female comparison. Score of three factor eating questionnaire (TFEQ) were divided by three factors according to Stunkard, (Stunkard & Messick, 1985) scoring guidelines. Values with different superscript letters are statistically significant (P≤0.05)

5.2 Blood glucose response

Figure 5.2 shows the serum blood glucose change over 180 min following the consumption of treatments.

There was an effect of treatment (P<0.0001), time (P<0.0001), and a treatment by time interaction (P<0.0001) on blood glucose over 180 min. Fasting serum blood glucose levels were similar among the treatments at 0 min (Table 5.2). The consumption of L significantly lowered the blood glucose at 30 min compared to the W (P= 0.0004, Figure 5.2). The relative reduction in maximum blood glucose concentration (Cmax) by L was 11% compared to W. There was no difference in the blood glucose between W and M and between L and M at any time point over 180 min (Figure 5.2). Blood glucose level after W consumption was significantly higher at 30 and 45 min compared to C (P<0.0001& P=0.0002 respectively, Figure 5.2). Consumption of M showed significantly higher blood glucose at 15 and 30 min compared to the C (P=0.0107 & P<0.0001, Figure 5.2). Blood glucose level after consumption of L closely resembled the C over 3 hours (Figure 5.2). There was an effect of treatment on blood glucose $tAUC_{0\text{-}180\ min}$ (P=0.0013) (Figure 5.4), $iAUC_{0\text{-}180\ min}$ (P=0.0001) (Figure 5.5) and net $iAUC_{0\text{-}180\ min}$ (P=0.0002) (Figure 5.6) Both L and M significantly reduced the blood glucose $tAUC_{0\text{-}180\ min}$, $iAUC_{0\text{-}180\ min}$ and net $iAUC_{0\text{-}180\ min}$ compared to the W (Table 5.3). The relative glycemic response reduction was 65% and 47 % for L and M

respectively compared to the W. No significant difference was observed between L and C, and M and C for blood glucose $tAUC_{0\text{-}180\ min}$, $iAUC_{0\text{-}180\ min\ and\ net}$ $iAUC_{0\text{-}180\ min}$ (Table 5.3).

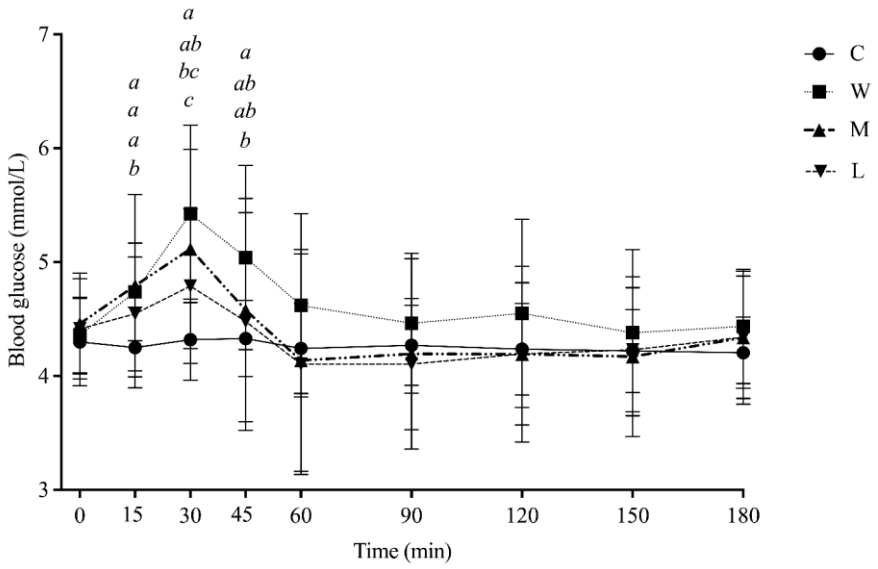

Figure 5.2 Blood glucose concentration over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment(P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

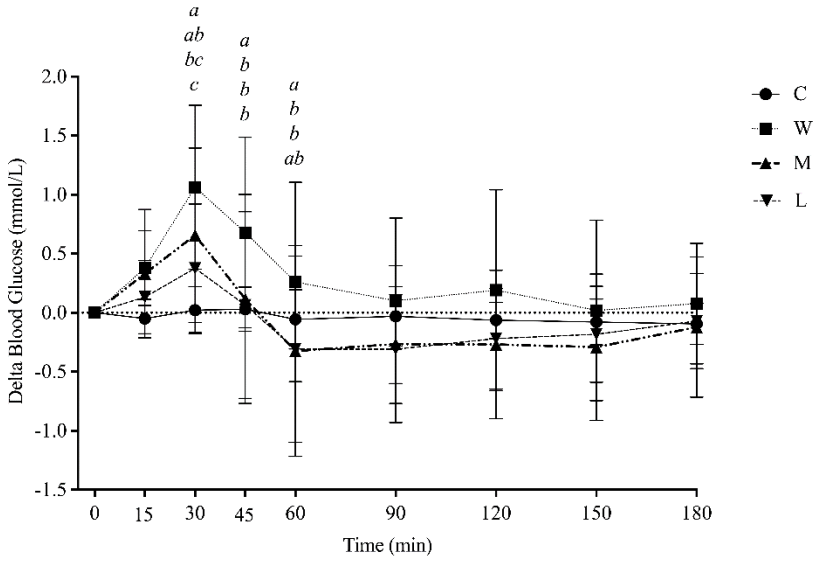

Figure 5.3 Blood glucose concentration over 180 min: change from the baseline (Δ)

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

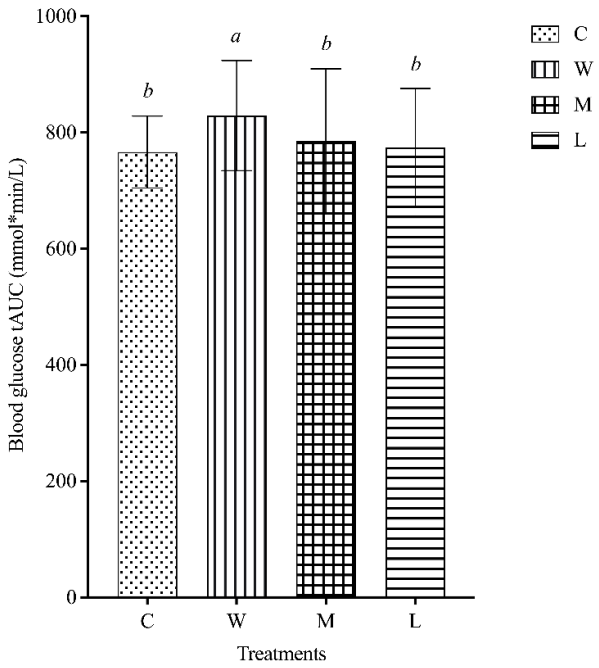

Figure 5.4 Blood glucose total area under the curve (tAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0013). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

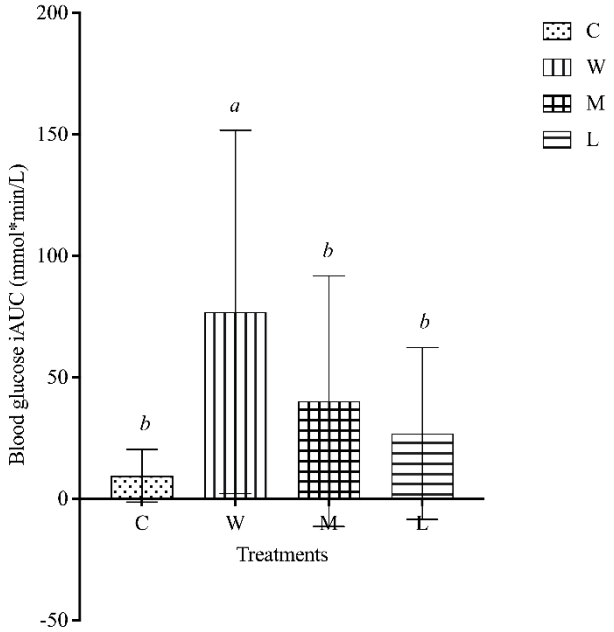

Figure 5.5 Blood glucose incremental area under the curve (iAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment(P<0.0001). Values with different superscript letters are statistically significant (P ≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

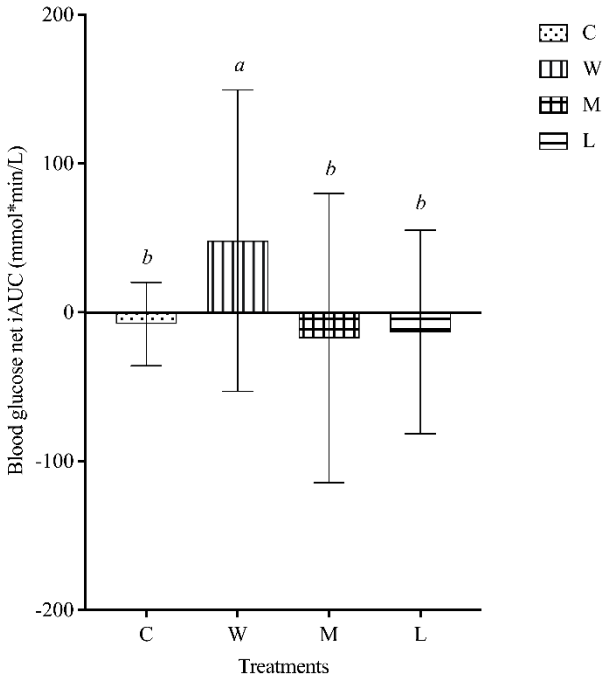

Figure 5.6 Blood glucose net incremental area under the curve (net iAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0002). Values with different superscript letters are statistically significant (P ≤0.05). L, 100% lentil flour pizza; A, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

Table 5.2 Blood glucose at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min

Treatment	Mean Blood Glucose mmol/l ± SD								
	0 min	15 min	30 min	45 min	60 min	90 min	120 min	150 min	180 min
W	4.4±0.3	4.7±0.4^{ab}	5.4±0.8^{a}	5.0±0.8^{a}	4.6±0.8^{a}	4.5±0.6	4.6±0.8	4.4±0.7	4.4±0.5
L	4.4±0.4	4.6±0.5^{ab}	4.8±0.7^{bc}	4.5±1.0^{ab}	4.1±1.0^{a}	4.1±0.6	4.2±0.6	4.2±0.6	4.3±0.5
M	4.5±0.4	4.8±0.8^{a}	5.1±0.9^{ab}	4.6±1.0^{ab}	4.1±1.0^{ab}	4.2±0.8	4.2±0.8	4.2±0.7	4.3±0.6
C	4.3±0.4	4.3±0.4^{b}	4.3±0.4^{c}	4.3±0.3^{b}	4.2±0.4^{ab}	4.3±0.4	4.2±0.4	4.2±0.4	4.2±0.3

Values are Means ± SD, n = 24. Two-way ANOVA with Tukey–Kramer post hoc test. Values with different superscript letters are statistically significant (P< 0.05). Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

Table 5.3 Mean blood glucose response over 180 min.

	Treatment	Means ± SD
BG over 180 mmol/L	C	4.26±0.36[b]
	W	4.67±0.73[a]
	M	4.44±0.84[ab]
	L	4.36±0.69[b]
	P (treatment):	0.0004
	P (time):	<0.0001
	P (treatment × time):	<0.0001
ΔBG over 180 min mmol/L	C	-0.04±0.19[b]
	W	0.31±0.74[a]
	M	-0.02±0.73[b]
	L	-0.06±0.55[b]
	P (treatment):	<0.0001
	P (time):	<0.0001
	P (treatment × time):	<0.0001
BG tAUC, (mmol*min/L)	C	766.17±61.90[b]
	W	833.17±97.70[a]
	M	785.75±123.89[b]
	L	773.82±101.94[b]
	P(treatment):	<0.0013
BG iauc (mmol*min/L)	C	9.4±10.85[b]
	W	76.86±74.82[a]
	M	40.1±51.53[b]
	L	26.87±35.49[b]
	P(treatment):	<0.0001
BG net iauc (mmol*min/L)	C	-7.85±27.98[b]
	W	48.14±101.25[a]
	M	-17.3±91.65[b]
	L	-20.73±66.38[b]
	P(treatment):	<0.0002

Values are Means ± SD, n = 24. Two-way ANOVA and 1-way ANOVA with Tukey–Kramer post hoc test Values with different superscript letters are statistically significant (P ≤0.05). tAUC, iAUC, and net iAUC represent total, incremental and net incremental areas under the curve, respectively. P indicates P values for the effect of a treatment (P treatment), time (P time), and a treatment by time interaction (P treatment × time) interaction. L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.3 Food intake

5.3.1 *Ad libitum* food intake

There was no effect of treatment (P=0.07), session (P=0.62) or a session by treatment interaction (P=0.55) on *ad libitum* food intake at 180 min. There was an effect of treatment (P=0.04) and sex (P=0.0039) but, no effect of a sex by treatment interaction (P=0.38) on *ad libitum* food intake. While there was no significant effect of treatment on *ad libitum* food intake at 180 min in males

(P=0.93), the *ad libitum* food intake was significantly higher after C in females (P=0.0039) compared to all the caloric treatments (Table 5.5).

5.3.2 Cumulative food intake

There was an effect of treatment on cumulative food intake over 180 min (P<0.0001). However, no effect of session (P=0.98) or a treatment by session interaction (P=0.36) on cumulative food intake was observed. Cumulative food intake was significantly lower after C compared to the all the caloric treatments (P≤0.05). All caloric treatments (L, W and M) led to similar cumulative food intake (Figure 5.8).

5.3.3 Cumulative water intake

There was no effect of treatment (P=0.67), or session (P=0.44) on cumulative water intake. But there was a marginal effect of treatment by session interaction on cumulative water intake (P=0.05) (Table 5.4).

5.3.4 Caloric compensation index

There was no effect of treatment (P=0.84), session (P=0.61) or treatment by session interaction (P=0.38) on caloric compensation index (Table 5.4).

5.3.5 Pleasantness of *Ad libitum* meal

The pleasantness (Hedonic) of macaroni and cheese meal was not affected by the type of treatment (P=0.61), session (P=0.29), or session by treatment interaction (P=0.67). There was also no effect of type of treatment (P=0.59), session (P=0.08), or session by treatment interaction (P=0.58) on the pleasantness (VAS) of the meal.

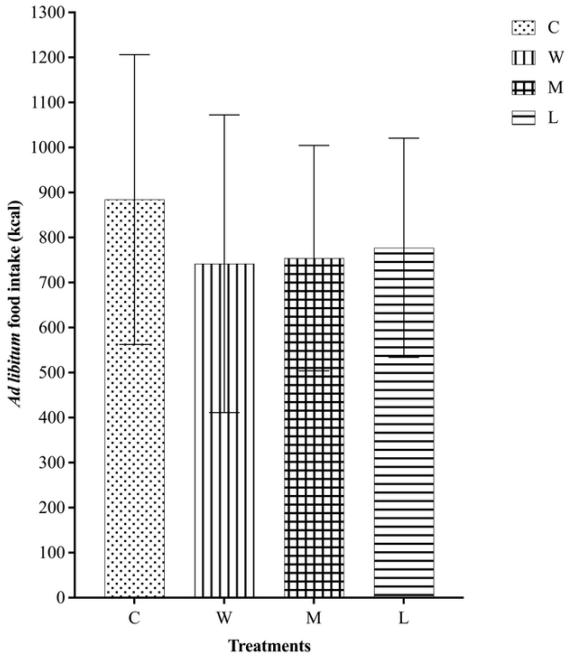

Figure 5.7 *Ad libitum* food intake at 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), no effect of session (P=0.9805) or session by treatment interaction (P=0.36). Values with different superscript letters are statistically significant (P≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

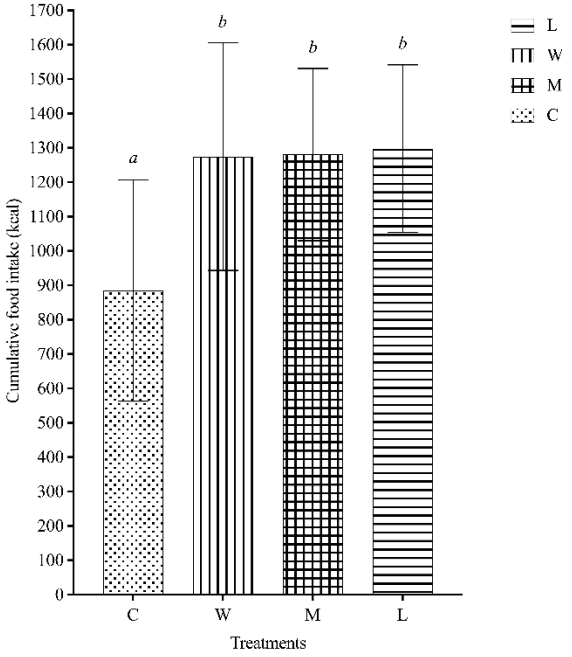

Figure 5.8 Cumulative food intake over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), no effect of session (P=0.98) or session by treatment interaction (P=0.36). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

Table 5.4 *Food and water intake at 180 min, cumulative food intake, caloric compensation and test meal pleasantness*

	Treatment	Means ± SD
Ad libitum food intake (kcal)	C	884.4±321.9
	W	741.8±330.7
	M	754.2±250.4
	L	777.2±243.3
	P(treatment):	0.07
	P(session):	0.62
	P (session × treatment):	0.55
Cumulative food intake (kcal)	C	884.4±321.1[a]
	W	1274.4±330.7[b]
	M	1280.7±250.4[b]
	L	1297.8±243.3[b]
	P(treatment):	0.0001
	P(session):	0.98
	P (session × treatment):	0.36
Cumulative water intake (g)	C	630.05±130.99
	W	650.09±138.02
	M	634.54±104.71
	L	642.80±119.89
	P(treatment):	0.67
	P(session):	0.44
	P (session × treatment):	0.05
Caloric compensation index (%)	C	0
	W	27±61.3
	M	25±48.6
	L	21±51.6
	P(treatment):	0.84
	P(session):	0.61
	P (session × treatment):	0.38
Ad libitum meal pleasantness (VAS)	C	76.04±20.54.
	W	71.61±21.62
	M	71.40±18.49
	L	72.38±18.45
	P(treatment):	0.59
	P(session):	0.08
	P (session × treatment):	0.58
Ad libitum meal food intake (Hedonic)	C	7.33±1.24
	W	6.92±1.50
	M	7.04±1.40
	L	7.13±1.33
	P(treatment):	0.61
	P(session):	0.29
	P (session × treatment):	0.67

Values are Means ± SD, n = 24. 1-way ANOVA with Tukey–Kramer post hoc test. Values with different superscript letters are statistically significant (P≤0.05). P indicates P values for the effect of a treatment (P treatment), session (P session), and treatment by session interaction P (session × treatment). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

Table 5.5 *Ad libitum* food intake - treatment by sex comparison

	Sex	Treatment	Means ± SD
Ad libitum food intake (kcal)	Males	C	990.14±326.52
		W	931.83±304.0
		M	868.84±188.88
		L	900.34±326.52
		P (treatment):	0.93
		P (session):	0.97
		P (session × treatment):	0.63
	Females	C	778.72±292.69[a]
		W	551.86±239.87[b]
		M	639.49±258.34[b]
		L	654.01±188.33[a*b]
		P (treatment):	0.0019
		P (session):	0.52
		P (session × treatment):	0.09

Values are Means ± SD, n = 24. 1-way ANOVA with Tukey–Kramer post hoc test. Values with different superscript letters are statistically significant and * indicates the marginal effect (P≤0.05). P indicates P values for the effect of a treatment (P treatment), session (P session), and treatment by session interaction P (session × treatment). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.4 Subjective appetite

5.4.1 Average appetite (AA) over three hours

There was no difference in AA (Figure 5.9) between the treatments at 0 min (P>0.05). There was an effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001) on AA over three hours. AA decreased drastically after the consumption of the caloric treatments at 15 min followed by a gradual increase until 180 min (Figure 5.9). All caloric treatments (L, W & M) led to reduced AA compared to C (Table 5.6). All caloric treatments (L, W & M) resulted in similar AA (Figure 5.9).

There was an effect of treatment on $tAUC_{0\text{-}180\ min}$ for AA (p<0.0001) (Figure 5.10). There was no effect of session (P=0.13) or session by treatment (P=0.46) on $tAUC_{0\text{-}180\ min}$ for AA (Table 5.6). All caloric treatments (L, W & M) led to reduced $tAUC_{0\text{-}180\ min}$ for AA compared to C (P≤0.05). $tAUC_{0\text{-}180\ min}$ for AA was not significantly different between the caloric treatments (L, W & M) (P>0.05) (Figure 5.10).

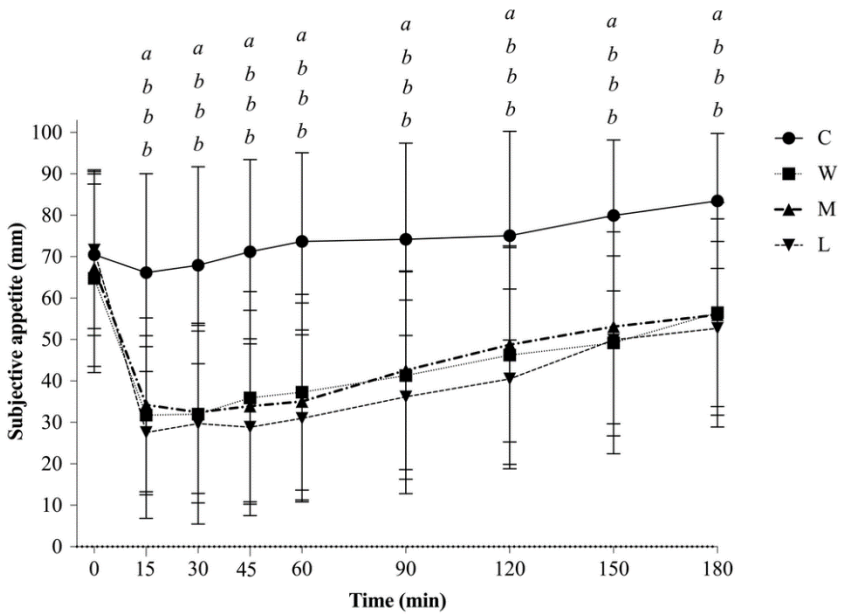

Figure 5.9 Average appetite over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

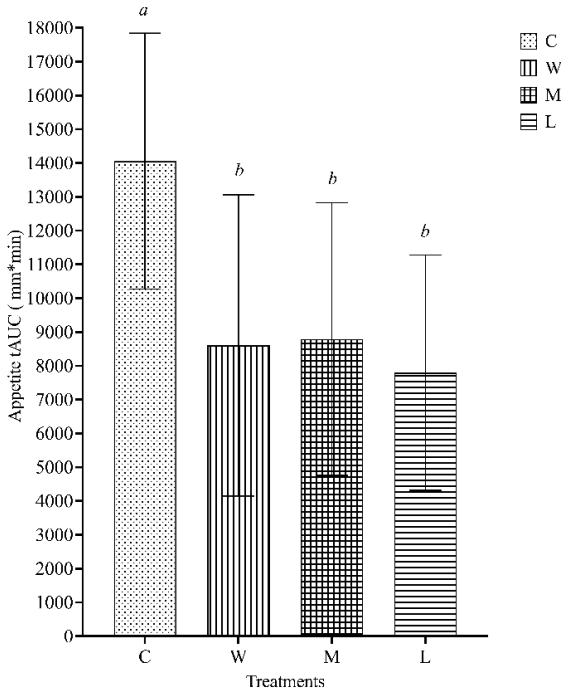

Figure 5.10 Average appetite total area under the curve (tAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), no effect of session (P=0.13) or a session by treatment interaction (P=0.47). Values with different superscript letters are statistically significant (P ≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.4.2 Desire to eat (DTE)

There was an effect of a treatment (P<0.0004), time (P<0.0001), and a treatment by time interaction (P<0.0001) on DTE over three hours. There was no difference in DTE between the treatments at 0 min (P>0.05) (Figure 5.11). DTE reduced drastically after the consumption of treatments at 15 min followed by a gradual increase until 180 min (Figure 5.11). All caloric treatments (L, W & M) led to lower DTE compared to C (Table 5.6). All caloric treatments (L, W & M) resulted in similar DTE (Figure 5.11).

There was an effect of treatment on $tAUC_{0\text{-}180\ min}$ for DTE (P<0.0001) (Figure 5.12). There was no effect of session (P=0.57) or session by treatment interaction (P=0.87) on $tAUC_{0\text{-}180\ min}$ for DTE. All caloric treatments (L, W & M) reduced DTE $tAUC_{0\text{-}180\ min}$ compared to C (P≤0.05). DTE $tAUC_{0\text{-}180\ min}$ was not significantly different between the caloric treatments (L, W & M) (P>0.05) (Figure 5.12).

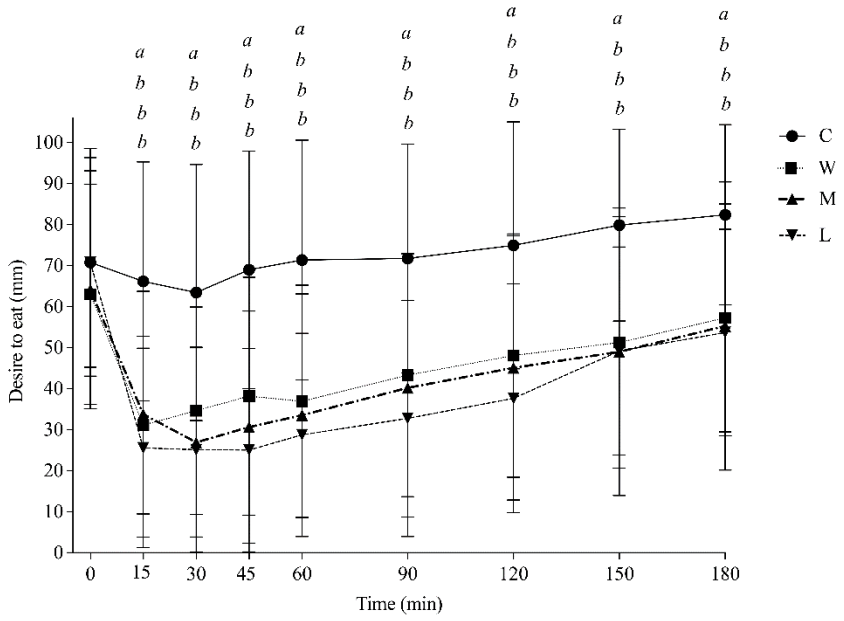

Figure 5.11 Desire to eat (DTE) over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

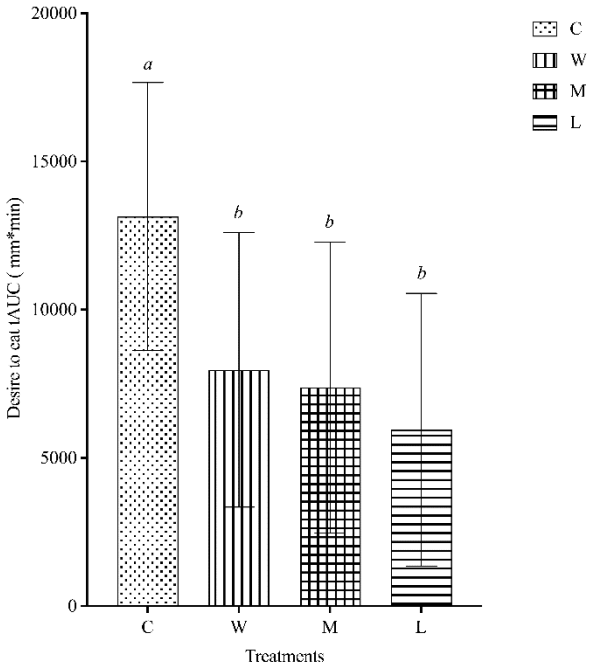

Figure 5.12 Desire to eat total area under the curve (tAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), no effect of session (P=0.57) or a session by treatment interaction (P=0.87). Values with different superscript letters are statistically significant (P ≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.4.3 Hunger

There was an effect of treatment (P<0.0001), time (P<0.0001), and a treatment by time interaction (P<0.0001) on hunger over three hours. There was no difference in hunger between the treatments at 0 min (P>0.05) (Figure 5.13). Hunger reduced drastically after the consumption of caloric treatments at 15 min followed by a gradual increase until 180 min (Figure 5.13). All caloric treatments (L, W & M) led to lower hunger compared to C (Table 5.6). All caloric treatments (L, W & M) resulted in similar hunger (Figure 5.13).

There was an effect of treatment on $tAUC_{0\text{-}180\ min}$ for hunger (P<0.0001) (Figure 5.14). There was no effect of session (P=0.24) or a session by treatment interaction (P=0.72) on $tAUC_{0\text{-}180\ min}$ for hunger. All caloric treatments (L, W & M) reduced hunger $tAUC_{0\text{-}180\ min}$ compared to C (P≤0.05). Hunger $tAUC_{0\text{-}180\ min}$ was not significantly different between the caloric treatments (L, W & M) (P>0.05) (Figure 5.14).

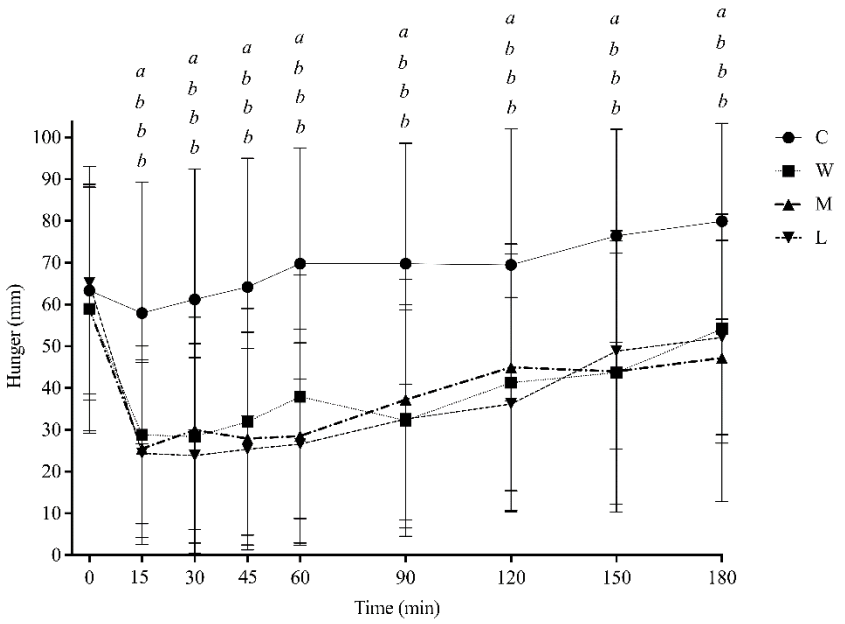

Figure 5.13 Hunger over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

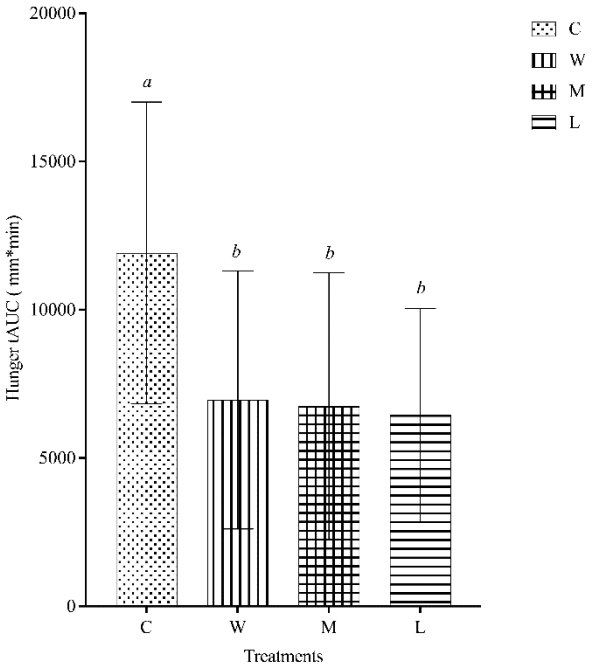

Figure 5.14 Hunger total area under the curve (tAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), no session (P=0.24) or a session by treatment interaction (P=0.72). Values with different superscript letters are statistically significant (P≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.4.4 Fullness

There was an effect of treatment (P<0.0001), time (P<0.0001), and a treatment by time interaction (P<0.0001) on fullness over three hours. There was no difference in fullness between the treatments at 0 min (P>0.05) (Figure 5.15). Fullness increased drastically after the consumption of treatments at 15 min followed by a gradual decrease until 180 min (Figure 5.15). All caloric treatments (L, W & M) led to higher fullness compared to C (Table 5.6). All caloric treatments (L, W & M) resulted in similar fullness (Figure 5.14).

There was an effect of treatment (P<0.0001), session (P=.0044), and no session by treatment interaction (P=0.34) on $tAUC_{0\text{-}180\ min}$ for fullness (Figure 5.16). All caloric treatments (L, W & M) increased fullness $tAUC_{0\text{-}180\ min}$ compared to C (P≤0.05). Fullness $tAUC_{0\text{-}180\ min}$ was not significantly different between the caloric treatments (L, W & M) (P>0.05) (Figure 5.16)

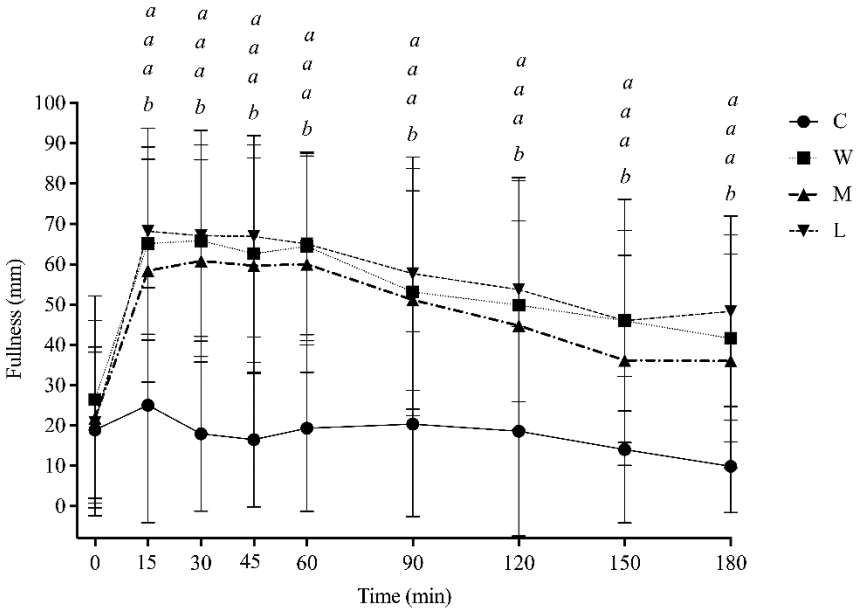

Figure 5.15 Fullness over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

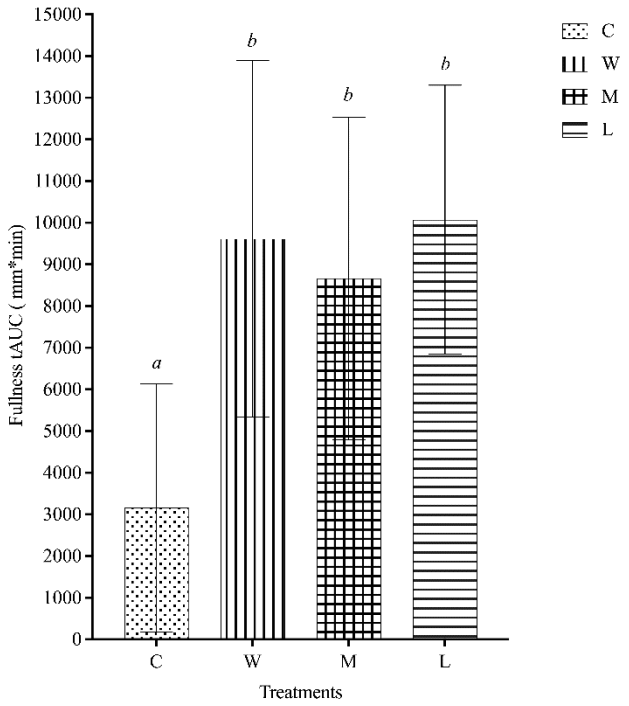

Figure 5.16 Fullness total area under the curve (tAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), session (P=0.0044) and a no session by treatment interaction (P=-0.34). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.4.5 Prospective food consumption (PFC)

There was an effect of a treatment (P<0.0001), time (P<0.0001), and a treatment by time interaction (P<0.0001) on PFC over three hours. There was no significant difference in PFC between the treatments at 0 min (P>0.05) (Figure 5.17). PFC dropped drastically after the consumption of treatments at 15 min followed by a gradual increase until 180 min. All caloric treatments (L, W & M) led to lower PFC compared to C (P≤0.05) (Table 5.6). All caloric treatments (L, W & M) resulted in similar PFC (Figure 5.17).

There was an effect of treatment (P=0.0142) and no effect of session (P=0.21) or a session by treatment interaction (P=0.63) on $tAUC_{0\text{-}180\ min}$ for PFC. All caloric treatments (L, W & M) reduced PFC $tAUC_{0\text{-}180\ min}$ compared to C (P≤0.05). PFC $tAUC_{0\text{-}180\ min}$ was not significantly different between the caloric treatments (L, W & M) (P>0.05) (Figure 5.18).

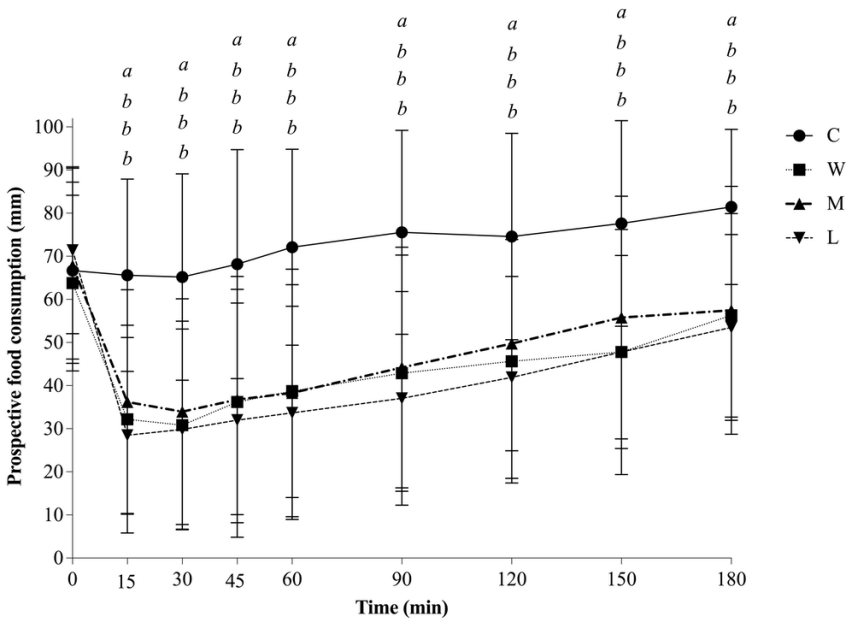

Figure 5.17 Prospective food consumption over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), time (P<0.0001) and a treatment by time interaction (P<0.0001). Values with different superscript letters are statistically significant (P <0 .05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

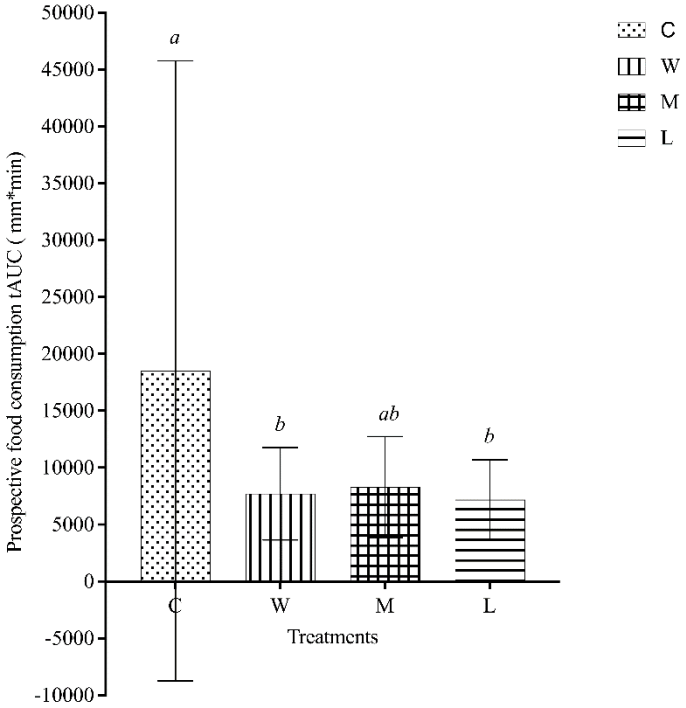

Figure 5.18 Prospective food consumption total area under the curve (tAUC) over 180 min.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0142), no effect of session (P=0.21) or session by treatment interaction (P=-0.63). Values with different superscript letters are statistically significant (P < 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

Table 5.6 Subjective appetite measures over 180 min.

	Treatment	Means ± SD
Appetite	C	73.57±21.94[a]
	W	43.89±25.76[b]
	M	44.83±25.96[b]
	L	40.89±25.17[b]
	P (treatment):	<0.0001
	P (time):	<0.0001
	P (treatment × time):	<0.0001
Appetite tAUC	C	13029.04±3510.33[a]
	W	7763.70±4000.63[b]
	M	7978.06±3800.09[b]
	L	7088.35±3213.22[b]
	P(treatment):	<0.0001
	P(session):	0.13
	P (session × treatment):	0.46
Desire to eat	C	72.16±27.74[a]
	W	44.83±29.10[b]
	M	42.04±32.24[b]
	L	38.89±29.92[b]
	P (treatment):	0.0004
	P (time):	<0.0001
	P (treatment × time):	<0.0001
Desire to eat tAUC	C	13143.06 ±4518.97[a]
	W	7975.03±4633.38[b]
	M	7372.50±4904.43[b]
	L	6657.09±3967.01[b]
	P(treatment):	<0.0001
	P(session):	0.57
	P (session × treatment):	0.87
Hunger	C	68.10±28.86[a]
	W	39.71±28.10[b]
	M	38.24±30.10[b]
	L	37.42±28.09[b]
	P (treatment):	<0.0001
	P (time):	<0.0001

	P (treatment × time):	<0.0001
Hunger tAUC	C	13053.13±4447.89[a]
	W	6596.78±4349.78[b]
	M	6756.09±4485.64[b]
	L	6449.53±3598.09[b]
	P(treatment):	<0.0001
	P(session):	0.24
	P (session × treatment):	0.72
Fullness	C	17.85±20.92[a]
	W	52.81±29.31[b]
	M	47.66±28.91[b]
	L	54.67±28.46[b]
	P (treatment):	<0.0001
	P (time):	<0.0001
	P (treatment × time):	<0.0001
Fullness tAUC	C	3157.28±2980.09[a]
	W	9614.4±4277.8[b]
	M	8663.94±3872.51[b]
	L	10072.14±3230.14[b]
	P(treatment):	<0.0001
	P(session):	0.0044
	P (session × treatment):	0.34
Prospective food consumption	C	71.89±23.18[a]
	W	43.83±36.75[b]
	M	46.70±28.67[b]
	L	41.91±26.67[b]
	P (treatment):	<0.0001
	P (time):	<0.0001
	P (treatment × time):	<0.0001
Prospective food consumption tAUC	C	18507.73±27255.38[a]
	W	7709.38±4068.27[b]
	M	8305.78±4483.29[b]
	L	7191.38±3489.18[b]
	P(treatment):	0.0142
	P(session):	0.21
	P (session × treatment):	0.63

Values are Means ± SD, n = 24. Two-way ANOVA and 1-way ANOVA with Tukey–Kramer post hoc test. Values with different superscript letters are statistically significant (P< 0.05). tAUC, represents total area under the curve. P indicates P values for the effect of a treatment (P treatment), time (P time), a treatment by time interaction (P treatment × time) interaction, session (P session) and a treatment by session interaction (P session × treatment). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.5 Subjective perception of energy level, tiredness, and wellness

5.5.1 Energy level

There was an effect of a treatment (P=0.04) on the energy level of participants but no effect of a time (P=0.12) or a treatment by time interaction (P=0.24) on the energy level was observed. All caloric treatments (L, W & M) resulted in higher energy level compared to C. Further, the energy levels followed by the consumption of caloric treatments (L, W & M) were not significantly different from each other (P>0.05) (Figure 5.19).

5.5.2 Tiredness

There was no effect of treatment (P=0.28) or time (P=0.13), but there was a marginal effect of treatment by time interaction (P=0.05) on tiredness followed by treatment consumption over 180 min (Figure 5.20).

5.5.3 Wellness

There was no effect of treatment on wellness (P=0.13) but there was an effect of time (P<0.0007), and treatment by time interaction (P=0.04) followed by treatment consumption over 180 min (Figure 5.21).

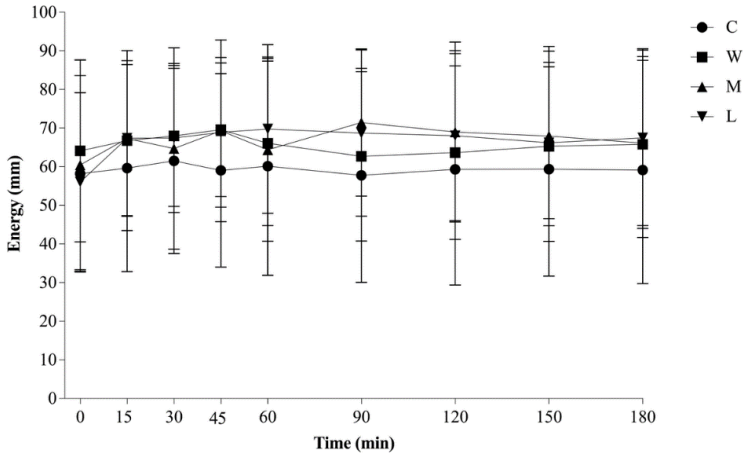

Figure 5.19 Subjective feeling of energy over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.04) and no effect of time (P=0.12) or treatment by time interaction (P=0.24). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

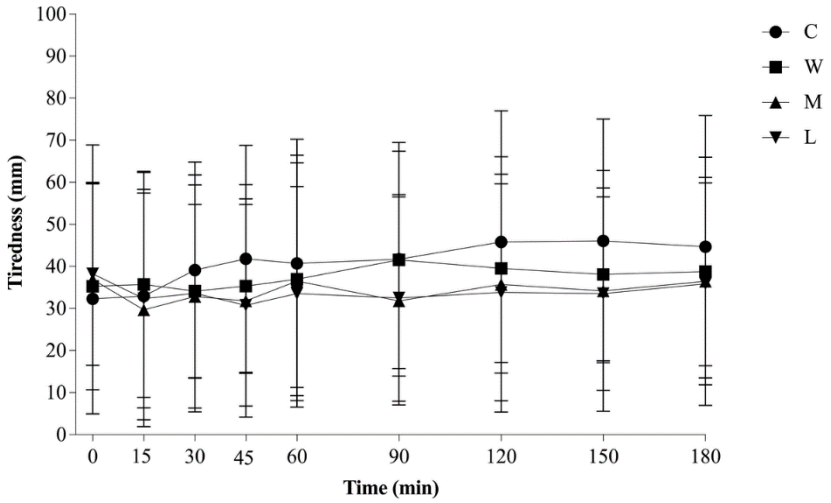

Figure 5.20 Subjective feeling of tiredness over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. No effect of treatment (P=0.28) or time (P=0.13), but effect of a treatment by time interaction (P<0.04). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

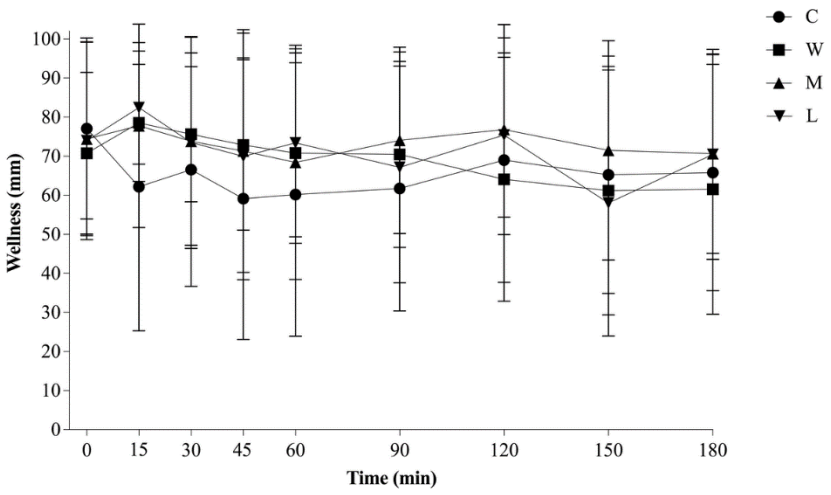

Figure 5.21 Subjective feeling of wellness over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment(P<0.03) or time (P<0.0043), but no effect of a treatment by time interaction (P=0.08). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.6 Subjective perception of physical comfort

5.6.1 Nausea

The subjective feeling of nausea was not affected by treatment (P=0.14) or time (P=0.81). But there was a marginal effect of treatment by time interaction (P=0.05) and session (P=0.03) on subjective feeling of nausea over 180 min (Figure 5.22).

5.6.2 Gas

There was no effect of treatment (P=0.29), time (P=0.38) or treatment by time interaction (P=0.15), on subjective feeling of gas, but it was affected by the treatment by session interaction (P=0.0025, Figure 5.23).

5.6.3 Diarrhea

There was no effect of treatment (P=0.68), time (P=0.28) or treatment by time interaction (P=0.45), on subjective feeling of diarrhea, but it was affected by the treatment by session interaction (P=0.0307) (Figure 5.24).

5.6.4 Stomach pain

There was no effect of treatment (P=0.28), time (P=0.81) or a treatment by time interaction (P=0.13) on subjective feeling of stomach pain. But there was an effect of session (P=0.03) (Figure 5.25).

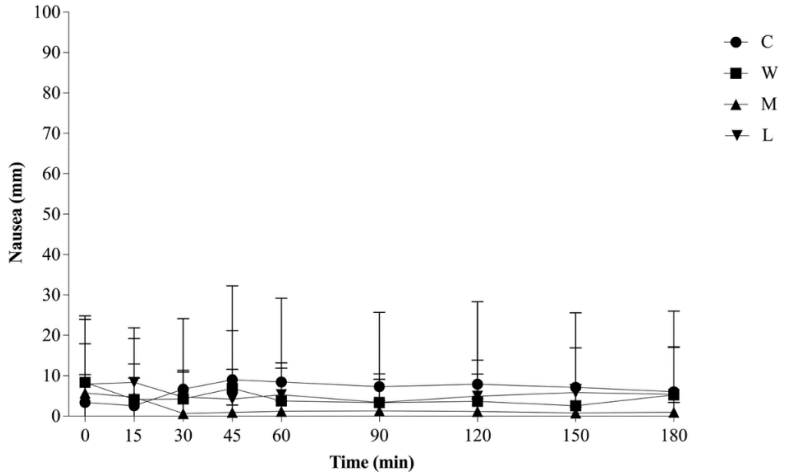

Figure 5.22 Subjective feeling of nausea over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. No effect of treatment (P=0.14) or time (P=0.81) but, marginal effect of treatment by time interaction (P=0.05) and session (P=0.03). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

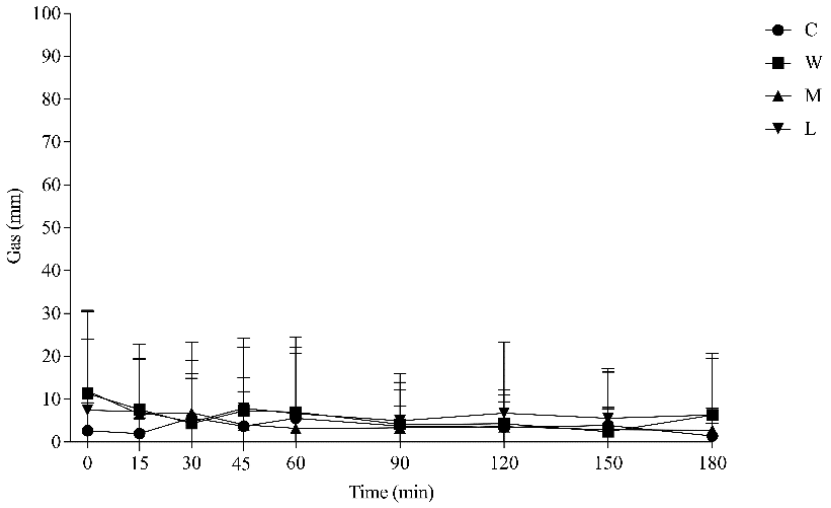

Figure 5.23 Subjective feeling of gas over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. No effect of treatment (P=0.29), time (P=0.38) or treatment by time interaction (P=0.15), but effect of a treatment by session interaction (P=0.0025). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

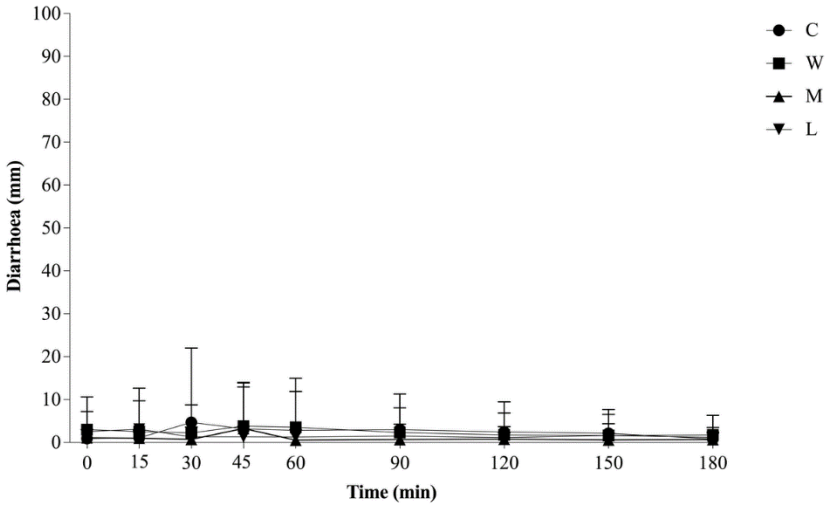

Figure 5.24 Subjective feeling of diarrhea over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. No effect of treatment (P=0.68) or time (P=0.28) and no effect of treatment by time interaction (P=0.45) and session (P=0.0307). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

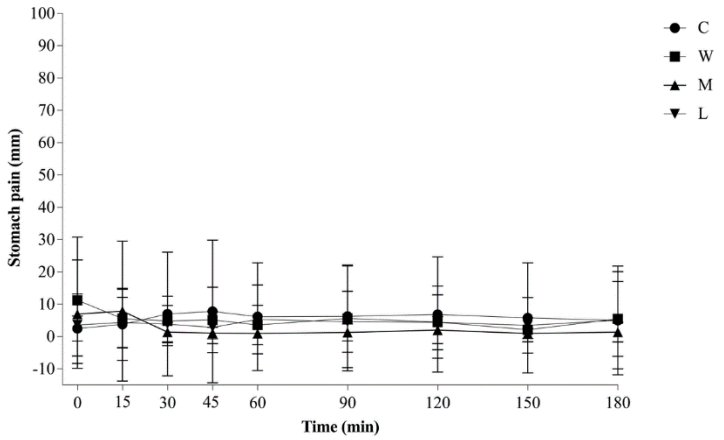

Figure 5.25 Subjective feeling of stomach pain over 180 min.

Values are Means ± SD; n=24. Two- way ANOVA with Tukey–Kramer post hoc test. No effect of treatment (P=0.28), time (P=0.81) or a treatment by time interaction (P=0.12). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.7 Sensory characteristics of treatments

The sensory properties of the pizzas formulated with wheat flour (W), lentil flour (L), mixture of wheat and lentil flour (M) were assessed using the 100mm VAS and 9-point hedonic scales.

5.7.1 Pleasantness (VAS)

There was a significant difference in pleasantness (P=0.007) among the treatments. W was perceived as the most pleasant treatment (76.75±17.4) among the other treatments. There was no significant difference in pleasantness between treatments L and M (Table 5.7).

5.7.2 Chewiness (VAS)

There was an effect of treatment on the chewiness of the pizzas (P=0.0001). All caloric treatments (L, W & M) were perceived as higher in chewiness than C. There was no significant difference in chewiness among the caloric treatments (L, W & M) (Table 5.7).

5.7.3 Bitterness (VAS)

There was an effect of treatment on the bitterness of the pizzas (P=0.0005). Treatments L and M were perceived as significantly more bitter compared to C. There was no significant difference in bitterness among the different type of pizzas (W, L & M) (Table 5.7).

5.7.4 Lentil flavor (VAS)

There was an effect of treatment on the lentil flavor of the pizzas (P=0.0001). Treatments L and M pizza were perceived as significantly high in lentil flavor than C and L and M pizza were similar in lentil flavor (Table 5.7).

5.7.5 Prospective Purchasing (VAS)

There was an effect of treatment on prospective purchasing (P=0.0054). W was rated significantly higher for prospective purchasing compared to all other treatments. There was no significant difference between L and W in prospective purchasing (Table 5.7).

5.7.6 Taste (9-point Hedonic Scale)

There was an effect of treatment on the taste of pizzas (P=0.0009). Treatment W was perceived as the tastiest (7.17±1.27) among other caloric treatments. However, there was no difference in taste between L and M pizza (Table 5.7).

5.7.7 Texture (9-point Hedonic Scale)

There was an effect of treatment on the texture of pizzas (P=0.0019). W had the highest rating for texture compared to other treatments (6.88±1.23). There was no difference in texture between W and M (Table 5.7).

5.7.8 Flavor (9-point Hedonic Scale)

There was an effect of treatment on flavor (P=0.0014). The flavor of W was liked most compared to other pizzas. There was no difference in flavor between W and M (Table 5.7)

5.7.9 Aftertaste (9-point Hedonic Scale)

There was an effect of treatment on the aftertaste (p=0.0008). The hedonic rating for aftertaste was higher for W (7.00±1.29) compared to L and M. There was no difference in aftertaste between L and M (Figure 5.34).

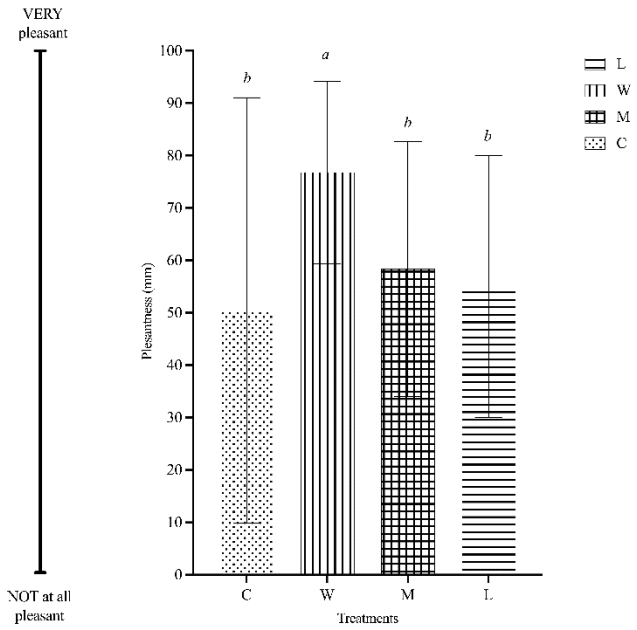

Figure 5.26 Pleasantness of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0070), no effect of session (P=0.91) or session by treatment interaction (P=-0.83). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

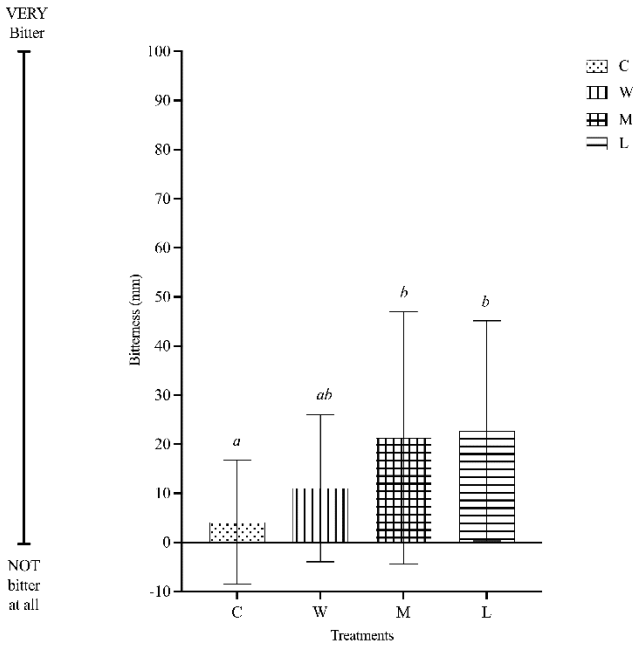

Figure 5.27 Bitterness of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment(P<0.0005), no effect of session (P=0.19) or session by treatment interaction (P=-0.58). Values with different superscript letters are statistically significant (P≤0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

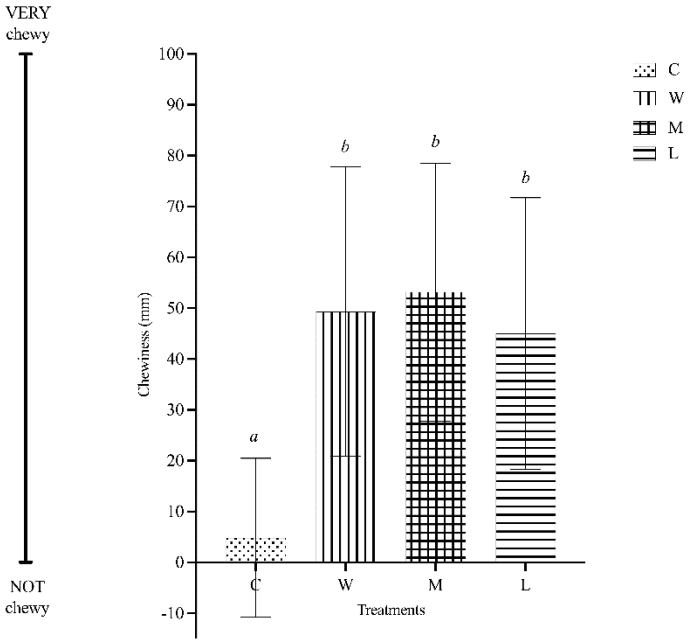

Figure 5.28 Chewiness of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0001), no effect of session (P=0.59) or session by treatment interaction (P=0.88). Values with different superscript letters are statistically significant (P < 0.05). LF, 100% lentil flour pizza; WF, 100% wheat flour pizza; MF, 50% lentil flour and 50% wheat flour pizza; W, water.

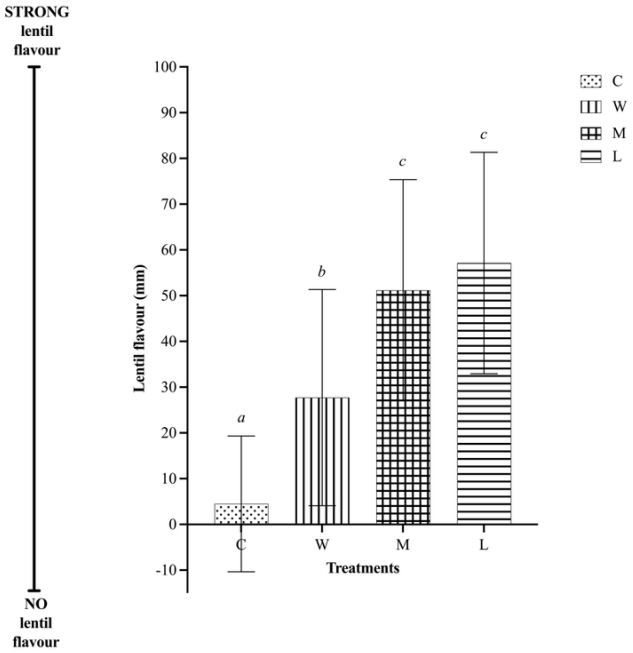

Figure 5.29 Lentil flavor of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment(P<0.0001), no effect of session (P=0.97) or session by treatment interaction (P=0.50). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

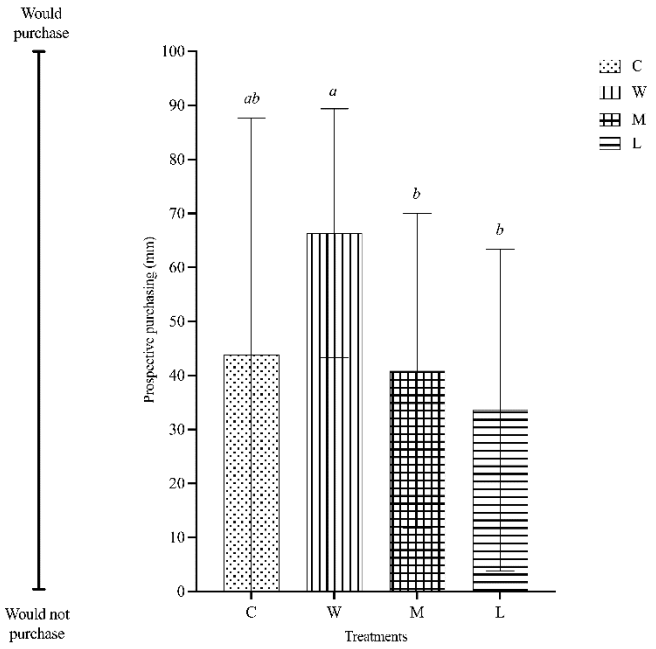

Figure 5.30 Prospective purchasing of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P=0.0054), no effect of session (P=0.69) or session by treatment interaction (P=0.27). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

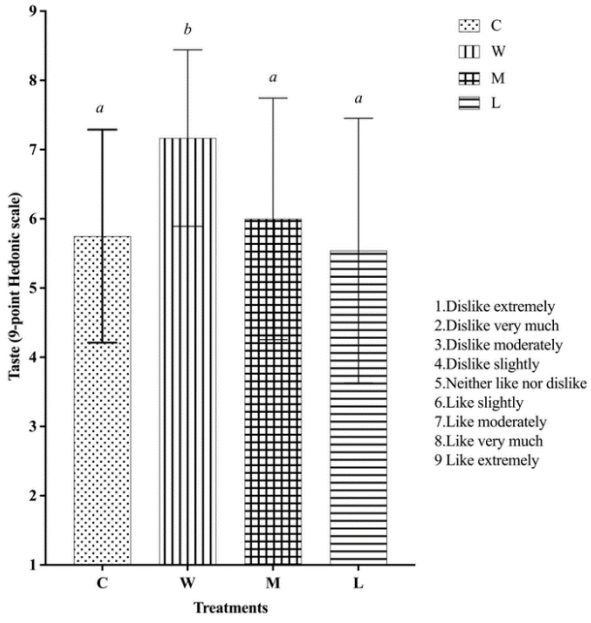

Figure 5.31 Taste of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0009), no effect of session (P=0.88) or session by treatment interaction (P=0.26). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

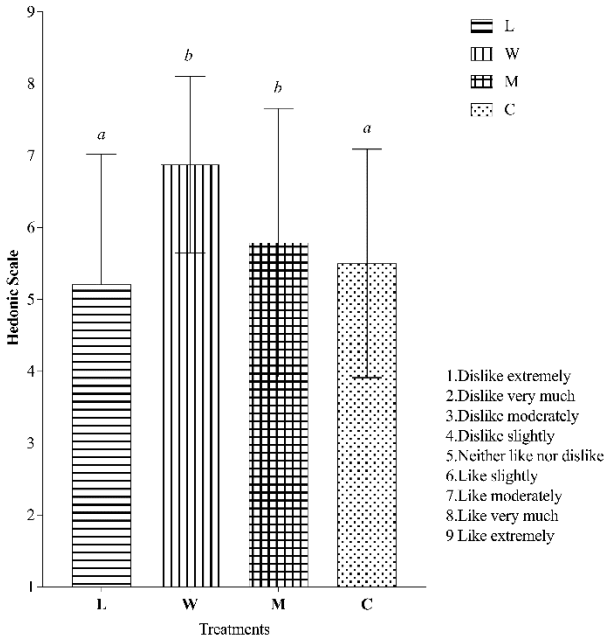

Figure 5.32 Texture of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0019), no effect of session (P=0.80) or session by treatment interaction (P=0.77). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

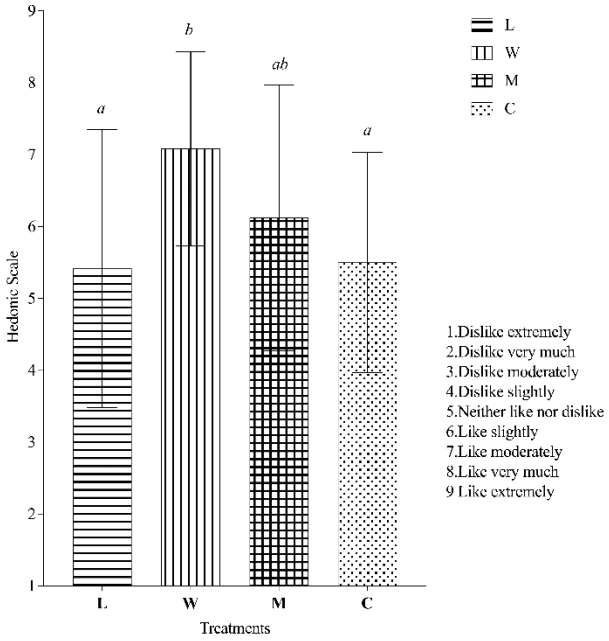

Figure 5.33 Flavor of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0014), no effect of session (P=0.97) or session by treatment interaction (P=0.83). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

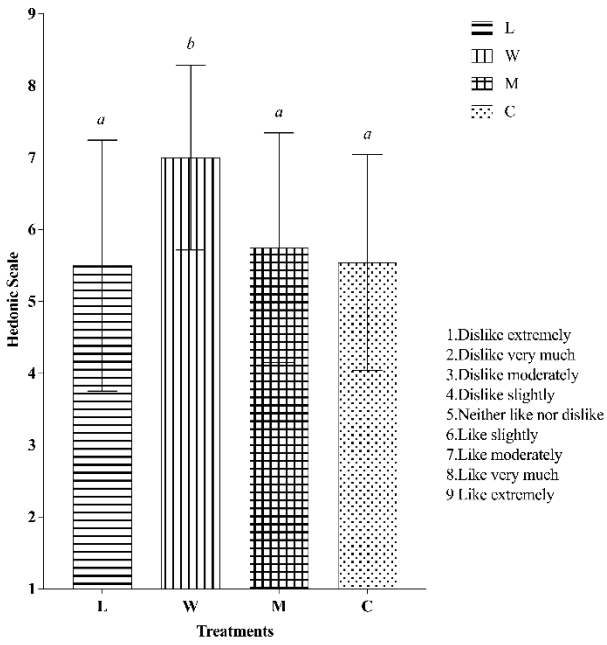

Figure 5.34 Aftertaste of the treatments.

Values are Means ± SD; n=24. One- way ANOVA with Tukey–Kramer post hoc test. Effect of treatment (P<0.0008), no effect of session (P=0.67) or session by treatment interaction (P=0.51). Values with different superscript letters are statistically significant (P< 0.05). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

Table 5.7 Sensory perception of the treatments

	Treatment	Means ± SD
Pleasantness	C	50.47±40.6[a]
	W	76.75±17.4[b]
	M	58.39±24.3[ab]
	L	54.97±24.99[a]
	P(treatment):	0.0070
	P(session):	0.91
	P (session × treatment):	0.83
Chewiness	C	4.85± 15.61[a]
	W	47.56±29.84[b]
	M	53.15±25.34[b]
	L	45.04±26.67[b]
	P(treatment):	0.0001
	P(session):	0.59
	P (session × treatment):	0.88
Bitterness	C	4.13±12.67[a]
	W	10.73±15.12[ab]
	M	21.27±25.65[b]
	L	22.73±22.42[b]
	P(treatment):	0.0005
	P(session):	0.19
	P (session × treatment):	0.08
Lentil flavor	C	4.49±14.84[a]
	W	27.72±23.64[b]
	M	51.18±24.17[c]

	L	57.14±24.20[c]
	P(treatment):	0.0001
	P(session):	0.97
	P (session × treatment):	0.50
Prospective purchasing	C	43.83±43.81[ab]
	W	65.05±24.14[a]
	M	40.87±29.11[b]
	L	33.59±29.72[b]
	P(treatment):	0.0001
	P(session):	0.69
	P (session × treatment):	0.27
Taste	C	5.75 ±1.54[a]
	W	7.17±1.27[b]
	M	6.00±1.74[a]
	L	5.54±1.91[a]
	P(treatment):	0.0009
	P(session):	0.88
	P (session × treatment):	0.26
Texture	C	5.50±1.59[a]
	W	6.88±1.23[b]
	M	5.79±1.86[b]
	L	5.21±1.82[a]
	P(treatment):	0.0019
	P(session):	0.80
	P (session × treatment):	0.77
Flavor	C	5.50±1.53[a]
	W	7.08±1.35[b]
	M	6.13±1.85[ab]
	L	5.42±1.93[a]
	P(treatment):	0.0014
	P(session):	0.97
	P (session × treatment):	0.83
Aftertaste	C	5.54±1.50[a]
	W	7.00±1.29[b]
	M	5.75±1.59[a]
	L	5.50±1.74[a]
	P(treatment):	0.0008
	P(session):	0.67
	P (session × treatment):	0.51

Values are Means ± SD, n = 24. 1-way ANOVA with Tukey–Kramer post hoc test. Values with different superscript letters are statistically significant (P≤0.05). P indicates P values for the effect of a treatment (P treatment), session (P session) and session by treatment interaction (P session × treatment). L, 100% lentil flour pizza; W, 100% wheat flour pizza; M, 50% lentil flour and 50% wheat flour pizza; C, water.

5.8 Correlations

Correlations between mean values of body weight, fat free mass, food intake, appetite and blood glucose are shown in Table 5.7

There was a positive correlation between food intake and bodyweight (r=0.28, P=0.045), fat free mass (r=0.39, P=0.001), appetite (r=0.52, P=0.0088) and subjective average appetite at 180 min. There was a positive correlation between appetite and body weight (r=0.33, P=0.0012) and fat-

free mass (r=0.38, P=0.0001). There was no correlation amongst other combination of variables assessed in table 5.8

Table 5.8 Relationships between dependent and independent measures

Correlation of variables		r	P
Food intake	Body weight	0.29	**0.0045**
Food intake	Fat free mass	0.39	**0.0001**
Food intake	Appetite	0.5	**0.0088**
Food intake	Appetite at 180 min	0.4	**0.0473**
Food intake	Blood glucose	0.15	0.4870
Food intake	Blood glucose at 180 min	0.03	0.8900
Appetite	Body weight	0.3	**0.0012**
Appetite	Fat free mass	0.34	**0.0001**
Appetite	Blood glucose	-0.56	0.1168

Pearson's correlation coefficient test for correlation among variables. n=24, $P \leq 0.05$ indicates significant difference.

Chapter 6: Discussion

6.1 General discussion

Lentils have gained popularity among consumers as well as the food industry due to its excellent nutritional composition and functional properties. There is a growing market for lentil flour, and it is being used in a variety of innovative food applications (Pulse Canada, 2022a). Lentil flour is produced in various particle sizes depending on the need of the food industry. Learning from an *in vitro* study that a larger particle size of lentil flour correlates with a lower glycemic response piqued interest in investigating the effect of lentil flour consumption with a similar particle size to wheat flour consumption on glycemic response in humans (Kathirvel et al., 2019).

In the current study, treatments (pizza) were formulated with lentil flour and all-purpose wheat flour with a particle size of less than 80µm (Appendix 10) which falls under the superfine particle size category (Kathirvel et al., 2019). Pizza was selected as the treatment because it is considered as culturally appropriate and a realistic meal for Canadians. According to Technomic's 2020 Canadian Pizza consumer Trend Report, pizza consumption has increased since 2018 and over 80% of Canadians consume pizza at least once a month (Technomic,2020). Health Canada Draft Guidance Document on Food Health Claims Related to the Reduction in Post-Prandial Glycaemic Response specifies that the amounts of reference and test food given in the study must be consistent with its serving size and intended pattern of consumption (Health Canada, 2013). Treatments were formulated in accordance with Health Canada's draft guidance document for post-Prandial glycaemic response reduction, to ensure consistency with other commercially available pizzas and to account for approximately 50g of available carbohydrate (Health Canada, 2016). In the current study, participants were served 150g of pizza which contained 111.5g of pizza crust equating nearly two servings of pizza crust as per the Health Canada's Table of Reference Amounts of Food for nutrient facts tables (Reference amount for pizza crust is 55g) (Health Canada, 2016).

According to the researcher's best knowledge, this is the first study which controlled the particle size of the flour in the treatments in assessing the effect of lentils on glycemic response in humans. In the current study, lentil and mixed pizza meals significantly lowered the blood glucose $iAUC_{0\text{-}180\ min}$ compared to 100% wheat pizza. Lentil and mixed pizza lowered blood glucose $iAUC_{0\text{-}180\ min}$ by 65% and 47% compared to wheat flour pizza respectively. In addition, lentil flour pizza consumption led to nearly 11% reduction in peak blood glucose at 30 min relative to wheat pizza.

Therefore, the finding of the current study supported the hypothesis that the partial or complete replacement of wheat flour with a processed lentil flour of similar particle size into a formulated pizza improve blood glucose control. Only very few studies have studied the effect of lentils in the form of lentil flour incorporated into the treatment by replacing commonly consumed high glycemic carbohydrate sources such as wheat flour in a culturally appropriate realistic meal. The current finding is aligned with the findings reported by Moravek and colleagues in 2018. They showed that incorporating green and red lentils with the dose of 25g available carbohydrate into muffin led to 12% and 15.7 % reduction in blood glucose $iAUC_{0\text{-}120\ min}$ compared to wheat flour muffin in healthy young adults (n=24). However, the effect was dependent on lentil variety, only red lentil muffin significantly improved the postprandial blood glucose compared to the control muffin. Despite the similarities in treatment context (baking and energy content) and study population with the current study, limited information on amount of lentil flour incorporated and the information on particle size of the flours are available. (Moravek et al., 2018). Further, the current finding reaffirms the findings of earlier investigation in which lentils were tested as part of a complex meal. Jenkins and colleagues administered lentil as a part of the meal consisted of tomato and butter, and a control whole meal bread with bread, cottage cheese and tomato. Treatment and control were matched for the major macronutrients such as carbohydrate, fat and protein. The glucose area under the $curve_{0\text{-}240\ min}$ ($AUC_{0\text{-}240\ min}$) was significantly lower following lentil meal, compared to the wholemeal bread breakfast. Further, the blood glucose $AUC_{0\text{-}240\ min}$ after the lentils was only 29% that of the full bread meal (Jenkins et al., 1982). The current study also reemphasizes the Jenkins' statement that though processing methods such as milling, heat treatment affects the macronutrients' structure and its composition, slow-release nature of legumes is preserved. (Jenkins et al., 1982). A few contrast results also were reported in the literature. When Mollard and colleagues administered treatments with a dose of 40 g available carbohydrate from pulses with a total of about 100 g available carbohydrate. There was no significant difference in postprandial blood glucose net $iAUC_{0\text{-}260\ min}$ between pulse treatments including lentils and a macaroni and cheese control. The contradictory result could be attributed to the presence of cheese only in control meal while it was absent in the treatment meal (Mollard, Wong, Luhovyy, & Anderson, 2011). Cheese lowers the glycemic index the in the control macaroni and cheese meal (Henry, Lightowler, Kendall, & Storey, 2006). In another study, the mean blood glucose $AUC_{0\text{-}180\ min}$ for the treatment smoothie contained half cup pureed lentil was not significantly differed from

the control smoothie. It could be attributed to 14% higher carbohydrate present in the lentil-based smoothie and 7g of more fat in the control smoothie (Erickson & Slavin, 2016). Though we compared the findings of similar studies which assessed the effect of lentil consumption on postprandial blood glucose with the current study's findings, as discussed in the literature review (Chapter 2), there needs to be more consistency in the methodology followed by those studies. They were heterogeneous in the form of the lentil (whole/processed) used in the treatment, the amount of lentil included in the test meal, the vehicle used (lentils alone or as a part of the complex meal), the amount of available carbohydrate in the treatment and study population etc. It is evident from the finding of the current study that the observed glycemic response is likely to be due to the difference in macronutrient composition of wheat and lentil flour not due to the particle size of the flour. The differences observed cannot be attributed to the fat or water content, as these factors were kept constant throughout the different pizza treatments. This suggests that the glycemic variability of the lentil pizza meal seen may be a result of the either meals' fibre and/or protein content. Though researchers suggested number of possible different mechanisms in the explanation of how the nutritional composition of lentils contribute to the attenuation of glycemic response, exact responsible component/s and mechanisms through which glucose attenuation effect of lentil expressed is not known. In previous studies, it was believed that the dietary fibre, and nature of the carbohydrate present in the lentil are responsible for its desirable effect on postprandial blood glucose. The dietary fibre and resistant starch present in the lentils are act during the digestive, transit or during the absorptive phases of metabolism and modify those process in favor of body’s effort to lower the elevated blood glucose after a meal (Jenkins, Wolever, Taylor, Barker, & Fielden, 1980). Dietary fibre and starch can bind enzymatic digestion products, delaying their release and absorption at the brush border (Jenkins, Wolever, Taylor, Barker, & Fielden, 1980). Higher amylose to amylopectin ratio found in lentils leads to slower digestion and absorption (Faris, Takruri, Shomaf, & Bustanji, 2009). In addition, amylose starch undergoes retrogradation more quickly, converting portion of the starch into resistant starch (type 2) that cannot be digested by digestive enzymes in the small intestine (Zafar et al., 2013). Another plausible mechanism is antinutritional factors present in the lentil disturb carbohydrate metabolism. Though many of these compounds are not heat resistant, few compounds such as amylase inhibitor may not be altered during cooking (Jenkins et al., 1982). Alternately, the type of the protein and its interactions with starch may have an impact on blood glucose levels. Despite

the above plausible mechanisms proposed by the investigators, a study by Fabek *et al., reported that* lentil protein is likely to be the responsible component for the acute glycemic lowering ability of lentils not the lentil fibre (Fabek et al., 2016*).*

There was an effect of treatment on subjective appetite ($P \leq 0.05$). Appetite after the water treatment was higher compared to caloric treatments, for which explanation is unnecessary. There was no difference in appetite scores or appetite sensations among different type of pizzas. All the caloric treatments were matched for weight, volume, energy, and energy density. It is interesting that despite of varying in macronutrient composition, all caloric treatments suppressed the appetite equally. Lentil and mixed pizza had 9g and 4g of more protein compared to wheat pizza respectively. Another component that enhances satiety is dietary fibre. Dietary fibre content of lentil and mixed pizza were 12.7 and 7.6g respectively where wheat pizza had only 2.5 grams of dietary fibre. The *ad libitum* food intake was not affected by type of treatment ($P=0.07$). The energy content of the caloric treatments (L, M & W) and the length of time used to measure the appetite may have impacted the observed appetite and *ad libitum* meal intake findings. All caloric treatments (L, M & W) had around 520kcal, which might be sufficient to suppress the appetite for three hours. Appetite measures were assessed only up to 180 min; however, an extended measure of appetite (more than three hours) would have also produced different results as the appetite did not return to what it had been at 0 min at 180 min. When compared to lentil pizza, wheat pizza had higher ratings for taste and pleasantness in the current study, which influenced appetite and subsequent food intake at 180 min. According to Mattes & Vickers, 2018, how much participants like the taste or pleasantness of the treatment may impact the appetite and *ad libitum* food intake results (Mattes & Vickers, 2018). A bitter smoothie produced significantly higher mental hunger and less mental fullness than control smoothie in healthy adults while other factors which are known to influence the satiety were being controlled (Mattes & Vickers, 2018). In addition, the less liked smoothie led to an intake of 77 kcal greater than the preferred smoothie. Though there are not many studies available to show that pleasantness of the treatment impacts the satiety and subsequent food intake, it is more likely that higher ratings for the pleasantness and taste for wheat pizza might have masked the satiating property of the lentil in the current study. The observed appetite and *ad libitum* food intake response in the current study was consistent with the finding of Clark et al., 2019. There was no significant different in subjective appetite sensation $tAUCs_{0\text{-}120\ min}$ between wheat flour muffin and green lentil flour muffin. The green lentil muffin contained

61.8 grams of green lentils equating nearly 0.75 cups of cooked lentils and provided 505 ± 6.2 kcal energy. The wheat flour muffin provided 543 ± 1.5 kcal of energy. The treatments used in the study were matching for energy content and volume. The treatments used in this study are in align with the treatments of current study in terms of food matrix, energy content and cooking method (baking). Although green lentil muffin lowered the appetite, it didn't reduce the ad libitum food intake or 24-hour energy intake (Clark et al., 2019). However, the amount of lentils present in the lentil pizza was nearly 36grams higher than the amount of lentils in the muffin treatment. Erickson & Slavin, 2016 also didn't observe any significant differences in average subjective appetite or food intake when substituting ice-cream for an isocaloric half-cup of red lentil puree in a smoothie matrix (Erickson & Slavin, 2016). It could be attributed to the liquid form of treatment which is known as less satiating than solid foods. Mollard et al., 2011 whose findings were disagreement with current findings, reported that lentil meal with 597.3 kcal energy content suppressed the appetite compared to the isocaloric macaroni and cheese meal. Also, the lentil treatment significantly reduced food intake (energy, weight, and volume) at the pizza meal, and cumulative energy intake, food weight and volume intake were significantly reduced by lentil treatment. The amount of lentil in the meal was as approximately 1.6 cups (332.9g) which is similar to the amount of lentil in the current study. Clark et al., 2019 found that consumption of one bowl of chili which contained 61.8g of cooked lentil reduced the subjective appetite sensation score compared to the rice flour chili. Also, lentil flour chili improved the appetite sensations $AUC_{0\text{-}240}$ min such as fullness, prospective food consumption, desire to eat with the exception of hunger sensation. But it failed to suppress the appetite. The relationships between the variables presented in Table 5.8 indicate a positive correlation between mean subjective appetite over 180 min and FI (r=0.5, P=0.009). The subjective appetite at 180 min was the predictor of FI (r=0.4, P=0.047). There was a positive relationship between body weight and FI and subjective appetite, and between fat-free mass and FI and subjective appetite (Table 5.8).

Compared to lentil pizza, wheat pizza had better hedonic ratings for taste, texture, flavour, and aftertaste and higher VAS scores for pleasantness and potential purchasing. Even though there was a significant difference in taste between lentil pizza and wheat pizza statistically, the actual interpretation of hedonic ratings reveals that participants liked the taste of wheat pizza moderately (7.17±1.27) and they liked the taste of lentil pizza slightly (5.54±1.91). Also, the higher texture preference for wheat pizza compared to lentil pizza shows the importance of wheat flour in the

texture of pizza. The current study's objectives didn't allow many modifications such as adding vital gluten or hydrocolloids in the lentil pizza recipe to enhance the texture of the lentil pizza.

Limitations of the study

The limitations of the study must be considered when interpreting the results of the study. This study was conducted only in healthy individuals with the BMI between 20 and 25. As a result, these findings cannot be extrapolated to the individuals whose BMI falls out of this range. Further research is required to determine the effect of lentil on blood glucose and appetite in overweight /obese individuals, individuals with metabolic disorders, individuals who are taking medications, women with irregular menses and post-menopausal women.

Insulin was not measured due to time constraints. However, data on insulin response is critical to ensuring that a decrease in blood glucose concentration is not accompanied by an abnormally high level of insulin in comparison to the reference food (Health Canada, 2013). Further, this study only used subjective appetite measures to assess appetite. However, physiological measures such as appetite-regulating hormones, energy expenditure, and substrate oxidation rates will be required in future studies to better understand the differences in appetite responses. Also, this is an acute study, the findings cannot be extrapolated to show how eating lentils affects long-term blood glucose or appetite. Moreover, the current study doesn't reveal the mechanism responsible for glycemic lowering potential of lentils.

Health Claims

The majority of the requirements outlined in the postprandial glycemia draft document except the missing insulin data were met in the current study (Appendix 9). Insulin was not analyzed due to time constraints.

Although the current study largely complied with the requirements in the satiety claim draft document, there were a few conditions that this study was unable to meet. Participants received all the preload and test meal treatments only at the beginning and not at the end of the study period due to the time and cost constraints (Appendix 8). The current study also was unable to blind the participants for the treatments which is common in whole food studies. Also, due to the need to access the electronic devices, and timers, the participants in the current study were not able to be completely blinded to the time thus, they were aware when their next meal was. There was no

preliminary research done due time constraints to show that the altering of the food did not change its palatability.

Strengths

This study followed a repeated measures single blinded randomized control cross over study design. Randomized control trials are the accepted gold standard, producing the highest level of evidence and incorporating repeated measures minimize the individual differences in eating behaviour and perceptions of subjective appetite (Bondemark & Ruf, 2015). Healthy males and females were included in the study to obtain data, which are generalizable to the general population. The treatment plan accounted for reality and palatability, consumption feasibility and cultural relevance. This study controlled for particle size by using wheat and lentil flour of similar particle size. Incorporating flours with similar particle size minimize variations in glycemic response due to particle size.

The VAS scale was used in this study to assess appetite sensations. Numerous reviews have supported the validity and reliability of research studies that employed VAS to evaluate appetite sensations, and these studies have shown a high degree of reproducibility (Flint, Raben, Blundell, & Astrup, 2000). Menstrual phase of the female participants was taken into consideration while scheduling participants to control the influence of menstrual phase on appetite, metabolism, and energy intake (Dye & Blundell, 1997). The methodology employed in the study almost satisfied the requirements of Health Canada draft guidance document on post-prandial glucose response reduction and satiety.

6.2 Future Directions

The current study doesn't reveal what macronutrient or macronutrients are responsible for the glycemic lowering ability of the lentils. Therefore, future research studies should be carried out to explain the responsible macronutrients and physiological mechanisms underlying lentil's potential glycemic lowering ability. Also, there is a need to assess whether the observed acute glycemic lowering ability of lentils is retained with long-term consumption.

The particle size tested in this study belongs to the superfine particle size category. As different product types require flours with different particle size flours it is important to study whether the

observed effect can be translated to the other particle size flours (coarse and regular) and to show all the products made from lentil could carry the lower glycemic response claim.

Though previous studies showed that lentil intake led to increased satiety and reduced ad libitum food intake, current study didn't show any of those effects. It could be partly explained by the different in taste and pleasantness among the caloric treatments or altered physiochemical and functional properties of lentil flour during the baking process in current study. Therefore, a study similar to the current research but with treatments matched in sensory characteristics, especially pleasantness and taste, will help to learn whether the observed result is due to changes in the functional properties of lentil flour during the baking process.

Though sensory analysis was not the primary objective of the current study, it is undeniable that sensory acceptance of the product is important to be successful in the market. Therefore, this warrants a study investigates the sensory acceptance of the lentil-based pizza.

Finally, it would be interesting to see how lentil flour incorporated into different type of products which put through different types of processing methods impacts the glycemic response.

6.3 Conclusion

The ingestion of a pizza meal formulated with lentil flour resulted in a lower glycemic response compared to a pizza meal formulated with wheat flour of similar particle size. Pizza meals formulated with lentil or wheat flour or their mixture resulted in a similar subjective appetite & physical comfort, and a similar level of *ad libitum* food intake at 180 min or cumulatively over 3 hours. The sensory perception of pizza formulated with lentil flour was lower with compared to the pizza formulated with wheat flour only, but still, it is in acceptable range.

References

Adebamowo, C. A., Cho, E., Sampson, L., Katan, M. B., Spiegelman, D., Willett, W. C., & Holmes, M. D. (2005). Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *International Journal of Cancer*, *114*(4), 628–633. https://doi.org/10.1002/ijc.20741

Agriculture and Agri-Food. (2022, August 25). Canada: Outlook for Principal Field Crops, 2022-08-22. Retrieved October 14, 2022, from Government of Canada website: https://agriculture.canada.ca/en/canadas-agriculture-sectors/crops/reports-and-statistics-data-canadian-principal-field-crops/canada-outlook-principal-field-crops-2022-08-22

Agurs-Collins, T., Smoot, D., Afful, J., Makambi, K., & Adams-Campbell, L. L. (2006). Legume intake and reduced colorectal adenoma risk in African-Americans. *Journal of National Black Nurses' Association: JNBNA*, *17*(2), 6–12. Retrieved from https://pubmed.ncbi.nlm.nih.gov/17410754/

Akcicek, E., Otles, S., & Esiyok, D. (2005). Cancer and its prevention by some horticultural and field crops in Turkey. *Asian Pacific Journal of Cancer Prevention: APJCP*, *6*(2), 224–230. Retrieved from https://pubmed.ncbi.nlm.nih.gov/16101341/

Alberta Pulse Growers. (2022). Pulses and the Gluten-Free Diet. Retrieved October 14, 2022, from Alberta Pulse Growers website: https://albertapulse.com/resource/pulses-gluten-free-diet/

Al-Tibi, A. M., Takruri, H. R., & Ahmad, M. N. (2010). Effect of Dehulling and Cooking of Lentils (Lens Culinaris, L.)on Serum Glucose and Lipoprotein Levels in Streptozotocin-Induced Diabetic Rats. *Malaysian Journal of Nutrition*, *16*(3), 409–418. Retrieved from https://pubmed.ncbi.nlm.nih.gov/22691994/#:~:text=It%20is%20concluded%20that%20c

ooked

American Diabetes Association. (2001). Postprandial Blood Glucose. *Diabetes Care*, *24*(4), 775–778. https://doi.org/10.2337/diacare.24.4.775

Anderson, G. H., Liu, Y., Smith, C. E., Liu, T. T., Nunez, M. F., Mollard, R. C., & Luhovyy, B. L. (2014). The acute effect of commercially available pulse powders on postprandial glycaemic response in healthy young men. *British Journal of Nutrition*, *112*(12), 1966–1973. https://doi.org/10.1017/s0007114514003031

Aronoff, S. L., Berkowitz, K., Shreiner, B., & Want, L. (2004). Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Diabetes Spectrum*, *17*(3), 183–190. https://doi.org/10.2337/diaspect.17.3.183

Augustin, L. S. A., Chiavaroli, L., Campbell, J., Ezatagha, A., Jenkins, A. L., Esfahani, A., & Kendall, C. W. C. (2015). Post-prandial glucose and insulin responses of hummus alone or combined with a carbohydrate food: a dose–response study. *Nutrition Journal*, *15*(1). https://doi.org/10.1186/s12937-016-0129-1

Aune, D., De Stefani, E., Ronco, A., Boffetta, P., Deneo-Pellegrini, H., Acosta, G., & Mendilaharsu, M. (2009). Legume intake and the risk of cancer: a multisite case–control study in Uruguay. *Cancer Causes & Control*, *20*(9), 1605–1615. https://doi.org/10.1007/s10552-009-9406-z

Barbana, C., Boucher, A. C., & Boye, J. I. (2011). In vitro binding of bile salts by lentil flours, lentil protein concentrates and lentil protein hydrolysates. *Food Research International*, *44*(1), 174–180. https://doi.org/10.1016/j.foodres.2010.10.045

Barbana, C., & Boye, J. I. (2011). Angiotensin I-converting enzyme inhibitory properties of lentil

protein hydrolysates: Determination of the kinetics of inhibition. *Food Chemistry*, *127*(1), 94–101. https://doi.org/10.1016/j.foodchem.2010.12.093

Bilandzic, A., & Rosella, L. (2017). The cost of diabetes in Canada over 10 years: applying attributable health care costs to a diabetes incidence prediction model. *Health Promotion and Chronic Disease Prevention in Canada*, *37*(2), 49–53. https://doi.org/10.24095/hpcdp.37.2.03

Bondemark, L., & Ruf, S. (2015). Randomized controlled trial: the gold standard or an unobtainable fallacy? *The European Journal of Orthodontics*, *37*(5), 457–461. https://doi.org/10.1093/ejo/cjv046

Bourré, L., Frohlich, P., Young, G., Borsuk, Y., Sopiwnyk, E., Sarkar, A., … Malcolmson, L. (2019). Influence of particle size on flour and baking properties of yellow pea, navy bean, and red lentil flours. *Cereal Chemistry*, *96*(4), 655–667. https://doi.org/10.1002/cche.10161

Bresciani, A., & Marti, A. (2019). Using Pulses in Baked Products: Lights, Shadows, and Potential Solutions. *Foods*, *8*(10), 451. https://doi.org/10.3390/foods8100451

Brownlee, M. (2005). The Pathobiology of Diabetic Complications: A Unifying Mechanism. *Diabetes*, *54*(6), 1615–1625. https://doi.org/10.2337/diabetes.54.6.1615

Canadian Cancer Society. (2022). Reduce your risk. Retrieved October 18, 2022, from Canadian Cancer Society website: https://cancer.ca/en/cancer-information/reduce-your-risk

Canadian Cancer Statistics Advisory Committee in collaboration with the Canadian Cancer Society, Statistics Canada, & Public Health Agency of Canada. (2021). *Canadian Cancer Statistics*. Toronto, ON: Canadian Cancer Society. Retrieved from Canadian Cancer

Society website: http://cancer.ca/Canadian-Cancer-Statistics-2021-EN

Clark, S. L., Ramdath, D. D., King, B. V., O'Connor, K. E., Aliani, M., Hawke, A., & Duncan, A. M. (2019). Food Type and Lentil Variety Affect Satiety Responses but Not Food Intake in Healthy Adults When Lentils Are Substituted for Commonly Consumed Carbohydrates. *The Journal of Nutrition*, *149*(7), 1180–1188. https://doi.org/10.1093/jn/nxz050

Cokkizgin, A. (2013). Lentil: Origin, Cultivation Techniques, Utilization and Advances in Transformation. *Agricultural Science*, *1*(1), 55–62. https://doi.org/10.12735/as.v1i1p55

Conte, P. (2018). Technological and Nutritional Challenges, and Novelty in Gluten-Free Breadmaking: a Review. *Polish Journal of Food and Nutrition Sciences*, *69*(1), 5–21. https://doi.org/10.31883/pjfns-2019-0005

Cryne, C. N., Veenstra, J. M., Deschambault, B. R., Benali, M., Marcotte, M., Boye, J. I., … Duncan, A. M. (2012). Spray-dried pulse consumption does not affect cardiovascular disease risk or glycemic control in healthy males. *Food Research International*, *48*(1), 131–139. https://doi.org/10.1016/j.foodres.2012.02.018

Dabai, F. D., Walker, A. F., Sambrook, I. E., Welch, V. A., Owen, R. W., & Abeyasekera, S. (1996). Comparative effects on blood lipids and faecal steroids of five legume species incorporated into a semi-purified, hypercholesterolaemic rat diet. *British Journal of Nutrition*, *75*(4), 557–571. https://doi.org/10.1079/bjn19960159

Dastgiri, S., Mahdavi, R., TuTunchi, H., & Faramarzi, E. (2006). Prevalence of obesity, food choices and socio-economic status: a cross-sectional study in the north-west of Iran. *Public Health Nutrition*, *9*(08). https://doi.org/10.1017/s1368980006009827

de la Hera, E., Ruiz-París, E., Oliete, B., & Gómez, M. (2012). Studies of the quality of cakes

made with wheat-lentil composite flours. *LWT*, *49*(1), 48–54. https://doi.org/10.1016/j.lwt.2012.05.009

Diabetes Canada. (2022). Understanding diabetes. Retrieved October 13, 2022, from Diabetes Canada website: https://www.diabetes.ca/about-diabetes

Dilawari, J. B., Kamath, P. S., Batta, R. P., Mukewar, S., & Raghavan, S. (1981). Reduction of postprandial plasma glucose by Bengal gram dal (Cicer arietinum) and Rajmah (Phaseolus vulgaris). *The American Journal of Clinical Nutrition*, *34*(11), 2450–2453. https://doi.org/10.1093/ajcn/34.11.2450

Dimitriadis, G. D., Maratou, E., Kountouri, A., Board, M., & Lambadiari, V. (2021). Regulation of Postabsorptive and Postprandial Glucose Metabolism by Insulin-Dependent and Insulin-Independent Mechanisms: An Integrative Approach. *Nutrients*, *13*(1), 159. https://doi.org/10.3390/nu13010159

Dye, L., & Blundell, J. E. (1997). Menstrual cycle and appetite control: implications for weight regulation. *Human Reproduction*, *12*(6), 1142–1151. https://doi.org/10.1093/humrep/12.6.1142

Erickson, J., & Slavin, J. (2016). Satiety Effects of Lentils in a Calorie Matched Fruit Smoothie. *Journal of Food Science*, *81*(11), H2866–H2871. https://doi.org/10.1111/1750-3841.13499

Escobedo, A., & Mojica, L. (2021). Pulse-based snacks as functional foods: Processing challenges and biological potential. *Comprehensive Reviews in Food Science and Food Safety*, *20*(5), 4678–4702. https://doi.org/10.1111/1541-4337.12809

Eyaru, R., Shrestha, A. K., & Arcot, J. (2009). Effect of various processing techniques on

digestibility of starch in Red kidney bean (Phaseolus vulgaris) and two varieties of peas (Pisum sativum). *Food Research International*, *42*(8), 956–962. https://doi.org/10.1016/j.foodres.2009.06.007

Fabek, H., Rajadurai, A., Arshad, M.U., Smith, C.E., Bailo, B.G., Kubant, R. and Anderson, G.H. (2016).Acute Effects of Lentil Fractions on Satiety and Glycemic Responses Before and After a Meal in Healthy Young Men. The FASEB Journal, 30: 893.4-893.4. https://doi.org/10.1096/fasebj.30.1_supplement.893.4

Faris, M. A.-I. E., Takruri, H. R., & Issa, A. Y. (2012). Role of lentils (Lens culinaris L.) in human health and nutrition: a review. *Mediterranean Journal of Nutrition and Metabolism*, *6*(1), 3–16. https://doi.org/10.3233/s12349-012-0109-8

Faris, M. A.-I. E., Takruri, H. R., Shomaf, M. S., & Bustanji, Y. K. (2009). Chemopreventive effect of raw and cooked lentils (Lens culinaris L) and soybeans (Glycine max) against azoxymethane-induced aberrant crypt foci. *Nutrition Research*, *29*(5), 355–362. https://doi.org/10.1016/j.nutres.2009.05.005

Figoni, P. (2004). *How Baking Works*. John Wiley & Sons Incorporated.

Filippatos, T. D., Panagiotakos, D. B., Georgousopoulou, E. N., Pitaraki, E., Kouli, G.-M., Chrysohoou, C., … ATTICA Study Group. (2016). Mediterranean Diet and 10-year (2002-2012) Incidence of Diabetes and Cardiovascular Disease in Participants with Prediabetes: The ATTICA study. *The Review of Diabetic Studies : RDS*, *13*(4), 226–235. https://doi.org/10.1900/RDS.2016.13.226

Flint, A., Raben, A., Blundell, J., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies.

International Journal of Obesity, *24*(1), 38–48. https://doi.org/10.1038/sj.ijo.0801083

Frankenfeld, C. L., & Wallace, T. C. (2020). Dietary Patterns and Nutritional Status in Relation to Consumption of Chickpeas and Hummus in the U.S. Population. *Applied Sciences*, *10*(20), 7341. https://doi.org/10.3390/app10207341

Friedenreich, C. M., Courneya, K. S., Neilson, H. K., Matthews, C. E., Willis, G., Irwin, M., … Ballard-Barbash, R. (2006). Reliability and Validity of the Past Year Total Physical Activity Questionnaire. *American Journal of Epidemiology*, *163*(10), 959–970. https://doi.org/10.1093/aje/kwj112

Fujikawa, T. (2021). Central regulation of glucose metabolism in an insulin-dependent and -independent manner. *Journal of Neuroendocrinology*, *33*(4). https://doi.org/10.1111/jne.12941

Ganesan, K., & Xu, B. (2017). Polyphenol-Rich Lentils and Their Health Promoting Effects. *International Journal of Molecular Sciences*, *18*(11), 2390. https://doi.org/10.3390/ijms18112390

Gerich, J. E. (2003). Clinical Significance, Pathogenesis, and Management of Postprandial Hyperglycemia. *Archives of Internal Medicine*, *163*(11), 1306. https://doi.org/10.1001/archinte.163.11.1306

Goñi, I., & Valentín-GamazoC. (2003). Chickpea flour ingredient slows glycemic response to pasta in healthy volunteers. *Food Chemistry*, *81*(4), 511–515. https://doi.org/10.1016/s0308-8146(02)00480-6

Health Canada. (2012, April 29). Canadian Nutrient File Search Engine Online. Retrieved October 13, 2022, from Government of Canada website: https://food-nutrition.canada.ca/cnf-

fce/index-eng.jsp

Health Canada. (2016, November 28). Nutrition Labelling - Table of Reference Amounts for Food. Retrieved October 14, 2022, from Government of Canada website: https://www.canada.ca/en/health-canada/services/technical-documents-labelling-requirements/table-reference-amounts-food/nutrition-labelling.html#a

Health Canada. (2017, February 9). Heart Disease in Canada. Retrieved October 13, 2022, from Government of Canada website: https://www.canada.ca/en/public-health/services/publications/diseases-conditions/heart-disease-canada.html

Health Canada. (2019, December 17). Canada's food guide: Healthy eating recommendations. Retrieved October 13, 2022, from Government of Canada website: https://food-guide.canada.ca/en/healthy-eating-recommendations/

Health Canada. (2020, November 12). Prevalence of Chronic Diseases and Risk Factors among Canadians aged 65 years and older. Retrieved from www.canada.ca website: https://www.canada.ca/en/services/health/publications/diseases-conditions/prevalence-chronic-disease-risk-factors-canadians-aged-65-years-older.html

Henry, C. J. K., Lightowler, H. J., Kendall, F. L., & Storey, M. (2006). The impact of the addition of toppings/fillings on the glycaemic response to commonly consumed carbohydrate foods. *European Journal of Clinical Nutrition*, *60*(6), 763–769. https://doi.org/10.1038/sj.ejcn.1602380

Hiyoshi, T., Fujiwara, M., & Yam, Z. (2017). Postprandial hyperglycemia and postprandial hypertriglyceridemia in type 2 diabetes. *Journal of Biomedical Research*, *33*(1). https://doi.org/10.7555/jbr.31.20160164

Hossain, M. I., Islam, M. M., Wahed, M. A., Khatun, M., & Kabir, I. (2009). Lentil-based high protein diet is comparable to animal-based diet in respect to nitrogen absorption and nitrogen balance in malnourished children recovering from shigellosis. *Asia Pacific Journal of Clinical Nutrition*, *18*(1), 8–14. Retrieved from https://pubmed.ncbi.nlm.nih.gov/19329389/

Ipsos. (2010). *Factors Influencing Pulse Consumption in Canada* (pp. 1–102). Retrieved from https://www1.agric.gov.ab.ca/$Department/deptdocs.nsf/all/sis13117/$FILE/v3_factors_influencing_pulse_consumption_final_report_feb24_2010.pdf

Jahan-Mihan, A., Luhovyy, B. L., El Khoury, D., & Anderson, G. H. (2011). Dietary Proteins as Determinants of Metabolic and Physiologic Functions of the Gastrointestinal Tract. *Nutrients*, *3*(5), 574–603. https://doi.org/10.3390/nu3050574

Jain, M. G., Hislop, G. T., Howe, G. R., & Ghadirian, P. (1999). Plant Foods, Antioxidants, and Prostate Cancer Risk: Findings From Case-Control Studies in Canada. *Nutrition and Cancer*, *34*(2), 173–184. https://doi.org/10.1207/s15327914nc3402_8

Jarpa-Parra, M. (2017). Lentil protein: a review of functional properties and food application. An overview of lentil protein functionality. *International Journal of Food Science & Technology*, *53*(4), 892–903. https://doi.org/10.1111/ijfs.13685

Jenkins, D. J. A., Kendall, C. W. C., Augustin, L. S. A., Mitchell, S., Sahye-Pudaruth, S., Blanco Mejia, S., … Josse, R. G. (2012). Effect of Legumes as Part of a Low Glycemic Index Diet on Glycemic Control and Cardiovascular Risk Factors in Type 2 Diabetes Mellitus. *Archives of Internal Medicine*, *172*(21), 1653. https://doi.org/10.1001/2013.jamainternmed.70

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., … Goff, D. V. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, *34*(3), 362–366. https://doi.org/10.1093/ajcn/34.3.362

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H. M., & Fielden, H. (1980). Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. *British Medical Journal*, *281*(6240), 578–580. https://doi.org/10.1136/bmj.281.6240.578

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Griffiths, C., Krzeminska, K., Lawrie, J. A., … Bloom, S. R. (1982). Slow release dietary carbohydrate improves second meal tolerance. *The American Journal of Clinical Nutrition*, *35*(6), 1339–1346. https://doi.org/10.1093/ajcn/35.6.1339

Jeong, D., Han, J.-A., Liu, Q., & Chung, H.-J. (2019). Effect of processing, storage, and modification on in vitro starch digestion characteristics of food legumes: A review. *Food Hydrocolloids*, *90*, 367–376. https://doi.org/10.1016/j.foodhyd.2018.12.039

Jeske, S., Bez, J., Arendt, E. K., & Zannini, E. (2019). Formation, stability, and sensory characteristics of a lentil-based milk substitute as affected by homogenisation and pasteurisation. *European Food Research and Technology*, *245*(7), 1519–1531. https://doi.org/10.1007/s00217-019-03286-0

Joshi, M., Timilsena, Y., & Adhikari, B. (2017). Global production, processing and utilization of lentil: A review. *Journal of Integrative Agriculture*, *16*(12), 2898–2913. https://doi.org/10.1016/s2095-3119(17)61793-3

Kahlon, T. S., Berrios, J. D. J., Chiu, M. C., & Pan, J. L. (2014). Relative Bile Acid Binding

Potential of Extruded Lentil Snacks. *Food and Nutrition Sciences*, *05*(04), 361–365. https://doi.org/10.4236/fns.2014.54043

Kastorini, C.-M., Milionis, H. J., Esposito, K., Giugliano, D., Goudevenos, J. A., & Panagiotakos, D. B. (2011). The Effect of Mediterranean Diet on Metabolic Syndrome and its Components. *Journal of the American College of Cardiology*, *57*(11), 1299–1313. https://doi.org/10.1016/j.jacc.2010.09.073

Kathirvel, P., Yamazaki, Y., Zhu, W., & Luhovyy, B. L. (2019). Glucose release from lentil flours digested in vitro: The role of particle size. *Cereal Chemistry*, *96*(6), 1126–1136. https://doi.org/10.1002/cche.10223

Koloverou, E., Esposito, K., Giugliano, D., & Panagiotakos, D. (2014). The effect of Mediterranean diet on the development of type 2 diabetes mellitus: A meta-analysis of 10 prospective studies and 136,846 participants. *Metabolism*, *63*(7), 903–911. https://doi.org/10.1016/j.metabol.2014.04.010

Leathwood, P., & Pollet, P. (1988). Effects of slow release carbohydrates in the form of bean flakes on the evolution of hunger and satiety in man. *Appetite*, *10*(1), 1–11. https://doi.org/10.1016/s0195-6663(88)80028-x

Lentils.org. (2015, June 9). Lentil Production. Retrieved October 14, 2022, from Lentils.org website: https://www.lentils.org/about-lentils/lentil-production

Liu, W., Jin, Y., Wilde, P. J., Hou, Y., Wang, Y., & Han, J. (2020). Mechanisms, physiology, and recent research progress of gastric emptying. *Critical Reviews in Food Science and Nutrition*, 1–14. https://doi.org/10.1080/10408398.2020.1784841

Lobos, D. R., Vicuña, I. A., Novik, V., & Vega, C. A. (2017). Effect of high and low glycemic

index breakfast on postprandial metabolic parameters and satiety in subjects with type 2 diabetes mellitus under intensive insulin therapy: Controlled clinical trial. *Clinical Nutrition ESPEN*, *20*, 12–16. https://doi.org/10.1016/j.clnesp.2017.04.082

Luhovyy, B. L., Hamilton, A., Kathirvel, P., & Mustafaalsaafin, H. (2017). The Effect of Navy Bean Flour Particle Size on Carbohydrate Digestion Rate Measured in Vitro. *Cereal Foods World*, *62*(5), 208–213. https://doi.org/10.1094/cfw-62-5-0208

Luhovyy, B. L., Mollard, R. C., Panahi, S., Nunez, M. F., Cho, F., & Anderson, G. H. (2015). Canned Navy Bean Consumption Reduces Metabolic Risk Factors Associated with Obesity. *Canadian Journal of Dietetic Practice and Research*, *76*(1), 33–37. https://doi.org/10.3148/cjdpr-2014-030

Lytvyak, E., Straube, S., Modi, R., & Lee, K. K. (2022). Trends in obesity across Canada from 2005 to 2018: a consecutive cross-sectional population-based study. *Canadian Medical Association Open Access Journal*, *10*(2), E439–E449. https://doi.org/10.9778/cmajo.20210205

Malcolmson, L., Boux, G., Bellido, A.-S. ., & Frohlich, P. (2013). Use of Pulse Ingredients to Develop Healthier Baked Products. *Cereal Foods World*, *58*(1), 27–32. https://doi.org/10.1094/cfw-58-1-0027

Mandalari, G., Merali, Z., Ryden, P., Chessa, S., Bisignano, C., Barreca, D., … Waldron, K. W. (2016). Durum wheat particle size affects starch and protein digestion in vitro. *European Journal of Nutrition*, *57*(1), 319–325. https://doi.org/10.1007/s00394-016-1321-y

Marchini, M., Carini, E., Cataldi, N., Boukid, F., Blandino, M., Ganino, T., … Pellegrini, N. (2021). The use of red lentil flour in bakery products: How do particle size and substitution

level affect rheological properties of wheat bread dough? *LWT*, *136*(1), 110299. https://doi.org/10.1016/j.lwt.2020.110299

Maskus, H., Bourré, L., Fraser, S., Sarkar, A., & Malcolmson, L. (2016). Effects of Grinding Method on the Compositional, Physical, and Functional Properties of Whole and Split Yellow Pea Flours. *Cereal Foods World*, *61*(2), 59–64. https://doi.org/10.1094/cfw-61-2-0059

Mattes, M. Z., & Vickers, Z. M. (2018). Better-liked foods can produce more satiety. *Food Quality and Preference*, *64*, 94–102. https://doi.org/10.1016/j.foodqual.2017.10.012

Meigs, J. B., Nathan, D. M., D'Agostino, R. B., & Wilson, P. W. F. (2002). Fasting and Postchallenge Glycemia and Cardiovascular Disease Risk: The Framingham Offspring Study. *Diabetes Care*, *25*(10), 1845–1850. https://doi.org/10.2337/diacare.25.10.1845

Melini, F., Melini, V., Luziatelli, F., & Ruzzi, M. (2017). Current and Forward-Looking Approaches to Technological and Nutritional Improvements of Gluten-Free Bread with Legume Flours: A Critical Review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(5), 1101–1122. https://doi.org/10.1111/1541-4337.12279

Miao, L., Deng, G.-X., Yin, R.-X., Nie, R.-J., Yang, S., Wang, Y., & Li, H. (2019). No causal effects of plasma homocysteine levels on the risk of coronary heart disease or acute myocardial infarction: A Mendelian randomization study. *European Journal of Preventive Cardiology*, *28*(2), 227–234. https://doi.org/10.1177/2047487319894679

Mills, P. K., Beeson, W. L., Abbey, D. E., Fraser, G. E., & Phillips, R. L. (1988). Dietary habits and past medical history as related to fatal pancreas cancer risk among adventists. *Cancer*,

61(12), 2578–2585. https://doi.org/10.1002/1097-0142(19880615)61:12<2578::aid-cncr2820611232>3.0.co;2-0

Mills, P. K., Beeson, W. L., Phillips, R. L., & Fraser, G. E. (1989). Cohort study of diet, lifestyle, and prostate cancer in Adventist men. *Cancer*, *64*(3), 598–604. https://doi.org/10.1002/1097-0142(19890801)64:3<598::aid-cncr2820640306>3.0.co;2-6

Mollard, R. C., Luhovyy, B. L., Panahi, S., Nunez, M., Hanley, A., & Anderson, G. H. (2012). Regular consumption of pulses for 8 weeks reduces metabolic syndrome risk factors in overweight and obese adults. *The British Journal of Nutrition*, *108 Suppl 1*, S111-22. https://doi.org/10.1017/S0007114512000712

Mollard, R. C., Wong, C. L., Luhovyy, B. L., & Anderson, G. H. (2011). First and second meal effects of pulses on blood glucose, appetite, and food intake at a later meal. *Applied Physiology, Nutrition, and Metabolism*, *36*(5), 634–642. https://doi.org/10.1139/h11-071

Mollard, R. C., Wong, C. L., Luhovyy, B. L., Cho, F., & Anderson, G. H. (2014). Second-meal effects of pulses on blood glucose and subjective appetite following a standardized meal 2 h later. *Applied Physiology, Nutrition, and Metabolism*, *39*(7), 849–851. https://doi.org/10.1139/apnm-2013-0523

Mollard, R. C., Zykus, A., Luhovyy, B. L., Nunez, M. F., Wong, C. L., & Anderson, G. H. (2011). The acute effects of a pulse-containing meal on glycaemic responses and measures of satiety and satiation within and at a later meal. *British Journal of Nutrition*, *108*(3), 509–517. https://doi.org/10.1017/s0007114511005836

Monnier, L., & Colette, C. (2009). Target for Glycemic Control: Concentrating on glucose. *Diabetes Care*, *32*(suppl_2), S199–S204. https://doi.org/10.2337/dc09-s310

Monro, J. A., & Shaw, M. (2008). Glycemic impact, glycemic glucose equivalents, glycemic index, and glycemic load: definitions, distinctions, and implications. *The American Journal of Clinical Nutrition*, *87*(1), 237S243S. https://doi.org/10.1093/ajcn/87.1.237s

Mudryj, A. N., Yu, N., Hartman, T. J., Mitchell, D. C., Lawrence, F. R., & Aukema, H. M. (2012). Pulse consumption in Canadian adults influences nutrient intakes. *British Journal of Nutrition*, *108*(S1), S27–S36. https://doi.org/10.1017/s0007114512000724

Murty, C. M., Pittaway, J. K., & Ball, M. J. (2010). Chickpea supplementation in an Australian diet affects food choice, satiety and bowel health. *Appetite*, *54*(2), 282–288. https://doi.org/10.1016/j.appet.2009.11.012

Naseer, S., Arshad, M. U., Imran, A., Arshad, M. S., Saeed, F., Ahmad, A., … Naeem, U. (2019). Acute effect of pulses based functional muffins on food intake, postprandial glucose and appetite regulation in healthy females. *Progress in Nutrition*, *21*(1-S), 378–387. https://doi.org/10.23751/pn.v21i1-S.6782

Nirmalan, N., & Nirmalan, M. (2017). Hormonal control of metabolism: regulation of plasma glucose. *Anaesthesia & Intensive Care Medicine*, *18*(10), 502–507. https://doi.org/10.1016/j.mpaic.2017.06.019

Obesity Canada. (2020, August 4). Epidemiology of Adult Obesity. Retrieved October 13, 2022, from Obesity Canada website: https://obesitycanada.ca/guidelines/epidemiology/

Osundiji, M. A., & Evans, M. L. (2013). Brain Control of Insulin and Glucagon Secretion. *Endocrinology and Metabolism Clinics of North America*, *42*(1), 1–14. https://doi.org/10.1016/j.ecl.2012.11.006

Ottawa: Diabetes Canada. (2022). *Diabetes in Canada: Backgrounder* (pp. 1–5). Retrieved from

https://www.diabetes.ca/DiabetesCanadaWebsite/media/Advocacy-and-Policy/Backgrounder/2022_Backgrounder_Canada_English_1.pdf

Papandreou, C., Becerra-Tomás, N., Bulló, M., Martínez-González, M. Á., Corella, D., Estruch, R., … Salas-Salvadó, J. (2019). Legume consumption and risk of all-cause, cardiovascular, and cancer mortality in the PREDIMED study. *Clinical Nutrition*, *38*(1), 348–356. https://doi.org/10.1016/j.clnu.2017.12.019

Papanikolaou, Y., & Fulgoni, V. L. (2008). Bean Consumption Is Associated with Greater Nutrient Intake, Reduced Systolic Blood Pressure, Lower Body Weight, and a Smaller Waist Circumference in Adults: Results from the National Health and Nutrition Examination Survey 1999-2002. *Journal of the American College of Nutrition*, *27*(5), 569–576. https://doi.org/10.1080/07315724.2008.10719740

Pasqualone, A., Costantini, M., Coldea, T. E., & Summo, C. (2020). Use of Legumes in Extrusion Cooking: A Review. *Foods*, *9*(7), 958. https://doi.org/10.3390/foods9070958

Patterson, C. A., Curran, J., & Der, T. (2017). Effect of Processing on Antinutrient Compounds in Pulses. *Cereal Chemistry Journal*, *94*(1), 2–10. https://doi.org/10.1094/cchem-05-16-0144-fi

Phillips, T., Zello, G. A., Chilibeck, P. D., & Vandenberg, A. (2015). Perceived Benefits and Barriers Surrounding Lentil Consumption in Families with Young Children. *Canadian Journal of Dietetic Practice and Research*, *76*(1), 3–8. https://doi.org/10.3148/cjdpr-2014-025

Pulse Canada. (2022a). Pulse Flours. Retrieved December 5, 2022, from Pulse Canada website: https://pulsecanada.com/processing/pulse-flour

Pulse Canada. (2022b). What is a Pulse? Retrieved October 13, 2022, from Pulse Canada website: https://pulsecanada.com/pulse/what-is-a-pulse

Ramdath, D., Wolever, T., Siow, Y., Ryland, D., Hawke, A., Taylor, C., … Aliani, M. (2018). Effect of Processing on Postprandial Glycemic Response and Consumer Acceptability of Lentil-Containing Food Items. *Foods*, *7*(5), 76. https://doi.org/10.3390/foods7050076

Rebello, C. J., Greenway, F. L., & Finley, J. W. (2014a). A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. *Obesity Reviews*, *15*(5), 392–407. https://doi.org/10.1111/obr.12144

Rebello, C. J., Greenway, F. L., & Finley, J. W. (2014b). Whole grains and pulses: a comparison of the nutritional and health benefits. *Journal of Agricultural and Food Chemistry*, *62*(29), 7029–7049. https://doi.org/10.1021/jf500932z

Saputo food service. (n.d.). Seizing opportunities in the pizza industry | Saputo Foodservice. Retrieved October 14, 2022, from www.saputofoodservice.ca website: https://www.saputofoodservice.ca/en/resources/seizing-growth-opportunities-pizza-industry

Schäfer, G., Schenk, U., Ritzel, U., Ramadori, G., & Leonhardt, U. (2003). Comparison of the effects of dried peas with those of potatoes in mixed meals on postprandial glucose and insulin concentrations in patients with type 2 diabetes. *The American Journal of Clinical Nutrition*, *78*(1), 99–103. https://doi.org/10.1093/ajcn/78.1.99

Schwartz, M. W., Seeley, R. J., Tschöp, M. H., Woods, S. C., Morton, G. J., Myers, M. G., & D'Alessio, D. (2013). Cooperation between brain and islet in glucose homeostasis and diabetes. *Nature*, *503*(7474), 59–66. https://doi.org/10.1038/nature12709

Shafaeizadeh, S., Muhardi, L., Henry, C., van de Heijning, B., & van der Beek, E. (2018). Macronutrient Composition and Food Form Affect Glucose and Insulin Responses in Humans. *Nutrients*, *10*(2), 188. https://doi.org/10.3390/nu10020188

Shams, H., Tahbaz, F., Entezari, M.H., & Abadi, A. (2008). EFFECTS OF COOKED LENTILS ON GLYCEMIC CONTROL AND BLOOD LIPIDS OF PATIENTS WITH TYPE 2 DIABETES. *ARYA atherosclerosis*, *4*, 215-218.

Singh, M., Manickavasagan, A., Shobana, S., & Mohan, V. (2020). Glycemic index of pulses and pulse-based products: a review. *Critical Reviews in Food Science and Nutrition*, *61*(9), 1567–1588. https://doi.org/10.1080/10408398.2020.1762162

Siva, N., Johnson, C. R., Richard, V., Jesch, E. D., Whiteside, W., Abood, A. A., … Thavarajah, D. (2018). Lentil (*Lens culinaris* Medikus) Diet Affects the Gut Microbiome and Obesity Markers in Rat. *Journal of Agricultural and Food Chemistry*, *66*(33), 8805–8813. https://doi.org/10.1021/acs.jafc.8b03254

Siva, N., Thavarajah, D., Johnson, C. R., Duckett, S., Jesch, E. D., & Thavarajah, P. (2017). Can lentil (Lens culinaris Medikus) reduce the risk of obesity? *Journal of Functional Foods*, *38*, 706–715. https://doi.org/10.1016/j.jff.2017.02.017

Statistics Canada. (2019). *Overweight and obese adults, 2018. [Fact sheet]*. Retrieved from https://www150.statcan.gc.ca/n1/en/pub/82-625-x/2019001/article/00005-eng.pdf?st=o9JTKWzT

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*, *29*(1), 71–83. https://doi.org/10.1016/0022-3999(85)90010-8

Szablewski, L. (2011). Glucose Homeostasis – Mechanism and Defects. *Diabetes - Damages and Treatments*, *12*. https://doi.org/10.5772/22905

Technomic. (2020). *Technomic's 2020 Canadian Pizza Consumer Trend Report*. Retrieved from https://www.technomic.com/canadian-pizza

Tiwari, B. K., Gowen, A., & McKenna, B. (2011). *Pulse Foods*. Academic Press.

Toh, D. W. K., Koh, E. S., & Kim, J. E. (2019). Lowering breakfast glycemic index and glycemic load attenuates postprandial glycemic response: A systematically searched meta-analysis of randomized controlled trials. *Nutrition*, *71*, 110634. https://doi.org/10.1016/j.nut.2019.110634

Tovar, Juscelino., Granfeldt, Yvonne., & Bjoerck, I. M. (1992). Effect of processing on blood glucose and insulin responses to starch in legumes. *Journal of Agricultural and Food Chemistry*, *40*(10), 1846–1851. https://doi.org/10.1021/jf00022a024

Venn, B. J., Perry, T., Green, T. J., Skeaff, C. M., Aitken, W., Moore, N. J., … Williams, S. (2010). The Effect of Increasing Consumption of Pulses and Wholegrains in Obese People: A Randomized Controlled Trial. *Journal of the American College of Nutrition*, *29*(4), 365–372. https://doi.org/10.1080/07315724.2010.10719853

Wolever, T. M., & Bolognesi, C. (1996). Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index. *The Journal of Nutrition*, *126*(11), 2807–2812. https://doi.org/10.1093/jn/126.11.2807

Wong, C. L., Mollard, R. C., Zafar, T. A., Luhovyy, B. L., & Anderson, G. H. (2009). Food Intake and Satiety Following a Serving of Pulses in Young Men: Effect of Processing, Recipe,

and Pulse Variety. *Journal of the American College of Nutrition*, *28*(5), 543–552. https://doi.org/10.1080/07315724.2009.10719786

Wood, J. A., & Malcolmson, L. J. (2011). Pulse milling technologies. *Pulse Foods*, 193–221. https://doi.org/10.1016/b978-0-12-382018-1.00008-3

World Health Organization. (2021, April 13). Noncommunicable Diseases. Retrieved October 13, 2022, from World Health Organisation website: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

World Health Organization. (2022, February 3). Cancer. Retrieved October 13, 2022, from World Health Organization: WHO website: https://www.who.int/health-topics/cancer#tab=tab_1

Zabor, E. C., Kaizer, A. M., & Hobbs, B. P. (2020). Randomized Controlled Trials. *CHEST*, *158*(1), S79–S87. https://doi.org/10.1016/j.chest.2020.03.013

Zafar, T. A., Al-Hassawi, F., Al-Khulaifi, F., Al-Rayyes, G., Waslien, C., & Huffman, F. G. (2013). Organoleptic and glycemic properties of chickpea-wheat composite breads. *Journal of Food Science and Technology*, *52*(4), 2256–2263. https://doi.org/10.1007/s13197-013-1192-7

Zucco, F., Borsuk, Y., & Arntfield, S. D. (2011). Physical and nutritional evaluation of wheat cookies supplemented with pulse flours of different particle sizes. *LWT - Food Science and Technology*, *44*(10), 2070–

Appendix 1 Study poster

Department of Applied Human Nutrition

Male and Female Participants Needed!

Would you like to participate in a research at MSVU Appetite Lab?

The purpose of this study is to learn more about new snack products formulated with lentil and wheat and their effect on blood sugar.

Requirements:

19-35-years-old healthy males and females
Non-smokers

Involves:

Information session
4 food sessions over 4 weeks
3.5 Hours per each food session
Blood collection
Eating pizza & meal @ food sessions

To thank you for your participation, you will receive $120 for completed study.
At three sessions you will be testing different types of pizza crust and the end of each visit, we will serve you with a hot meal!
You can also have chocolate milk or fruit juice after each session!

If you are interested in participating, please contact Appetite.Study@msvu.ca or leave a message at 902-457-6568 and we will contact you

Appetite.Study@msvu.ca 902-457-6568 @msvufoodstudy

Appendix 2 Telephone screening questionnaire (part 1)

Telephone Screening Questionnaire (part 1)

Study title: The Effect of Pulse Flours on Satiety and Food Intake
Study title: The Effect of Pulse Flours on Blood Glucose

TO BE KEPT SEPARATELY FROM DATA FORMS

Name:
_____

Month and year of birth _____/_____ (_____ y: calculated by recruiter)

To be completed by Staff: Eligible to participate: Yes ____ No____
If not eligible, this concludes the conversation and the form is to be shredded.

If eligible, continue with the remaining parts 1 and 2.

Address:

_____

_____

_____

Cell phone: _____ Can we send text messages? _____ Yes ___ No

Home phone: (_____) _____ What time would be convenient to call you? _____

E-mail: _____@_____

Participant ID assigned: _____

Appendix 3 Telephone screening questionnaire (part 2)

Telephone Screening Questionnaire (part 2)

Study title: The Effect of Pulse Flours on Satiety and Food Intake

Study title: The Effect of Pulse Flours on Blood Glucose

ID: _____

Weight (circle the correct unit): _____ lbs kg Height :_____cm (Calculated by recruiter) BMI:_____ kg/m^2

Do you regularly consume breakfast? Yes__ No___

Are you physically active? Yes ___ No___

Can you briefly recall your daily normal physical activity routine?

_____

_____

_____

Do you consume cannabis? Yes___ No___ Do you smoke tobacco? Yes__ No__

Do you have any allergies to any foods? Yes __ No__

If yes, are you allergic to any of the following wheat, lentil, dairy, eggs, or other foods? Yes ___ No___

Other foods? _____

Are you on a special diet? Yes ____ No____ If yes, please specify: _____

How many alcoholic beverages do you consume per day? _____ per week? _____

Do you have any major disease or medical conditions? Yes ____ No____ If yes, please specify: _____

_____

Do you take medications? Yes ____ No____ If yes, please specify: _____

_____

Do you like snack bars? Yes ____ No____

Do you like pizza? Yes ____ No____ If yes, please rank these types of pizza with (1) the first choice, (2) second choice, and (3) third choice: Pepperoni (Cheese, pepperoni) _____, Deluxe (cheese, pepperoni, peppers, mushrooms) _____, Three Cheese (mozzarella, cheddar, parmesan) _____

Do you like macaroni & cheese (e.g., Kraft Dinner)? Yes ___ No ___

To be completed by Staff: Eligible to participate: Yes ____ No____

Screening scheduled at: _____

Appendix 4 Information sheet & consent form

Information Sheet and Consent Form

Study title: The Effect of Lentils on Blood Glucose, Satiety and Food Intake

Investigators:

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Introduction:

You are invited to participate in the research study listed above. This form provides you with information about the study so you can make an informed decision about if you would like to

participate. It will enable you to understand the purpose of the study, the risks and benefits of participating, and what you will be asked to do should you choose to participate. We will keep you informed of any new information that may influence your willingness to continue to participate in the study. A member of the research team will be available to answer any questions you may have. You may decide not to participate and you may withdraw from the study at any time. Your participation in the study is entirely voluntary.

Funding Source:

Funding for this project is provided by the Mount Saint Vincent University Internal Research Grant.

Background and Purpose of Research:

The consumption of pulses (beans, lentils, peas and chickpeas) provide multiple benefits for health including healthier body weight, lower risk of diabetes and improved nutrient intake. Pulses are rich source of protein, dietary fibre, iron, potassium, vitamins B1, B2, B3 and folate. Previous studies with human participants have shown the benefits of pulses on satiety, blood glucose control and nutrient intake. However, the consumption of pulses in Canada is low. Canadian pulse industry offers the variety of products derived from pulses including pulse flours. There are many potential uses for these flours including gluten-free foods and special food products high in fibre and protein. This project will examine the effect of baked products formulated with lentil and wheat flours on blood glucose control in young healthy adults. This study will have 20 male and female participants.

Invitation to Participate:

You are being invited to take part in this study. If you chose to take part and meet eligibility criteria, you will be asked to eat a treatment of pizza crust, or plain water four times (four sessions) with each session one week apart. At each session, your blood will be collected by a registered nurse and your appetite will be measured using simple paper scales after eating the treatment. Each session will take up to three and a half hours of your time.

Eligibility:

To participate in this study, you must be considered overall healthy, and not being underweight, overweight or obese, and not having any diseases. You must also be between the ages of 19 and

35. You must be a non-smoker (including e-cigarettes) and you cannot be taking certain medications and consume cannabis products. You will not be able to participate if you have allergies to any food or if you usually skip breakfast. To find out if you can take part in this study, you will be asked to fill out questionnaires, which ask questions about your age, if you smoke, exercise, your health, if you are on any medications and your eating habits. Your height and weight will be measured. Female participants will be asked to attend experimental sessions during the same time of their menstrual cycle and therefore will be asked to provide information about their menstrual cycle at the screening session. The study will take place in the Department of Applied Human Nutrition, Room 211 in the Centre of Applied Research, Mount Saint Vincent University, 47 College Rd, Halifax, NS.

Procedure:

You will be asked to fast for at least 10 hours overnight before each session and arrive to our laboratory between 8 am and 10 am in the morning. However, you may drink water until one hour before arriving to the laboratory. Please note that you will have to arrive exactly the same time at each of four sessions. Upon arrival, we will ask you about your recent food intake, stress level, sleep and physical activity. You will then be asked to have a blood sample taken to measure your fasting blood glucose levels. Registered nurse will insert a tiny cannula (tubing) into your arm vein so we will not need to poke you every time we need to take a blood sample. This cannula will stay in your vein for two hours. If your blood glucose will be higher than normal fasting level (higher than 6 mmol/L), we will repeat the test within 30 min and if it still be high then we will need to reschedule the session. Similarly, if you had experienced stress, unusual food intake, alcohol consumption, did not sleep enough or had extra physical activity in the day before your session, we will need to reschedule your session as well. If your fasting blood glucose level is normal and you did not experience any unusual stress, sleep deprivation, extra physical activity, excessive food intake and alcohol consumption in previous day, the session will commence. We will ask you to complete the questionnaire related to your appetite and physical comfort, and then will take you to the feeding cubicles and will serve you with a baked product with a glass of water (in three sessions) or just the glass of water (in one session). You will have up to 12 minutes to consume your food and water and evaluate their taste. You will be asked to consume the whole amount of food and water provided. Then you will be taken back to the Appetite Lab and you can read, study

or use your computer during the next 112 minutes. Please note that you may not browse any content related to food or eating. Registered nurse will be taking 9 blood samples over the next 3 hours, at 10, 20, 30, 45, 60, 90, 120,150, and 180 minutes. Our research assistants will ask you to complete the same questionnaires related to your appetite and physical comfort at the same time points. At 180 min, we will ask you to proceed to the feeding cubicles and will serve you with a macaroni & cheese meal. You will be asked to eat until you feel comfortable full. The new tray with macaroni & cheese meal and new bottle with spring water will be provided every 8 minutes and the previous tray and water bottle with the left over (if any) will be taken back by our research assistant. Once you completed your meal, we will ask you to fill out the questionnaire rating your appetite and physical comfort. Then you will be offered to drink a glass of chocolate milk or fruit juice of your choice and you are free to go.

An Example of a Potential Time and Activity Schedule for Each Session:

Time	Activity
8:45	Arrive at the laboratory
8:50	Fill in Sleep, Stress, and VAS questionnaires and take fingerpick blood sample, IV catheter is inserted by nurse and first blood sample is drawn.
9:00 - 9:12	Star consuming the treatment at 0 min
9:15 - 12:00	The nurse will collect blood samples and you will complete VAS questionnaires at 15, 30, 45, 60, 90, 120 min, 150 and 180 min
12:00 – 11:20	Eat the lunch meal until you feel comfortably full
12:20	The session is completed. You will receive a glass of chocolate milk or fruit juice

VAS= Visual analogue scale

Voluntary Participation and Early Withdrawal:

Participation in this study is voluntary. You may choose to stop being in the study at any time without any negative consequences.

Risks:

There is a very little risk of food poisoning. All of the foods that you will be asked to consume will be prepared using practices to ensure food safety. Therefore, your risk of developing a food borne illness from participation in this study is very minimal. All blood samples gathered will be done so by a trained Nurse, and all samples will be collected using aseptic techniques in a hygienic environment. Each sample will be 5.0 ml, for a total of less than 70 ml, far less than that taken when donating blood. (Standard donation is approximately 450 ml as per Canadian Blood Services: https://www.blood.ca/en/blood/donating-blood/donation-process). After the overnight fast you may feel faint or dizzy, however the risk of this is minimal.

Benefits:

You will not benefit directly from taking part in this study. However, the study results will advance nutritional science and may lead to practical dietetic recommendations.

Confidentiality and Privacy:

Confidentiality will be respected and no information that shows your identity will be released or published without your permission unless required by law. Your name, personal information and signed consent form will be kept in a locked filing cabinet in the investigator's office. Your results will not be kept in the same place as your name. Your results will be recorded on data sheets and in computer records that have an ID number for identification but will not include your name. Your results, identified only by an ID number, will be made available to the study sponsor if requested. Only study investigators will have access to your individual results. If you withdraw from the study, your consent form and any other paperwork associated with your ID number will be destroyed.

Publication of Results:

The results of the study may be presented at scientific meetings and published in a scientific journal. If the results are published, no information about individuals will be reported.

New Findings:

If anything is found during the course of this research which may change your decision to continue, you will be told about it.

Compensation:

You will be paid $30 per experimental session with a total of $120 for completed study. Payment will be in the form of cash and can be paid either after each session or after the end of the study. Please note that in order to process your compensation the collection of your personal information including your full name, current mailing address and signature is required by the university's financial services department. This information is collected, stored and accessible to the financial services department alone other than the initial collection and is not linked to the current study in any way.

Injury Statement:

If you begin to feel sick following participation in the study, please seek medical advice as soon as possible. We will provide your medical specialist with information about the food you have consumed during the session. You can send the email to Dr. Luhovyy (Bohdan.Luhovyy@msvu.ca), call his cell phone 902-221-3810 and leave your question and contact information and we will contact you back at the earliest convenience.

Rights of Participants:

Before agreeing to take part in this research study, it is important that you read and understand your role as described here in this study information sheet and consent form. You waive no legal rights by taking part in this study. If you have questions about how this study is being conducted and wish to speak with someone not involved in the study, you may contact the Chair of the University Research Ethics Board (UREB) c/o MSVU Research Office, at 457-6350 or via e-mail at research@msvu.ca

The ethical components of this research study have been reviewed by the University Research Ethics Board and found to be in compliance with Mount Saint Vincent University's Research Ethics Policy.

Dissemination of findings:

A summary of results will be made available for you to pick up in one year after the study is done.

Copy of informed consent for participant:

You will be given a copy of this informed consent to keep for your own records.

Consent form

TO BE KEPT SEPARATELY FROM DATA FORMS

Study title: The Effect of Pulse Flours on Blood Glucose

Participant ID assigned: _____

PARTICIPANT AUTHORIZATION:

I have read or had read to me this information and authorization form and have had the chance to ask questions which have been answered to my satisfaction before signing my name. I understand the nature of the study and I understand the potential risks. I understand that I have the right to withdraw from the study at any time without any problems. I have received a copy of the Information and Authorization Form for future reference. I understand that to receive compensation I will need to provide personal information including full name, current mailing address and a signature on the compensation form which will be returned to the university's financial services department. I freely agree to participate in this research study.

Would you like to receive a summary of the results when they are available? Yes ___ No ____.

Would you like to be contacted for future research? Yes ___ No____.

Name of Participant: (Print)

_____

Date: _____ Time: _____ Participant ID:

_____

Sex _____ M _____ F, Age ____ (y). Month and year of birth (mm/yyyy) _____

If you would like to receive the summary of the results and/or be contacted for future research, please print your address below:

_____
_____

Participant Signature: _____

Date: _____ Time: _____

STATEMENT BY PERSON PROVIDING INFORMATION ON STUDY AND OBTAINING CONSENT

I have explained the nature and demands of the research study and judge that the participant named above understands the nature and demands of the study. I have explained the nature of the consent process to the participant and judge that they understand that participation is voluntary and that they may withdraw at any time from participating.

Name: (Print) _____ Signature: _____ Position: _____

Date: _____ Time: _____

Appendix 5 Health and Activity questionnaire

ID_____ **Date: _____** **Time_____**

Treatment: _____ **Session: _____**

Health and Activity Questionnaire

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

1. Did you have a normal sleep last night?
   Yes___ No____

2. How many hours of sleep?
   _____

3. What time did you go to bed last night?
   _____

4. What time did you wake up?
   _____

5. Did you do any physical activities outside your normal routine?
   Yes ___ No___

6. Are you have feelings of illness or discomfort, other than hunger?
   Yes ___ No ___

If yes, please describe briefly:

_____

_____

Are you experiencing any unusual stress? (E.g. exams, reports, work deadlines, personal, etc…)

Yes___ No___

Are you on any medication?

Yes__ No__

If yes, what medication(s) are you taking?

Have you had something to eat or drink, other than water for the past 10-12 hours?

Yes___ No ___

What time did you have dinner? _____

Please describe your dinner last night (list all food and drink and give an estimate of the portion size):

_____

_____

_____

The following three questions relate to your food intake, activity and stress over the last 24 hours. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

How would you describe your food intake over the past 24 hours?

Much LESS _____ Much MORE

than usual than usual

How would you describe your level of activity over the last 24 hours?

Much LESS _____ Much MORE

than usual than usual

How would you describe your level of stress over the last 24 hours?

Much LESS _____ Much MORE

than usual than usual

To be completed by Staff:

Blood glucose reading: _____mmol/L

Assessed by: Name (print) _____

Appendix 6 VAS questionnaires (Motivation to eat & Physical comfort)

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

Visual Analogue Scale

Motivation to Eat

DATE: _____ Session _____

Treatment ID ____

ID: _____ Time point: ___ min

These questions relate to your "motivation to eat" at this time. Please rate yourself by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very WEAK	———————————————————	Very STRONG

2. How hungry do you feel?

Not hungry at all	———————————————————	As hungry as I have ever felt

3. How full do you feel?

Not full at all ——————————————————— Very full

4. How much food do you think you could eat?

NOTHING at all ——————————————————— A LARGE amount

Lentil and wheat flours in snack formulations: effect on satiety and food intake

Visual Analogue Scale

Physical Comfort

DATE: _____ Session _____

Treatment ID ____

ID: _____ Time point: ___ min

These questions relate to your "**stomach**" and **general feeling** at this time. Please rate yourself by placing a small "x" across the horizontal line at the point which best reflects your present feelings.

1. Do you feel nauseous?

NOT at all _____ VERY much

2. Does your stomach hurt?

NOT at all _____ VERY much

3. How well do you feel?

NOT well at all

VERY well

4. Do you feel like you have gas?

NOT at all VERY much

5. Do you feel like you have diarrhea?

NOT at all VERY much

Appendix 7 VAS and 9-point hedonic scales

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

Visual Analogue Scale

Pleasantness

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

This question relates to the palatability of the food you just consumed. Please rate the **pleasantness** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

How pleasant have you found the food?

NOT at all pleasant ——————————————————— VERY pleasant

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

Visual Analogue Scale

Sweetness

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

This question relates to the palatability of the food you just consumed. Please rate the **sweetness** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

NOT sweet ——————————————— VERY sweet

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

9-Point Hedonic Scale

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

Please put a checkmark on the most appropriate response

How much did you enjoy the **Taste** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

9-Point Hedonic Scale

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

Please put a checkmark on the most appropriate response

How much did you enjoy the **Texture** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

9-Point Hedonic Scale

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

Please put a checkmark on the most appropriate response

How much did you enjoy the **Flavour** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

9-Point Hedonic Scale

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

Please put a checkmark on the most appropriate response

How much did you enjoy the **Aftertaste** of the sample?

Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

Visual Analogue Scale

Chewiness

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

This question relates to the palatability of the food you just consumed. Please rate the **chewiness** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

NOT chewy ——————————————————— VERY chewy

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

Visual Analogue Scale

Lentil flavour

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

This question relates to the palatability of the food you just consumed. Please rate the **lentil flavour** of the food by placing a small "**x**" across the horizontal line at the point which best reflects your present feelings.

NO ——————————————————————— STRONG

lentil flavour lentil flavour

Lentil and wheat flours in pizza formulations: effect on blood glucose, satiety and food intake

Visual Analogue Scale

Prospective purchasing

DATE: _____ Session _____

Treatment ID ____

ID: _____ Actual time _____

This question relates to the palatability of the food you just consumed. **How likely would you purchase this product?** Place a small "**x**" across the horizontal line at the point which best reflects your present feeli

Would not purchase ——————————————————— Would purchase

Appendix 8 Compliance to Health Canada's draft guidance document for satiety

Study design and considerations		
	Satiety studies supporting a claim should include at least three experimental treatments as preloads: the test food, the control food or the reference food, and the energy-free control 5.2.2	√
	Each subject should get all preload and test meal treatments at the beginning and at the end of the study period where the test and reference/control foods are consumed frequently. It is recommended to allow for a reasonable wash out time between the different treatments. The duration of the study period must be defined and justified in the study protocol.5.2.3	√ ×
	Satiety studies should assess subjective ratings by the administration of the different scales of the VAS before and at selected time intervals after the administration of the preloads. 5.2.4	√
	The duration and intervals of the VAS measurements should be based on prior knowledge of the effects of the food or ingredient or established from preliminary tests and should be consistent with the type and consumption pattern of the food being tested 5.2.5	√
	The time of the day at which the test food is offered and the appropriateness of the food for that time of the day need to be considered 5.2.6	√
	Evidence for adequate statistical power and analyses should be provided when selecting a study design. Cross-over (within-subject) study designs are preferred 5.2.7	√
	The setting of the study should be reported, and the description of the experimental design should include identification of the randomization procedures 5.2.8	√
	Double blinding is the preferred method to avoid bias. When assessing the time to the next meal, study participants should also be time-blinded so that they are not influenced by pre-conceived notions of when the next meal should occur and by the associated subjective sensations of satiety 5.2.9	×

	It is recommended that antecedent levels of energy depletion and physical activity experienced by the study subjects be standardized prior to testing to allow proper interpretation of results. 5.2.10	√
Characterization of the Test and Reference Foods	The food being tested, and the reference food should be identified and described in terms of constituents (macro- and micronutrients), physical form (solid, semi-solid, or liquid), and amounts. The amount of the food tested, and the reference food should match the serving size as stated in the Nutrition Facts table. Preliminary tests should be conducted to demonstrate that palatability was not altered by the manipulation of the food and that the test and the reference foods are similarly liked by the study participants.5.3.1	√×
	The choice and appropriateness of the reference foods should be justified 5.3.3	√
	The energy-free control, water, should match the test food in organoleptic characteristics (such as sweetness, palatability) only when the latter is in liquid form. For solid foods, the energy-free control preload could be plain water. 5.3.4	√
Study Population	The study population should be adult individuals who are generally healthy and should be clearly defined in terms of subject inclusion/exclusion criteria 5.4.1	√
	All confounders in the subject recruitment, screening, selection and analysis must be stated and justified.5.4.2	√
	The basis for sample size calculations should be the ability to detect at least 10% difference in satiety rating for the primary outcome between the test and control foods (for comparative claims), with a statistical significance at $p \leq 0.05$ and a power (β) of at least 80% 5.6.1	√
	Assessment of the satiety response should be done based on the total area under the curve (AUC) of a set of measurements for each of the parameters considered in the study with adjustment for the baseline by analysis of covariance (ANCOVA) 5.6.4	√

Appendix 9 Compliance to Health Canada's Draft Guidance Document for the reduction in postprandial glycaemia

Characterization of test and reference foods	The reference food should be the food without substitution 3.2	√
	The amounts of reference and test food given in the study must be consistent with its serving size and intended pattern of consumption 4.1.2	√
	The test food and the reference food should be sufficiently characterised in terms of physical properties and nutrient composition to allow for evaluation and for a comparison to be made. 4.1.3	√
	the food should be given as usually prepared because of the effects that factors such as cooking, physical form (whole versus puréed) and particle size of food can have on the glycaemic response	√
Study design and considerations	Evidence should be based on *in vivo* human studies. 4.1.1	√
	The quality of studies is assessed against applicable standards such as clearly defined objectives, and appropriate outcome measures, participant selection, methods and procedures and statistical analyses. 4.1.4	√
	The study population should be adult individuals who are generally healthy and should be clearly defined in terms of subject inclusion/exclusion criteria. 4.2.1	√
Outcome Measures for Post-Prandial Glycaemic Response	Substantiation of health claims for the reduction of post-prandial glycaemic response is obtained from acute (single meal) human intervention studies. 4.3	√
	Generally, measurements should be taken for at least 2 hours, with higher frequency (for example, at 15-minute intervals) in the first hour, and 30 minutes thereafter. 4.3.3	√
	The data on insulin concentrations following the consumption of the test food should be provided to show that the decrease in blood glucose concentrations is not accompanied by disproportionately increased levels of insulin, in comparison to the reference food. 4.3.4	×

	The glycaemic and insulinaemic responses should be measured as the incremental area under the response curves (iAUC) above the baseline, according to the trapezoidal method. Peak level (highest level) and time to peak for blood glucose or insulin are not sufficient to measure response, but can be used as supportive data, when correlating with area under the curve. 4.3.5	√ ×

Appendix 10 Particle size analysis of what and lentil flour used in the study

Masterizer	Wheat flour	Lentil flour
d (0.1), µm	15.7	5.2
d (0.5), µm	72.5	19.3
d (0.9), µm	151.9	148.5
D (4,3), µm	78.8	50.2