Mount St. Vincent University
Department of Applied Human Nutrition

The effects of snacks formulated with wheat and lentil flour of similar particle size on food intake and satiety in young adults

by
Morgan Beatrice King BSc, MSc (c)

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Lentils are sustainable and affordable food source for Canadians. The consumption of lentils is associated with nutritional benefits that may reduce the risk factors associated with chronic diseases. The objective of this study was to understand the short-term of effects partial and full replacement of wheat flour with lentil flour in snack bars in young healthy men. The hypothesis of this study was the replacement of wheat flour with lentil flour in the snack bars lead to a reduced energy intake and higher satiety. The study design was a single-blinded, within subject, randomized design with repeated measures. Twenty-five males aged 19-35 completed four randomized sessions with one treatment per session (water control (C), 100% lentil flour (LF), 50% lentil 50% wheat flour (MLW-F), or 100% wheat flour snack bar (WF). Participants filled out 100 mm VAS questionnaires assessing the appetite, physical comfort, and palatability of the treatments. Subsequently at 120 minutes, participants consumed an pizza meal. Subjective appetite was affected by treatment ($p=0.0001$), time ($p=0.0001$), and treatment by time interaction ($p<0.0001$). The highest appetite suppression was observed for the LF treatment compared to C, as revealed by average appetite scores and the total area above the curve over 120 minutes ($p<0.05$). This difference was driven by a significant effect of a treatment on such appetite components as the desire to eat (DTE) and hunger ($p<0.05$). There was no effect of treatment on food intake at 120 minutes or cumulatively over 120 minutes. There was an inverse relationship observed for metabolic equivalence (METS) and food intake ($r=-0.39$, $p=0.05$). In conclusion, the total replacement of WF with LF of the same particle size provided benefits for appetite control over 120 minutes. The partial replacement of WF with LF lead to the suppression of average subjective appetite compared to C. Although there was no reduction in subsequent FI after the treatment with LF, none of the caloric treatments contributed to a higher food intake cumulatively over 120 minutes.

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List of Abbreviations

A1C - hemoglobin A1C
ACC - Anterior insula cingulate Cortex
ACE- Angiotensin-Converting Enzyme
AgRP- Agouti-related protein
AMPK- AMP-Activated kinase
ANOVA- Analysis of Variance
Apo B- Apolipoprotein B-100
ARC - Arcuate nucleus
AUC/AAC- Area under/above the curve
BAT- Brown adipose tissue
BMI- Body mass index
C- Control
CART- Amphetamine regulated transcript
CFIA- Canadian Food inspection agency
CIGI- Canadian international grain institute
CKK- Cholecystokinin
CMA- Canadian Medical Association
CNF- Canadian Nutrient File
CNS- Central Nervous system
CVD - Cardiovascular disease
DM - Diabetes Mellitus

Introduction

Canada is one of the largest producers of pulses globally (1). In 2017, Canada produced 7.1 million tonnes of pulses which support approximately 130 pulse markets globally (1). Pulses are edible seeds from legume pods (2) such as chickpeas, peanuts, beans, and lentils (2). Lentils ( ) are a sustainable and affordable food for Canadians (1). The regular consumption of lentils could provide Canadians with nutritional and health benefits. Lentils are known for their high protein and fibre content (1). Currently, research on pulses have reported numerous potential health benefits on blood glucose control (3–6) and body weight (8,9). Additionally, lentils contain bioactive compounds (10) that may reduce risks for chronic disease such as for diabetes mellitus(3–6,11) cardiovascular disease (10,12–20), and obesity (8,9,21)

Pulses have raised research inquires on their effects on satiety and short-term food intake (2,4,24–30). Satiety and satiation are two different sensations that arise postprandial following food intake. Satiety is the postprandial sensation that follows postprandial up until the subsequent meal, whereas satiation is the feeling of fullness during food intake that terminates food intake (4). These sensations can be affected depending on the type of food consumed. In fact, studies have reported protein and carbohydrates in conventional foods can affect satiation and satiety (31–33,33–35). Since lentils are high in protein and fibre, the ingestion of foods formulated with lentil flour may provide benefits to control subjective appetite and short-term food intake. The aim of this research was to investigate how partial and full replacement of wheat flour with lentil flour of similar particle size affects the satiety and short-term food intake in young healthy adults.

1. Literature Review

1.1.0 Prevalence of chronic diseases and lifestyle modifications

Chronic diseases are incurable diseases that require medical management to mitigate negative effects on an individual's health. Unfortunately, prevalence of chronic diseases are quite high in Canada. Currently, chronic diseases are responsible for 67% of deaths in Canada's population (36). Some of the common chronic diseases in Canada are diabetes mellitus (DM), cardiovascular disease (CVD), and obesity (36). Chronic diseases develop due to socioeconomic, genetic, and lifestyle risk factors (37). Common lifestyle risk factors that contribute to chronic disease are unhealthy diet, physical inactivity, smoking, hypertension, hyperglycemia, and alcohol consumption (36). A study by Roberts et al. (2015) assessed the comorbidity factors that contributed to numerous chronic diseases in the Canadian population. The authors reported that high blood pressure, obesity, inactivity, high stress, eating fewer than five servings vegetables of fruit per day and smoking were highly associated with individuals with three or more chronic diseases (38). Lifestyle risk factors are controllable and can be modified through medical intervention or behavioural lifestyle modifications by an individual (22).

Currently, medical preventive interventions aim to change lifestyle factors such as physical activity and dietary interventions. The Canadian Medical Association's recommendations are to accumulate 150 minutes of exercise per week (39). The recommendations are set to improve cardio and weight maintenance and manage chronic diseases to meet individual targets including reducing the risk of dyslipidemia, and controlling blood sugars, and blood pressure (22). To date, most Canadians are not meeting the recommendations and are engaging in less than 150 minutes a week of moderate to vigorous

activity (40). Diet can be modified to an extent to improve health outcomes; however, the impact of income on the capabilities of purchasing healthy foods should not be neglected. Current lifestyle recommendation for Canadians from the Canada's Food Guide suggests including half a plate of fruits and vegetables in every meal (41). As of now, Canadians eat less than five servings fruits and vegetables in a day (40) and are well below the recommendations.

Overall, modifying lifestyle behaviours can contribute to an overall improvement in wellness. Between 2000 and 2012, Canadians' chronic disease rates have decreased overall, with diabetes being the exception (21). This indicates that the public interventions for lifestyle changes to prevent chronic disease are helping improve the populations' overall health.

Another aspect that influences the development of chronic disease are the social determinants of health (SDoH). SDoH play an integral role in overall health and may not be modifiable (e.g., healthy behaviours and coping skills being the exception). A detailed list of SDoH have been identified by the Government of Canada including income and social status, employment and working conditions, education and literacy, childhood experiences, physical environments, healthy behaviours, access to health care service, biology and genetic endowment, gender, culture, and race/racism, support, and coping skills (42). The factors listed above can negatively affect an individual's health. A substantial social determinant that impacts the development of chronic disease is income classification (38); individuals in a lower socioeconomic status group, regardless of age, had higher associations with one or more chronic diseases, For instance, a study by Williams et al. (2006) highlighted minimum wage workers in NS not being able to afford a conventional nutritious food basket (43). Since 2001, 28.9% of the NS population are living with two or more chronic diseases (44). Moreover, living with a chronic

disease, such as obesity, can increase the risk of obtaining other chronic conditions (i.e., T2DM and CVD) which are known as comorbidities (36).

1.2.0 Functional Snack Products

Government of Canada defines functional food products as those that are similar in appearance to, or may be a conventional food consumed as part of a usual diet, and demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional function (45). There must be substantial scientific evidence to support that the product has a health benefit and must directly apply to the specific target group (45). There are several categories of health claims for foods. Each health claim should be scientifically substantiated as required by Health Canada (45). For instance, in order to make a satiety health claim on food, the draft document prepared by Health Canada requires measuring the motivation to eat (MTE) using 100-150mm visual analog scale (VAS) right before a preload of food (time 0 minute) and after consumption of the preload. Furthermore, MTE should be measured at regular intervals (46). An meal that is either a buffet style or fixed meal at a pre-determined time is also included to measure the energy intake of participants (46). In addition, the food product should be comparative with other foods and the study design must include a negative energy control (water) to see the changes in subjective appetite over the whole period of time (46).

Over time there has been an interest in improving nutritional content in conventional food products (47). These functional food products take conventional food products and integrate other food ingredients with exceptional nutritional content that may provide health benefits to individuals. For instance, many Canadians do not regularly consume pulses, especially lentils,

despite their nutritional benefit. Including lentils or lentil flour in conventional food products could also increase the consumption of these pulses and enhance food products. Currently, there are few studies including lentils formulated into snack products. A number of studies have investigated the formulation of snack products using lentils such as snack bars (48), biscuits (49), muffins (24,26), crackers (50), angel food cake (26) and smoothies (30).

Overall, most studies have focused on the palatability of pulses in food products rather than the effects on satiety and food intake (22,24,48,49). A study by Ryland et al. (2010) assessed the palatability of various formulated snack bars of same serving size by replacing oats with micronized flaked lentils and was evaluated by a consumer panel and a descriptor panel. The authors reported that the panel preferred a sweeter fruit taste for the lentil bars (48). The fruit flavour and sweetness may have enhanced the palatability of the snack bars resulting in similar hedonic scores as the conventional snack bar. Other studies evaluating with lentil flour functional food products reported similar palatability acceptance and no statistical difference between products in terms of their sensory properties (22,49). As a result of these studies, lentil flour products may be accepted with careful consideration of additional ingredients that will increase sweetness (48,49), or masking (24) to make lentil flour product acceptable. To date, few studies include the effects of lentils in a food product on palatability, satiety, and food intake (30); all of which have conflicting results. Erickson and Slavin et al. (2016) reported no effects on satiety between their smoothie formulated with lentil and a non-lentil smoothie of the same caloric density. Comparatively, Clark et al. (2019) did find an impact on satiety, where muffin formulated with lentils were different from muffins with wheat flour of similar energy (24). A study by Mollard et al. (2012) using the meals with pulses of the same energy density and reported that the meal with lentils had the highest suppressing effect on subjective appetite

compared to the meals with chickpeas, navy beans and yellow peas (4,5). Although results are conflicting, lentils show promise to improve satiety when incorporated into conventional food products.

There have been other studies conducted on pulses other than lentils and their the effects of pulses in bread, moukoushe, pasta, and Indian breakfast foods effects on satiety and food intake have also been evaluated (25,27–29). For instance, a randomized study by Lee et al. (2006) evaluated the effects of equal-caloric enriched bread with lupin (1655 kJ) and short-term satiety. The authors found that enriched bread of same caloric density to control had higher perceived satiety and lower food intake postprandial (51). In contrast, two different other studies involving chickpea flour did not find a difference between chickpea flour and control products on subjective appetite (25,27). To summarize, pulses in general have the potential to affect satiety and food intake, however, studies involving pulses on functional food products have conflicting results. Therefore, further studies are needed to support the effects of pulses on satiety and food intake.

1.3.0 Lentils: potential effects on chronic diseases

1.3.1 Type 2 Diabetes Mellitus

Diabetes mellitus (DM) is a chronic disease in which individuals have impairment of glucose uptake into the cells due to lack of production of insulin or decreased insulin sensitivity (52). DM has two forms; type 1 and type 2. Type 1 DM is an autoimmune disorder in which patients' immune system catabolizes beta cells in the pancreas (52). Beta cells found in the liver produce insulin that aid in the uptake of glucose into cells (52). Loss of beta cells results in a reduction in insulin production (52). In consequence, glucose blood levels rise, and glucose

remains in the blood (52). A lack of glucose in the cell results in metabolic changes and shift to utilizing lipids as energy instead of glucose, which results in negative health consequences (52). Compared to T1DM, T2DM develops as a result of insulin receptors being desensitized to insulin (52). These receptors become desensitized due to mitochondrial dysfunction, that produces superoxide compounds (53). These superoxide compounds damages cells and results in desensitization to insulin (53).

Managing DM requires individuals to undergo nutrition education interventions targeting carbohydrate intake. Carbohydrate types can affect blood glucose levels in individuals with DM. The concept of Glycemic Index (GI), and associated methodology and education materials, was translated and implemented by Diabetes Canada through the new resource known as "Glycemic Index Education Portal". The resource helps navigate the effects of carbohydrate types and improve glucose control for those living with DM. For example, the portal recommends pulses to be added to a high-glycaemic meal to reduce the overall GI of a meal. The same resource defines GI as a scale that ranks a carbohydrate-containing food or drink by how much it raises blood glucose after it is eaten or drank, and introduces three categories of food based on their GI, including low GI (≤ 55 GI units), medium GI (56 to 69 GI units) and high GI (≥ 70 GI units) foods (11). Current guidelines for DM are to include a variety of foods, or dietary pattern (e.g., Mediterranean diet) that benefits glycemic control by replacing high glycemic food with low glycemic foods (11). These guidelines are to help reduce glycemic hemoglobin (A1C) by 1.0 to 2.0% (11).

According to the GI classification, lentils are low GI food (7). Research on lentils reported improved glycemic control in individuals living with DM (3,6). A study by Jenkins et al. (1983) compared the effects of starchy foods containing 50g of carbohydrates on blood

glucose in patients living with diabetes (3). The authors reported that food with the lowest blood glucose peak in diabetic patients were lentils (3). The GI of red lentils was 44 units, the lowest value compared to all other foods tested in this study, including 47 units for chickpeas, 71 units for black eye peas, 65 units for Romano beans, and 66 units for kidney beans (3). Subsequent studies have supported lentils improving blood glucose control in people living with DM and normoglycemic participants (4,5,54). A more recent study by Moravek et al (2017) analyzed the effects of partial replacement of rice and potatoes with cooked lentils. All treatments had similar content of available carbohydrate (50 g) (55); authors reported that the incremental area under the curve (iAUC) for blood glucose after consumption of 50% replacement of rice or potatoes with lentils was lower than the iAUC after consuming 100% rice or potatoes ($p<0.05$) (55). This study demonstrated that small red and green lentils known to have a low GI when mixed with a high GI food have the ability to lower blood glucose levels (55). Conversely, one study reported no difference in blood glucose response between their control smoothie and their lentil smoothie (30). However, in this study, the control smoothie was formulated with an extra amount of vanilla ice cream (replaced by lentil in the lentil smoothie), not with a carbohydrate-based ingredient that may explain the lack of observed effect on blood glucose. Thus, the amount of available carbohydrate was almost identical in the control smoothie (53.4 g) and in the smoothie with lentils (51.8 g) (29). To properly interpret the results of different studies, it is important to investigate the study design. For instance, the studies assessing the glycaemic index of food are designed to provide the treatments containing 50g of available carbohydrate (3). The other studies may use total or partial replacement approach when one ingredient is partially or totally replaced with the other ingredient. This approach is more practical for the development of

functional foods, especially snack products, that have a much lower amount of available carbohydrate than in the studies assessing GI of foods.

1.3.2 Cardiovascular Disease

Cardiovascular disease (CVD) is an umbrella term for all diseases that affect the cardiovascular system, including congestive heart failure (CHF), cerebral embolism, atherosclerosis, arrhythmia, and hypertension (20). The Atlantic provinces in Canada have the highest rates of CVD from 1999 (13.30%) to 2012 (6.93%) per 1000 Canadians (21). Overall, since 2012 heart disease diagnoses in the Canadian population have decreased (21). Dyslipidemia is an important risk factor for the development of CVD (20). As a result, recommendations for CVD are to lower blood lipids of low density and apo B and increase non-high density lipoproteins cholesterol (non-HDL-C) (20). Current recommendations to reduce risk of CVD disease are to adhere to low glycemic foods, and increase consumption of fibre and plant-based foods in a dietary pattern (20). The Canadian medical association continues to promote the pattern of Mediterranean diet as it includes a food pattern that lowers dyslipidemia (20). The Mediterranean diet recommends as part of that diet to consume pulses greater than 3 times per week (56)

The recommendations for the Mediterranean diet includes regular consumption of antioxidants and reducing cholesterol by high intakes of fibre-rich foods (12). Pulses, including lentils, contain both antioxidants (57) and high fibre (1). Furthermore, prevention of CVD includes the consumption of antioxidants. Lentils contain a high quantity of antioxidants and may reduce the risk for CVD by mitigating inflammatory responses (13). Studies have reported that antioxidants may result in reducing the oxidation of LDL (13,14). The overall reduction of

LDL oxidation and lower levels of total blood triglycerides are associated with a lower risk of CVD (14).

Hypertension is chronic abnormal high blood pressure greater than or equal to 140/90 mm Hg and is included in CVD disease (58). Hypertension can be controlled through dietary intervention by lowering sodium intake and increasing potassium intake (15). When hypertensive patients have a higher intake of potassium, magnesium, and calcium, this results in a decrease of blood pressure (15). In fact, an inverse association was observed between the intake of plant-based protein and blood pressure (17). A study by Hanson et al. (2013) investigated the effects of ground lentil added into the diet of

(18,60). Thus, there is the potential for lentil intake to contribute to a reduction in hypertension and CVD through blocking of the ACE enzyme. In addition to this, an study by Akillioglu et al. (2009) reported that heat treatment of pulses (beans, green lentils, and pinto beans) may result in a wide range of effectiveness (4.08-28,4%) in inhibiting ACE (18). Although studies indicate ACE inhibitory activity of lentils, further studies are needed to be conducted to support the ACE inhibitory activity from the consumption of lentils.

1.3.3 Overweight and Obesity

Obesity Canada Network reports that 30% or more adults in Canada live with obesity (61). Obesity is a complex chronic disease that goes beyond food intake regulation and involves psychological and SoDH (22). Food intake regulation is one aspect of the development of this complex chronic disease. Not only is obesity a complicated chronic disease, but it is also a risk factor for other major chronic diseases such as T2DM, CVD, and chronic hypertension. Currently, the Canadian Medical Association recommendations for primary prevention and treatment for overweight and obesity is lifestyle interventions (22). Lifestyle interventions recommended to individuals include increasing physical activity and changing nutrition habits to prevent obesity (22). Nutritional recommendations are aimed to increase the consumption of nutrient-dense foods rather than energy-dense food to regulate food intake (22,41). Foods with a higher satiating power and the enhancement of satiety (i.e., foods high in fibre and protein), may evoke the cessation of hyperphagia (23,31,32). Essentially, consuming lentils, a food high in protein and fibre, may decrease food intake short term. Thus, it has been reported that individuals that regularly consume pulses are associated with a lower body weight (8,9). However, the long-term effect of food on body weight is only possible with the combination of short-term episodic

signals such as satiety, and long-term factors including resting metabolic rate and physical activity (62).

Pulses have the potential to affect short term food intake. A study by Mollard et al. (2012) reported that a treatment with lentil, pasta and tomato sauce resulted in a lower cumulative food intake compared to the treatment with pasta and sauce (4). In addition, a systematic review meta-analysis conducted by Kim et al. (2016) reported that lentil consumption was correlated with reduced energy intake during short-term food intake (9).

Overall, obesity is a complex chronic disease that cannot solely be treated and prevented through the manipulation of individuals' short-term energy intake. Although short term intake is a part of the prevention strategy, all SDoH factors must be accounted for reducing the risk of developing obesity.

1.4.0 Resistant carbohydrates

1.4.1 Resistant starch

Carbohydrates can be classified based upon their ability to be digested, with some being classified as resistant carbohydrates. These carbohydrates cannot not be broken down by human digestive enzymes. Resistant starches (RS) are compounds that belong to resistant carbohydrates (63). The other classification of starches introduced by Englyst includes rapidly-digestible (RDS), slowly-digestible (SDS) and resistant starches (RS) (64). There are four distinct forms of RS: RS1, RS2, RS3 and RS4 (65). RS1 is the predominate RS found in lentils (63). RS1 is not accessible for digestive enzymes and therefore cannot be digested to glucose and absorbed into the systemic bloodstream. Instead, RS is fermented in the colon with the help of microorganisms.

The fermentation process takes place approximately 5-7 hours postprandially (65), which eventually metabolizes approximately 30-70% of the RS (66).

The health benefits that come from RS are enhanced satiety, and lowering blood glucose and lipids (63). RS is similar to insoluble fibre in how it interacts with the digestive system, however, it gives similar benefits like soluble fibre (66). Pulses contain high amount of RS at approximately between 10-55% of total starch (67).

1.4.2 Resistant starch and blood glucose control

RS can impact blood glucose responses in normoglycemic individuals (68,69). A study by Raben et al. (1994) explored the effects of 50g of RS in 9 healthy males and found that it led to a lower blood glucose response than the equal amount of digestible starch (70). Part of the care in diabetic management for T2DM, is to increase insulin sensitivity to allow absorption of glucose into the cell (11). In a randomized, single blinded, control trial, insulin resistant participants were given either a 40g/day RS2 pill or placebo for 12 weeks (71). This study showed an improved insulin sensitivity after RS2 pills compared to placebo.

A crossover study conducted by Sandberg et al (2008) investigated the effects of four rye-based meals enriched with either rye kernel, and with or without RS, or rye with kernel and RS. The study reported lower iAUC blood glucose when consuming rye bread enriched with kernel and RS and when consuming rye bread with RS compared to white bread with no added resistant starch (68). The origin and the dose of RS should be considered for functional food formulation. In a study evaluating with snack products formulated with a high amylose maize flour (a source of RS), Luhovyy et al (2014) reported that a snack product incorporating a high dose of RS reduced the AUC for blood glucose compared to a snack formulated without RS (70) Effects of RS and fibre on blood glucose are difficult to separate in foods such as lentils, since

the food can contain both and is likely that they both contribute to lowering the glycemic responses (71).

1.4.3 Resistant starch and food intake

Another area in which research has explored the potential biological activity of RS is its effect on food intake and satiety. RS and fibre have been shown to influence blood glucose levels, subjective satiety, and overall food intake (68). As previously stated, a study by Sandberg et al. (2017) reported that rye bread with RS significantly reduced hunger over 120 minutes (68). Conversely, they did not find an effect of treatment on food intake (68). In a randomized, single blinded crossover study by Bodinham et al. (2010), 48g of RS lowered energy intake compared to placebo of same energy and containing rapidly digestible starch (72). However, in the study with snack products formulated with a low and high doses of RS, there was no difference between the treatment with or without RS on subjective appetite and FI (70). This indicates that the doses, food matrix, timing and the origin of RS may contribute to the effect (or lack of effect) RS has on satiety and FI.

1.4.4 Oligosaccharides

Oligosaccharides are carbohydrate compounds with three to ten monosaccharides linked by a glycosidic bond (73). Common oligosaccharides found within lentils are alpha-galactosides from the raffinose family, including raffinose, verbascose, and stachyose (54,74). Oligosaccharides can result in flatulence, because they are fermented in the colon by gut bacteria (54). Alpha-galactosides contain alpha-D 1,6 linkages that cannot be digested by human enzymes and therefore are considered resistant carbohydrates (75). While the effect of pulse-derived

oligosaccharides on satiety is not known, the other group of oligosaccharides such as oligofructose may contribute to enhanced satiety when consumed in a significant amount (16g/day) (76). However, the dose that could be practically consumed (e.g., 10g/day) is not associated with any benefits for enhancing satiety or reducing FI (76). In context of lentils, the effect on satiety could be attributed to the synergistic action of multiple carbohydrate fractions including dietary fibre, RS and oligosaccharides.

1.5.0 Bioactive compounds

1.5.1 Polyphenols and antioxidant compounds

Lentils contain various derivatives of phenolic compounds such as flavones, anthocyanins, flavanones, flavanols and flavanol oligomers, isoflavone, stilbenes, tannins and lignans which are further subclassifications of polyphenols are all found in lentils (77). The link between cardiovascular health and phenolic compounds have been researched with attention given to its ability to prevent cellular damage (15). Antioxidants reduce the damaging effects of reactive oxygen species (ROS) that are produced by the body (15). The ROS production is derived from the inflammation resulting in a cascade of the events leading to the damage of the cells (15). Antioxidants cease ROS cascades by donating a free electron to the ROS while maintaining chemical stability (14). Chronic diseases such as CVD (14), diabetes (53) and obesity produce inflammation in the body, which result in the production of ROS (78).

Current studies correlate antioxidant activity and reduction of chronic diseases, such as CVD (14). A significant risk reduction for CVD can be attained by reducing dyslipidemia (CVD medical guidelines) previously explained in section 1.3.2. Parohan et al. (2019) conducted a systematic review and meta-analysis and reported that the dietary intake of antioxidants can aid in reducing risk for CVD (79). Antioxidant properties have a promising contribution in reducing

inflammation that contributes to the development of chronic diseases. In addition, a long-term study reported that individuals that had higher intakes of phenolics had reduced their risk for CVD (80).

Lentils contain the most polyphenols compared to other pulses, such as black beans, peas, chickpeas, mung beans (81). Lentils contain the high concentrations of flavonoid, stilbenes, anthocyanidins and tannin and catechins equivalents (77). The antioxidant capacity of lentils and its potential for reducing ROS has been studied using DPPH scavenging assay (77). The phenolic content of lentils range from 1.02 mg to 7.53 mg GAE/g (77), however, the content of polyphenols can be affected by the cooking methods of lentils. Cooking lentils by steaming can result in a 14-25% loss of free radical scavenging capacity (77). In addition, phenolics can be lost in water during the cooking process; in fact, 68% of phenolics can be lost during soaking and cooking in water (77). Hernandez-Salazsar et al (2010) demonstrated high antioxidant activity in lentils (82). The above study is consistent with other studies assessing the antioxidant activity in lentils (78,82–84). Overall, it is highly recommended to consume foods high in antioxidants such as lentils to reduce inflammation (85).

High intakes of dietary flavanols have been shown to reduce the risk for CVD by 20% (86). The free radical scavenging activities of flavanols found in lentils are similar to ascorbic acid (Vitamin C) (87). A long-term study on legume consumption and associated risk for CVD reported that frequent legume consumption resulted in lower systolic pressure, less overall hypertension, and lower levels of blood lipid levels of LDL in participants (88). In addition, other chronic diseases including diabetes and obesity are associated with the inflammation and production of ROS species. Therefore, the antioxidant activity of lentils may also contribute to the prevention of these chronic diseases.

1.5.2 Phytosterols

Lentils contain many different phytosterols (89,90). The main phytosterols found in lentils are beta-sitosterol, campesterol, stigmasterol, and delta-avenastertol (89). Health Canada has accepted the health claim on plant sterols and blood cholesterol lowering (91). For prevention and treatment of CVD, the level of LDL and apolipoproteins must be reduced (20). It has been reported that phytosterols have a higher affinity for micelles formed during digestion than cholesterol resulting in the reduction of cholesterol absorption and improving the excretion of biliary cholesterol (90). Additionally, individuals with a diet pattern that includes approximately 2g of plant sterols per day can reduce LDL serum levels from 6 to12% (15). In support of this, a cross-sectional study by Andersson et al (2004) observed a negative relationship between a high intake of phytosterols and the concentrations of total and low-density lipoprotein serum cholesterol in a free-living population (90).

A study by Yoshida et al (2006) analyzed the effects of phytosterol supplementation in healthy individuals and in patients with diabetes. The authors reported that overall LDL levels were lower after phytosterol supplementation in both groups (92). Lentils contain numerous phytosterols and therefore incorporating lentils into a daily diet pattern may reduce the level of LDL and thereby contribute to the reduction of risks associated with CVD (20).

1.6.0 Nutritional characteristics of lentils and varieties of lentils

Lentils ( ) have various genotypes. There are five lentil genotypes grown in Canada: , , , and (93). The two main exported genotypes of lentils in Canada are and (93). The lentils genotype also can determine the size of the lentil seed. For instance, , , and are 4-5mm and are a smaller size,

whereas is a larger lentil with the seeds of 6-7 mm (93). Most lentils are grown in Canada is in the province of Saskatchewan (94). Described below in Table 1 is a macronutrient comparison of raw lentils and boiled lentils from the Canadian Nutrient File (CNF). The nutrient content of lentil flour is similar to the one of raw lentil. Therefore, adding lentil flour can improve the nutrient density of many food products, including snacks.

Table 1. Moisture and macronutrients in raw and boiled lentils (per 100g)

Macronutrients	**Raw Lentils**	**Boiled Lentils**
Moisture, g	8.3	69.7
Carbohydrates, g	63	20
Fibre, g	11	4
Protein, g	25	9
Fat, g	1	0.38

Data source: Canadian Nutrient File (95,96).

Table 2. Comparison of Micronutrients: raw and boiled lentils (per 100g)

Micronutrient	**Raw Lentils**	**Boiled Lentils**
Calcium, mg	35	19
Potassium, mg	677	369
Phosphorus, mg	281	180
Magnesium, mg	47	36
Selenium, µg	0.1	2.8
Beta-carotene, µg	23	5
Folate, µg	479	181

Micronutrient	Raw Lentils	Boiled Lentils
Niacin, NE	6.28	2.41
Pantothenic acid, mg	2.14	0.64
Riboflavin, mg	0.21	0.073
Thiamin, mg	0.87	0.169
B-6, mg	0.54	0.178
B-12, µg	0	0
Vitamin C, mg	4.5	1.5
Vitamin D, IU	0	0
Vitamin K, µg	5.0	1.7
Tocopherol, Alpha, mg	0.49	0.11

Data source: Canadian Nutrient File (95,96).

Described above in Table 2 is the micronutrient comparison between raw and boiled lentils from the Canadian nutrient file (CNF). When lentils are boiled, water-soluble vitamins such as B complex and vitamin C are lost during the boiling process. Additionally, some minerals such as calcium, potassium, phosphorus and magnesium are lost during the boiling process of lentils. Lentils contain a wide variety of micronutrients that make their nutrient composition superior. Conversely, cultivation, genotype and the cooking of lentils may also inactivate bioactive compounds that may reduce the risk for chronic diseases (77,97). A study by Xu et al. (2008) reported boiling lentils can result in the loss between 55 and 60% of phenolics in water and longer cooking time and pressure in water can increase losses of phenolics from lentils (98). Moreover, boiling and steaming affected the overall antioxidant properties of lentils and reduced the free radical scavenging capacity (98).

1.7.0 Processing and food applications of lentil flour

1.7.1 Milling of lentils

There are two types of milling processes for lentils: dry milling and wet milling. Both milling processes can affect lentils nutritional composition. The traditional milling process of lentils is the wet milling process. Lentils are soaked between 3-14 hours and then dried after the soaking process (99). Subsequently, the broken particles from the seeds coat are removed by sieving, dehulling, and then eventually split, if needed, for the final product (100). Dry milling of lentils differs from wet milling, as lentil are milled with running water or oil, conditioned or tempered, and then dried (100).

The milling process of lentils can impact the nutritional content of the lentils. For instance, wet milling can impact the nutritional content of the lentils due to soaking in water, which can lead to the leaching of soluble nutrients into the milling water (100). Dehulling, the removal of the seeds coat, can impact the nutritional composition. However, dehulling is necessary as it increases the palatability and digestibility of lentils (101). A study by Wang et al (2008) reported increased content of protein and starch in lentils after dehulling (102). Moreover, dehulling lentils reduces the anti-nutritional compounds found in the seed coat like tannins, phytic acid, and saponins (101–103). The dehulling process highly impacts tannin concentrations in lentils (103). Additionally, dehulling can impact micronutrients levels in lentils such as calcium, iron and magnesium (102). Raw laird lentils contain 68.7mg, 9.18mg and 109mg of calcium, iron and magnesium, respectively (102). In comparison, dehulled laird lentils contain only 25.5mg, 5.93mg, and 83.5mg of calcium, iron and magnesium, respectively (102).

After the milling process, lentils can be further processed into flour. There many methods to produce pulse flour, some methods include stone, roller, impact, ball, disc, jet and classifier milling, hammer and pin milling (99). Hammer milling is the common method used to produce

lentil flour from raw lentils (99). In contrast, pin milling is used for conditioned or pre-cooked lentils (99). Depending on the type of milling, it may have an impact on the nutritional content of the flour. For instance, raw lentil flour contains approximately 2.5% ash, 0.7% lipids, 6.7% moisture, and 27.3% protein compared to cooked lentil flour which contains 2.4% ash, 0.3% lipids, 3.8% moisture, and 28.2% protein (104). Milling lentils into flour affects some physiochemical properties (105). Conditioning of pulses can significantly impact protein content; it has been suggested to reduce the losses of protein in pulse flour to have seed moisture below 10% (106). As for particle size of flours, Pulivarthi et al (2021) reported that the reduction in particle size did not impact protein, lipid, content of red lentil flour, except there was an impact on crude fibre compared to whole lentils (105). The higher damage occurs to the starch and fibre during the milling of lentil into the flour of a smaller particle size (104,105). Therefore, it is important to investigate how a particle size of lentil flours affects the magnitude of the physiological effects such as the effect on satiety or blood glucose response.

1.7.2 Lentil flour manufacturers

Canadian International Grains Institute reports that there are 14 companies manufacturers of pulses flours and fractions in Canada (107). Among the companies in Canada that currently provide commercially available lentil flours are Avena Foods limited Inc, CanMar Grain Products, K2 Milling Ltd, Northern Quinoa Corp. (107). Avena Foods limited sells a variety of lentil ingredients including , , and lentils (108).

1.7.3 Nutritional profile of lentil flour

The nutritional content of lentil flour is superior to white wheat flour when it comes to the protein and fibre content. Table 3 below summarizes the macronutrients composition between lentil flour and white wheat flour. Additionally, lentil flour is heavily enriched with micronutrients. Table 4 summarizes a comparison of micronutrients between lentil flour and white wheat flour. Lentil flour contains higher amounts of antioxidants such as alpha-tocopherol, vitamin C, and beta-carotene compared to white wheat flour. Moreover, lentil flour also contains higher concentrations of potassium compared to white wheat flour. The overall replacement of wheat flour with lentil flour greatly improves nutritional content of food products (109).

Table 3. Energy, moisture and macronutrients of lentil flour and wheat flour (per 100g)

Macronutrient	**Lentil Flour**	**White Wheat Flour**
Energy, kcal	367	366
Moisture, g	3.63	12.8
Carbohydrates, g	62	72.4
Fibre, g	12	2.7
Protein, g	30.5	12.2
Fat, g	1.04	2.24
Sugars, total	5.86	0.54

Data source: Canadian Nutrient File (110,111)

Table 4. Micronutrient composition of lentil flour and white wheat flour (per 100g)

Micronutrient	**Lentil Flour**	**Wheat flour**
Calcium, mg	55	14
Potassium, mg	982	103
Phosphorus, mg	493	120
Magnesium, mg	116	29
Selenium, µg	8.9	52.2
Beta-carotene, µg	25	0
Folate, µg	470	344
Niacin, NE	7.34	8.6
Pantothenic acid, mg	2.0	0.438
Riboflavin, mg	0.27	0.401
Thiamin, mg	0.52	0.714
B-6, mg	0.58	0.063
B-12, µg	0	0
Vitamin C, mg	6.8	0
Vitamin D, IU	0	0
Vitamin K, µg	23.9	0.3
Tocopherol, Alpha, mg	0.36	0.06

Data source: Canadian Nutrient File (110,111).

1.7.4 Particle size

According to the Canadian regulations for wheat flour, particle size must adhere to being <149 μm (112). Lentil flour particle size found in commercially available products can be similar to wheat flour particle size. Particle size for pulses is not standardized and the flour can be course (710–1,190 μm), regular (355-710 μm), fine (180-355 μm), very fine (80-180 μm , and superfine (<80 μm) (113). Avena Foods limited produce superfine flours that are less than <80 μm (108). The particle sizes of flours can vary depending on the milling process of the flours (113).

Particle size in flours should be taken into consideration as it can impact the carbohydrate availability. It has been reported that as the particle sizes decrease in pulse flour, the higher the glucose availability during a simulated digestion (113,114). As the flours decreases in particle size, these studies found that the content of RS in the flour decreased as well, resulting in more glucose availability (113,114). Pulse flours that were more course had less available carbohydrate compared to the finer particle sizes (113,114). Additionally, Kathirvel et al. (2019) reported these effects are still retained when pulse flours are cooked (113).

1.7.5 Applications of lentil flour

Lentil flour can be used as a replacement for wheat flour (109). Currently, there are many applications of lentil flours as a replacement of wheat flour in baked products, pasta, and noodles (108), as well as dressings, soups, dairy and meat products (109). Other examples of using lentil flour in baked products are muffins (24), angel food cake (26) and crackers (50). Baked goods are widely consumed food product which makes them an ideal vehicle to incorporate lentil flour (109). Formulation of the lentil flour must be considered as an increase of lentil flour can impact baked goods volume, texture, flavour and acceptability of the product

(109,115). For instance, a study reported significant increase of density and lower gas bubble fraction with bread enriched with lentil flour (10 and 20% lentil flour) compared to wheat bread (116).

Lentil flour in products arise different sensory attributes compared to standard conventional products with wheat flour (115). A study reported that the sour dough bread with 30% lentil flour had impacted some sensory properties resulting in a higher acidity intensity, and less elasticity compared to wheat bread (115). Regardless, they reported high hedonic scores on taste, indicating that the breads were still palatable and accepted by the panel (115). In a more recent study by Ryland et al (2019), they reported high acceptability of snack bars with 20% lentil flour when masked with sweetness and fruit flavour achieving the acceptability comparable to the conventional snack bar (48). Other studies had similar findings showing that a 20% replacement of wheat flour with lentil flour achieved acceptable sensory parameters (25,28,50).

Not only can lentil flour be used to replace wheat flour, but the protein in lentils may have some applications in replacing egg whites or dairy proteins. However, the replacement of egg whites in muffins and angel food cake with lentil flour resulted in lower acceptability scores (26). A major concern in replacing wheat flour with lentil flour is effects on sensory and physiochemical composition on food products. Lentil flour can increase density and reduce leavening in doughs (109). Generally, 20-30% of lentil flour is the acceptable amount that has minimal effects on physical structure in the baked goods (109). Other studies replace 20-30% of wheat flour with pulse flour to ensure no effect on physical structure of baked goods (25,27,48).

1.8.0 Regulations of satiety and food intake in humans

Satiety and food intake are complex and are driven by central, peripheral, hedonic, and homeostatic factors (117). Current research illustrates the complexity of central and peripheral mechanisms involved in appetite regulation. As a result, satiety-cascade theory was developed to describe the factors affecting satiety, including physiological, biochemical, sensory, and food processing factors (118). Food intake is regulated by a network of interactions that categorized into three levels, psychological, behavioural, and peripheral physiologic and metabolic events (117). Appetite reflects the process and operations of the satiety cascade (117).

1.8.1 Central mechanisms of food intake regulation

The regulation of food intake involves the higher cortical centres, hypothalamic nuclei and nucleus of solitary tract in brainstem. Thus, hedonic and sensory signals, including social and emotional factors, can act though the cortico-limbic system while peripheral factors including anorexigenic and orexigenic hormones and nutrients are able to convey signals through the incomplete areas of blood-brain barrier to the hypothalamic and brainstem centres (119,120). The information about stomach distention is conveyed through the to the solitary tract nucleus in the brainstem (119). The arcuate nucleus (ARC) in the hypothalamus guides feeding behaviour by releasing anorexic neuropeptides including pro-opiomelanocortin (POMC) and cocaine-and amphetamine-regulated transcript (CART), and orexigenic neuropeptides including agouti-gene-related protein (AgRP), and neuropeptide Y (NPY) (117,121). The ARC receives all peripheral response signals that are translated to neurochemical activity to create hunger, satiation, and satiety perceptions (117,118). There are two inhibitory food intake terms, which are satiation, the termination of food intake, and satiety, prolonged inhibition of the urge

to eat (117). The CNS drives the increased (hunger) or decreased (satiety) desires to eat (117,118). Satiation and satiety sensations arise as a result of ingestion of food that creates a physiological response that is translated into neurochemical activity in the CNS (117,118).

1.8.2 Peripheral mechanisms for food intake

Peripheral mechanisms involve mastication, gastric distention, gastrointestinal tract secretory hormones and chemical compositions of food (117)

Mastication is the first step in the process of breaking down foods. Mastication is the process that physically breaks down food through chewing and grinding of the teeth. Macronutrient composition, especially fibre in food, increases the mastication and gut hormone secretion resulting in an increase in subjective satiety (122). As ingested food spends time in the mouth, amylase is secreted and starts the process of carbohydrate breakdown that is continued in the small intestine by pancreatic amylase. The glucose ingestion stimulates beta-cells in the pancreas to release insulin. These endocrinological secretions and neurological signals triggered by glucose are then sent to the hypothalamus. Food with higher protein and fibre content takes longer to break down and increases mastication of food. A study by Haber et al (1977) reported that apples compared to apple puree and apple juice with the same amount of available carbohydrates (60g), resulted in a higher satiety likely do to their fibre content (32).

The secondary digestion process involves the stomach and the small intestine that breaks food down further for absorption. Some physical properties of food can influence the signalling from the stomach and small intestine, such as weight and size of ingested food (123). These factors influence the feelings of satiation and satiety as it results in more muscular distension in the stomach and proximal small intestine, creating the sensation of fullness. As a direct result of

muscular distention, neurological signals from distension are transferred to the CNS to inhibit desire to eat (124). In addition to the distention signals, the gastrointestinal tract secretes hormones during food intake (FI) to further decrease desire to eat (124). The endocrine hormones secreted from the stomach and small intestine that influence food intake are Peptide YY (PYY), ghrelin, cholecystokinin (CKK), and glucagon-like peptide 1 (GLP-1). Gastric hormone PYY suppresses desires to eat (125) by action on the neurological signals in the ARC to increase the secretions of pro-opiomelanocortin (POMC) (126). Moreover, during the suppression of hunger, also secrete CKK, which transmits signals to the ARC in the hindbrain via vagal afferent fibres to suppress the desire to eat (127). The small intestine also produces GLP-1, an anorexigenic protein that releases 15 minutes postprandial that acts on the CNS (128). The antagonistic hormone to PYY, CKK and GLP-1 is ghrelin. When ghrelin is interacting in the ARC, it stimulates the release of neuropeptide Y (NYP) and resulting in an increase desire to eat (129). A study by Cummings et al (2001) reported an 78% increase of blood ghrelin plasma 1-2 hours pre-prandial and with subsequent falling levels within one hour of postprandial (124). When ghrelin was added into the blood stream of participants, there was an increase in energy intake compared the placebo (130).

1.8.2.1 Sleep

Sleep is regulated by circadian rhythm. The circadian rhythm is an endogenous internal clock that is regulated by light and dark cycles. The circadian rhythm can also control the feeding and fasting times in relation to the daytime and nighttime cycles. The circadian rhythm can influence food intake by two mechanisms 1) sleep phase 2) and an active phase (129). The sleep phase is a period in which the body is prepared to utilize energy stores during a fasting period. The active

phase is the time frame in which energy stores are replenished during habitual time frames. These rhythms are regulated by the hypothalamus. The function of the circadian rhythm is to allow humans to predict or anticipate food availability (129). Disturbed sleep can result in dysrhythmia of the circadian clock and may affect food intake. However, a meta-analysis by Fenton et al (2021) on sleep and food intake found the overall studies had no difference between restricted sleeping and control on food intake or desires for particular macronutrients (131).

1.8.2.2 Stress

Stress can have an impact on food intake. Stress activates the parasympathetic nervous system and increases cortisol which can potentially influence satiety and appetite cues (124). Cortisol and corticosterone are made in the adrenal glands and are considered glucocorticoids (128) which influences the desire for food intake. Moreover, stress can increase emotional eating and influence what people desire to consume as well. Individuals under stress may indulge in more "comfort foods" while undergoing stressful situations. A study by Strengel (2018) reported that rats preferred food that was more palatable when under stress (128). The reasoning behind this is that palatable food can reduce feelings of stress individuals (128).

1.8.3 Role of hedonic factors

The human drive for food intake doesn't just rely on hunger and satiety cues from the central and peripheral mechanisms, hedonic preferences play a role in food intake. Hedonic eating is regulated by restrained or inhibition eating (132). Hedonic factors rely on the five sensory systems that can override the desires (or lack of) for food intake. Hedonic factors include vision, olfactory, gustatory, somatosensation and audition of food. These factors combined can impact food intake, perception, and preference of foods (133). Additionally, food intake can be

influenced by cognitive, emotional and reward systems (133). Four theories have been proposed regarding in how hedonic feeding takes place i) flavour hedonics (e.g., sweetness, saltiness, aftertaste); ii) sensory specific satiety (SSS) (e.g., olfaction, sight, taste); iii) negative gustatory alliesthesia (e.g., pleasantness of food decreases); and iv) hedonic hunger (e.g., consumption of food outside CNS and homeostatic drives) (134). The hedonic factors are regulated by the cortico-limbic areas including the nucleus accumbent, caudate nucleus, insula, orbitofrontal cortex (OFC), gustatory cortex, amygdala, and the hippocampus (133). The main two activated mechanisms are the OFC and the insula, which can drive food intake and the brain reward system. Perceptions of visual, olfactory and taste cues of palatable food can heighten insula and OFC activity (133). Moreover, the OFC also receives signals from the amygdala on emotional perception when ingesting food (133).

A study by Pribic et al. (2016) conducted a study on hedonic eating and monitored the effects of probing meals with functional magnetic resonance imaging. The authors reported that hedonic feeding influenced the connectivity of OFC and the anterior insula cingulate cortex (AAC). When the connectivity of these two regions of the brain decreases, the higher the satiety perception (135). Both the OFC and ACC are responsible for the feed reward system in the brain. When the ACC and OFC interconnect, they are responsible for the motivation for emotional desires and generating sensory perceptions (135).

Olfaction is the perception of odorant compounds entering and interacting with the olfactory mucosa (136). These interactions with the olfactory translate and communicate with the orbitofrontal cortex found in the brain to decrease response to consistent odours (137). Olfaction of food can increase desires for food and override the sensation of satiety. Interestingly, a study by O'Dogerty et al (1999) reported less OFC activation in all human subjects when olfactory

signals are saturated (137). When individuals begin food intake, olfactory sensations of food become less pleasant or alluring to consume and decrease the desires for food intake.

As part of the hedonic process, the gustatory cortex, insula and frontal operculum are responsible for the perception of taste (134). Gustatory is the perception of the taste of foods; it is found that food tastes more pleasant when individuals have a higher perception of hunger. The perception of pleasantness can decrease or becomes neutral when food is eaten till the feeling of satiation. When food taste is perceived, it can reduce the motivation to consume food (137,138).

The hedonic sensation of somatosensation can impact food intake. Somatosensation is the texture or tactile sensation of food. A study by Pitchard et al (2013) reported the effect of four meals of cottage (shepherds) pie of either texture modified food and/or energy dense modified food and food intake. The authors reported that both textured modified meals reduced food intake by 9% and 7 %, respectively, compared to the standard meal (139).

Each hedonic theory has intertwining factors that can influence food intake and lead to hyperphagia. However, many of the factors overlap and can be influenced by emotion, stress and homeopathic regulations. It is important to note that food palatability affects satiation and not satiety. A study by De Graaf et al (1999) reported that soup that was less palatable affected the onset of satiation, but not satiety (140).

1.8.4 Role of homeostatic factors

The human body constantly is adjusting to maintain a homeostatic energy balance. Homeostatic factors that influence food intake are directly related to energy homeostasis (141). Leptin is directly correlated with adipose tissues (117) and the leptin gene (ob gene) is responsible for regulating food intake and energy expenditure (142). Satiation and satiety

sensations are triggered by leptin and insulin receptor stimulation in the CNS and which inhibits desires to eat. Leptin has a vital role in neurological signalling. Simultaneously, activation of the leptin receptor also inhibits hunger transduction signals (121). In the hindbrain, leptin acts on the POMC neurons in the ARC, resulting in suppressed desires to eat (143,144). Furthermore, in the ARC, there is neurotransmitter AMP-activated kinase (AMPK) that acts on energy metabolism. When AMPK activates from the expression of agouti-related protein (AgRP) the neurotransmitter increases hunger sensations (145). The opposite occurs when there is an increased insulin concentration which inhibits AMPK activation and increases satiation and satiety sensations (145). Additionally, a series of neuropeptide mediators allow leptin and insulin to act on the ARC. These neuropeptides can either be orexigenic (increase regulation of adiposity signals) or anorexigenic (decrease regulation of adiposity signals) (121). For instance, NYP and AgRP are orexigenic because they inhibit leptin signalling and stimulate food intake (121). A study by Luquet et al. (2005) reported that the expression of NYP and AgRP are essential for feeding in adult mice (146). Mice that do not express the neuropeptide AgRP have a reduced FI (147). The anorexigenic neuropeptide compound found in the CNS is POMC; when POMC is expressed there is a release of melanocortins (146). The meleancortin alpha-melanocyte stimulating hormone (alpha-MSH) is secreted by POMC (146) which bind to melanocortin receptors (MC3/MC4) (121). The binding of the receptors results in CNS to suppress the desire to eat (121).

Secretions of leptin are directly proportionate to the adiposity tissue stores is modulated by the ARC (117,143). When there are losses or increases in adiposity cells are detected, it triggers a cascade of hormonal responses to maintain the body's homeostasis. There are two kinds of adipose tissues, white adipose tissue (WAT) and brown adipose tissue (BAT) (148).

When adiposity cells decrease, concentration levels of leptin are low, which stimulates increased production of NYP and AgRP neurotransmitter proteins to increase the desire to eat (121). Inversely, an increased leptin levels can increase the expression of POMC and cocaine-amphetamine regulated transcript (CART) that suppress appetite and decreases the desire to eat (121,141). A study by Balthasar et al (2004) reported that leptin acts on the POMC to maintain the homeostasis of body weight in mice (149).

White adipose tissue secretes adiponectin, an anorexigenic compound (149). Adiponectin is a component to the homeostatic regulation of appetite and is associated with metabolic syndrome (150). Additionally, adiponectin modulates the expression of AgRP in the CNS. Concentrations of adiponectin are negatively correlated with body mass by energy expenditure (150,151). Adiponectin levels are lower in obese individuals compared to non-obese (151). There was hypothesis of adiponectin and its influence on energy expenditure though crossing the blood brain barrier (150). There is potential that adiponectin crosses the blood brain barrier and influences body mass via energy expenditure (150).

The other half of homeostatic regulation is habitual physical activity (PA) that influences body composition (fat-free mass and adipocyte cells). Habitual physical activity has a long-term effect on body composition and resting metabolic rate (RMR) (152). Conversely, acute exercise interventions have little to no effect on hunger or daily energy intake immediately (117,153). In addition, high intensity exercise has no to little effect immediately on hunger (117). Overall, individuals with increased and decreased exercise continue to eat with their normal habitual pattern and evidence for PA has a weak effect on eating behaviour (117).

Body composition, influenced by habitual PA, is strongly associated with its influences on appetite and food intake (154). In fact, RMR, driven by fat-free mass and regulation of

habitual PA, can predict short-term food intake and hunger (155). Overall, habitual PA modulates fat-free mass (a driving factor for RMR), which is considered a predictor for food intake as RMR (155). Individuals who are more physically active may have better modulation of appetite and affect short term food intake (117,156,157). Modulation of food intake may result from increased leptin and insulin receptor sensitivity and delay gastric emptying that produces higher GLP-1 and PYY secretions (158). In addition, it is known that CKK, a short-term food regulator, increases post exercise (117); this could be another possible mechanism of appetite modulation. It is proposed that dual action of insulin sensitivity and delayed gastric emptying increases the sensitivity of satiety in those who habitually exercise (153). Currently, an inverted U relationship between PA and FI has been suggested (117). Where sedentary activity does not down-regulate FI, and increased PA improves satiety signalling (117). In fact, a reduction in energy expenditure does not reduce FI and could increase FI (117).

1.8.5 Short and long-term regulation of food intake

Short-term regulation is the daily adjustment of energy intake and is tied to anorexigenic and orexigenic neurological endocrinology mechanisms. One mechanism that regulates food intake is through gastric and proximal small intestine, which through their vagal afferent fibres produce distention signals that travel to the CNS to indicate fullness. As a result of the distention signals, appetite sensations are suppressed and cease desires for FI (124). In addition the small intestine secretes CKK and glucagon-like peptide-1 (GLP-1) which are endocrinological neuropeptides that regulate short-term satiation and satiety that in turn influence food intake (128). Small intestine secretions of CKK and GLP-1 are both processed by the hindbrain producing the perception of short-term satiety (159). The antagonistic short-term neuropeptide to CKK (160) and

GLP-1 is ghrelin (23). Ghrelin stimulates the desires to eat in individuals. Ghrelin stimulates NYP, which when it is expressed in the hypothalamus, signals the rest of the body to increase the desire to eat (161).

Long-term FI is regulated by the ARC which receives signals from adipocytes. When insulin is secreted it acts on the ARC creating neurotransmitter cascade to suppress desires to eat (162). In conjunction to insulin secretions, leptin secreted by adipocyte cells acts on the ARC suppressing the desire to eat (163). When adipocytes cells reduce in size, there is a reduced secretion of leptin, which signals the CNS to increase desire to eat (121). Although short-term regulators and long-term endocrines influence desires to eat independently, they also work synergistically. For instance, a study by Matson et al (2017) reported incidences of CCK-leptin injections that resulted in long term reduction of food intake in rats (164), which means that both long term and short-term food regulation are necessary to regulate FI.

1.8.6 Role of macronutrients

Macronutrients such as carbohydrates, proteins, and lipids, have a role in regulation of satiety. Foods can vary in macronutrient composition which in turn can increase or decrease subjective appetite sensations. These food products are described as either as energy dense or nutrient dense foods. Energy density describes energy content per one gram of food or kilocalories derived from macronutrients in a serving of food. The foods with a higher energy density are not as satiating as nutrient-dense foods. Holt et al (1995) researched common foods and their effects on satiety (31). The authors reported that common foods that contained higher concentrations of fibre and protein resulted in higher satiety, and foods that contained higher concentrations of lipids had minor effects on satiety (31).

1.8.6.1 Carbohydrates

Carbohydrates are macronutrients including sugars, starches and fibres. A phenomenon that affects appetite is the glucostatic theory suggesting that the glucose stores from the previous feeding period can affect food intake on a subsequent day (165). It has been reported that the amount of carbohydrates ingested from the previous day can influence feedings (166). Individuals with low intakes of carbohydrates have higher intakes of food on a subsequent day (166). When carbohydrates are ingested, there is an increase in secretions of CKK, PYY and insulin that signal to the CNS to reduce the desire to eat (124,125,160,162).

A contributing factor to satiety sensations is the fibre content found in a food or food product. The fibre hypothesis has three primary mechanisms that contribute to reduction in food intake (167). Fibre i) increases mastication effort and time in oral cavity, ii) increases gastric and intestinal distention and iii) reduces absorption capacity in the intestines (167). Hypothetically, intake with food that are higher in fibre has the potential to decrease total energy intake by a 10% decrease in total energy intake (167). In addition, the type of carbohydrate should be taken into consideration. Thus, GI of foods can affect appetite sensations (168). Anderson et al (2002) reported a higher glycemic response from high GI sugars results in suppression of appetite compared to low glycemic response from low glycemic sugars (34).

1.8.6.2 Protein

Protein content in food is the strongest contributing macronutrient factor towards satiety sensations. Protein increases the feeling of satiety and may reduce FI (31). Specifically, some certain amino acid, such as histamine, tryptophan, leucine, may mediate activity of neurotransmitters (169). The theory behind the process in which it affects satiety is the

aminosatatic theory. Proteins act directly and indirectly on neural pathways. The indirect neural pathway is conveyed via the vagus nerve from the orosensory areas, stomach, duodenum, ilium and liver to the solitary tract in the brain stem (165,169). Direct pathway involves gut hormones post-absorption that signal to the ARC and medulla oblongata in the brainstem (169). The digestion of proteins into amino acids enhances the secretion of GLP-1 and PYY (125,165). A study by Marmonier et al (2002) reported that when high-protein snacks are consumed, it resulted in longer delays in requesting next meal and higher feelings of satiety (33). State of protein (i.e., solid and liquid forms) also can make a difference in satiety effects (169). Proteins that are in a solid form increases mastication and may result in enhanced satiety (169). However, when proteins and carbohydrate were ingested in a liquid form, there was an insignificant difference between them on satiety (35). Overall, it is considered that protein can modulate FI stronger than carbohydrates by i) suppressing food intake and by more than their energy content alone, ii) delaying the return of hunger longer, iii) high protein diet supports lean body mass during energy restriction and iv) stimulates physiological and metabolic responses involved in intake regulation (170).

1.8.6.3 Fat

Fat is considered the least satiating macronutrient despite the assumptions that fat would be more satiating because of its highest caloric density of 9 kcal per gram. A study by Holt et al. (1995) reported that foods that contained high fat and carbohydrates, such as baked goods, increased the palatability of the foods resulting in hyperphagia in these food products (31). Overall, fat has the least satiety affect and can increase hyperphagia due to the increase perception of palatability (165).

Macronutrients individually are difficult to separate on their effects on satiety as most foods are a mix of carbohydrates, protein and fats, and rarely are consumed purely on their own. Each of the theories in combination adds to the mechanisms of satiety and satiation. In isocaloric meals, macronutrients regulate the feelings of satiety and appetite (165). Overall, it is assumed that the order from most to least satiety effect in macronutrients is protein>carbohydrates>lipids (165). For instance, lentil flour is a mix of macronutrient contents. However, it also contains the highest content of protein and fibre, the two main macronutrients known to influence satiety.

This literature review identified many factors that can play a role in the effects of lentil flour in satiety and food intake. The present work will investigate how the replacement of wheat flour with lentil flour known to have a higher content of resistant carbohydrates and proteins affect satiety and food intake in young healthy males.

2.0.0 Rationale

Despite nutritional and health benefits that lentils provide, and recommendations from the Canadian Medical Association guidelines for DM and CVD, lentils are not widely consumed by North Americans with only 13% of Canadians consume pulses (11,20,171). There is the potential to have lentils incorporated into a functional food product as consumers have vocalized an increased desire for functional foods (172). Foods's high in protein and dietary fibre have shown to affect subjective appetite. Recently, there have been recent studies incorporating whole pulses and pulse flours into baked products and have reported effects on subjective appetite (22–28,48,49). Lentil flour, a food part of the pulse family, contains high protein and dietary fibre content and may retain the satiating properties of whole lentils.

2.0.1 Study Aim

This study aimed to investigate the effects of lentil flour on satiety and food intake. Collectively from previous studies on pulses, lentil flour has the potential to influence satiety and food intake. This study investigated the effects of partial and total replacement of wheat flour with lentil flour snack bars with similar particle size on food intake and satiety in young males aged 19-35 years old in Nova Scotia.

2.0.2 Hypothesis

A conventional functional food product formulated with lentil flour may suppress subjective appetite and reduce food intake compared to the same product formulated with wheat flour of similar particle size due to the difference in their macronutrient composition.

2.0.3 Objectives

This study's primary objective was to investigate the effects of partial and full replacement of wheat flour with lentil flour on:

i. The effects of formulated snack bars on subjective appetite and subjective appetite components (desires to eat, hunger, fullness, and prospective consumption).

ii. The effect of formulated snack bars treatments on food intake.

Secondary objectives were to:

i. Investigate the effects of formulated snack bars on sensory characteristics.

ii. Study the effects of formulated snack bars on physical comfort.

iii. Explore the relationships between physical activity, appetite, and appetite components, physical activity and food intake, and subjective appetite and food intake.

2.1.0 Significance

2.1.1 Theoretical importance

Lentil flour has a higher fibre and protein content compared to white wheat flour as seen in Table 3. The macronutrient composition of lentils may elicit a higher subjective satiety and reduce short term food intake. Thus, the foods with a higher fibre and protein resulted in higher satiety scores (31). This can be explained by the fact that the food high in protein and dietary fibre increase physiological responses, resulting in neurological signalling to the hypothalamus to inhibit desires to eat (124). The aminostatic theory explains the mechanisms behind protein and its highly satiating properties.

Therefore, in theory, food products enriched with lentil flour would have a similar effect on the energy intake with an meal two hours later. Moreover, lentil flour is high in protein, which would contribute to enhanced subjective satiety (23,168).

Moreover, it is important to consider the impacts of the glycemic index of foods is on satiety (168). A longitudinal study by Zhu et al. (2022) reported food high in protein and low GI diet can suppress the feelings of hunger but does not affect food intake (173). Dosage of

carbohydrate availability is important to consider. In fact, a particle size of the flours can impact glucose availability (113,114). The inverse relationship was observed for lentil flour particle size and carbohydrate availability and this relationship was retained when thermally cooked (113). As pulse flours particle reduce in size, there is higher damage to starch content in the flours, increasing the glucose availability in those flours; thus, flours that are superfine pulse have higher glucose availability compared to course pulse flour (113,114). The higher the glucose that is available could potentially affect subjective appetite. If particle sizes in wheat and lentil flours are of different sizes from each other, the effects of the food products on subjective appetite could be a result of the difference in particle sizes and not from macronutrient composition. Therefore, to compare the effects of foods formulated with wheat or lentil flour, it is important that both types of flours have similar particle size. Hence the potential difference between flours will be due to their composition only. In addition, it is important that the palatability of the treatments are similar, as palatability affects satiation (140).

2.1.2 Practical importance

There is increased awareness of health and nutrition in the North American population that is driving the demand for snack products with nutritional health benefits (47). The incorporation of low GI plant based foods in a dietary pattern is recommended by the Canadian medical guidelines for DM, DM and obesity (11,20,22). If lentil flour, from a low GI plant-based protein, can be formulated into functional snack food products, it will help reduce the risk for chronic diseases in the larger Canadian population. Incorporating lentil flour into the snack products will provide the convenient way for the Canadian population to gain nutritional benefits from lentils to reduce the risk for chronic diseases. Lentils have been reported to improve glycemic control (3,174). Hypothetically, the ingestion a snack formulated with lentil flour

should elicit better glycemic control compared to a snack formulated with other ingredients derived from food with a higher GI than lentils. However, this study was designed to explore the effects of partial or total replacement of wheat flour with lentil flour on satiety and food intake, not the effects on blood glucose.

Not only will lentil flour potentially help with reducing the risk factors associated with chronic disease, but it will also contribute to a better appetite control due to its unique macronutrient composition. Lentils and lentil flour contain high protein and dietary fibre that contribute to the enhancement of satiety. If a snack is formulated with lentil flour, it would enhance satiety as a result of macronutrient composition. Enhancement of satiety could result in a lower short-term intake of food. Regardless of their effects on food intake, foods high in protein can reduce feelings of hunger, have higher compliance to diets during energy restriction, and improve mood and cognition (169). Moreover, lentil flours incorporated into snack products can help improve the intake of dietary fibre of consumers. Within the Canadian population, there is an insufficient intake of dietary fibre. Thus, obese and non-obese Canadians have an average intake of dietary fibre of 0.7g/100 kcal and 0.8g/100 kcal of their diet, respectively (175). The recommendation for daily fibre intake is 25 to 38 grams, and many Canadians do not meet these recommendations (176). Therefore, the incorporation of lentils in baked products may provide healthy snack product high in fibre and increase daily fibre intakes in Canadians. As of recently, the Canada's Food Guide includes recommending higher intakes of plant-based proteins (177). Including lentil flour into a conventional snack product, could act as a vehicle to increase consumption pulses in the Canadian population and provide a food that includes recommendations from the Canada's Food Guide.

2.2.0 Methodology

2.2.1 Visual analog scale

Visual analogue scale (VAS), is a psychometric scale that is used to assess satiety, hunger, prospective food consumption, physical comfort, and gastrointestinal wellness, was developed by Hill and Blundell (178). The VAS has a line of various lengths, typically 100 or 150mm, in which participants mark their subjective feeling (70,179). The preferred VAS length is 100mm (2,180). A study by Flint et al (2000) reported that the VAS was found to be predictive of "prospective consumption" and "desire to eat"(179). In addition, the Health Canada's draft document for satiety claims on food also requires to substantiate the effect of foods on satiety using a VAS scale of 100 or 150mm with exact word anchors assessing the desires to eat, hunger, fullness, and prospective consumption (45).

2.1.2 9-Point hedonic scale

The 9-point hedonic scale was created by Peryam 1957 in the US military to assess perceived taste intensities in foods (181). The 9-point hedonic scale is the golden standard throughout food sensory analysis. However, there has been debate whether a numerical or ordinal word scale produces a superior sensitive methodological approach. The use of ordinal words resulted in superior reproducibility compared to a numerical scale with provided stimuli (182). Judges from the panels could accurately reproduce the same responses with and an ordinal word scale to the same stimuli after a forgetting period (182). Furthermore, using categorical 9-point hedonic scale is more sensitive to acceptance of food products and is used with an untrained a population (183). Conversely, Kalva et al (2014) conducted a validation study on perception of various stimuli foods ranking them from most to least comparing the 9-

point hedonic scale and reported a ceiling effect (i.e., higher ratings on the upper end of the scale) with the tool (182). However, the 9-point hedonic scale is the standard tool to assess the acceptability and palatability of food products during food sensory analysis.

2.2.3 Energy intake assessment

Energy intake assessments can be measured in multiple ways; however, two distinct categories of energy intake assessment are to: (a) collect the data memorized by the individuals (e.g., 24-hour recalls, food frequency questionnaires, and diet records); and (b) collect the data in a strictly controlled environment (e.g., laboratory or hospital) where the amount and type of food consumed is recorded. In the first case, the energy and nutrient intake can be analyzed using a specialized computer software such as Food Processor SQL (ESHA Research, Salem, Oregon, U.S.A.) known to provide an accurate reflection of food intake in participants (184).

2.2.4 food intake

As part of standard procedures for satiety studies, the general methodology used for FI is food intake. means that participants can eat food freely until they are satiated. There are different variations in food intake protocols. The first is a single meal protocol in which participants only consume a standardized meal till they are comfortably full (154). The second style is buffet style which provides participants variation and variety of different foods that are logged (154). A study by Arvaniti et al (1999) assessed the reproducibility of food intake with buffet-style meals. The author reported no significant difference between the two meals, indicating reproducibility (185). In addition, buffet-style meals are closer to free-living condition compared to a single meal (186). However,

there is the tendency of overfeeding due to an increase in food choices and its limiting factor when using buffet-style feeding (186).

Comparatively, Gregersen et al (2008) assessed the reproducibility and power of food intake with repeated single meals. The authors reported that they found no differences in meal days between groups, meaning that the reproducibility of this approach was high. (187). Participants rated on visual appeal, smell, aftertaste, and overall palatability of the two test day meals were similar as well (187). Although feeding using a single meal does not represent free-living conditions, it is can indicate the satiation (187). Fixed food intake is an appropriate approach to investigate the effect of snack bars on short-term food intake.

2.3.0 Methods

2.3.1 Recruitment

The aim was to recruit 30 healthy, non-smoking, male participants (aged 19 -35), with normal BMI (18-24.9kg/m^2), from Halifax municipality. Individuals with allergies, medications, smokers or breakfast skippers were excluded. All females were excluded from the study as there are interactions of appetite ratings occurring at different menstrual cycle phases; hunger increase at luteal phase, therefore, adjustments in methods such as tracking of menstrual cycle and longer washout periods would need to be considered (154). Those with a higher BMI were excluded as those with higher a BMI may have a different effect on appetite ratings due to potentially have higher leptin resistance and lower adiponectin levels secreted by adipocytes (148). Participants were recruited via posters, social media (online) postings, and word of mouth. After initial recruitment, participants underwent undergo a telephone eligibility questionnaire and an information session where they provided an informed consent form and an anthropometric assessment. The recruitment of participants was affected due to multiple closures of the laboratory regarding to the COVID-19 pandemic. Recruitment and data collection for the study was also affected during this time as laboratory closures occur periodically throughout the pandemic (188). The study recruited forty-four healthy participants; seventeen participants were ineligible or declined to participate, twenty-eight participants were allocated to a randomized ID correlating to a randomized order or treatments. Three withdrew because of loss of follow through (not responding to emails) or contact or personal reasons. Twenty-five participants completed all four laboratory sessions.

2.3.2 Screening

The study design was a single-blinded, repeated measures, randomized trial aiming to collect data from 30 healthy adult males. Participants completed a telephone screening to assess eligibility. In addition, participants were measured using a stadiometer and body composition analyzer (TBF-300A, Tanita Corporation, Tokyo, Japan). After the screening sessions and if subjects met the criteria, they were scheduled for four experimental sessions. Participants were then asked to fill out an informed consent form and scheduled to return to the laboratory between 8-10 am following a 10+ hour fast. Participants were allowed to consume water up to 1 hour before each session. Participants were instructed to refrain from alcohol consumption, cannabis consumption and any unusual exercise the night prior to the sessions. Participants each session filled out a health and well-being questionnaire (Appendix B). Additionally, during one of the study sessions the participants were asked to fill out a Restraint Scale questionnaire (189), a Three-Factor Eating Questionnaire (TEFQ) (190), and a Past Year Total Physical Activity questionnaire (191). Moreover, the restrictive eating questionnaire was scored out of 18. The TFQ was scored using the guidelines by Stunkard et al. (1985).

2.3.3 Sample size

There have been several studies that assess the effects of food on satiety using a within-subject design with a fixed subsequent meal (2,4,5,22,25,49,51). Many of these studies use between 25-26 participants to detect a difference between treatments. Flint et al. (2000) conducted a study on reproducibility of VAS participants characteristics were normal weight males, that did not smoke, were not elite athletes or had any history of obesity (179). Moreover, Flint et al. 2000 reported 18 participants with a power of 0.8 is sufficient to detect difference as long as the study utilizes a fasted and mean appetite ratings (179). In addition, Gregersen et al.

(2008) used a fixed meal in the study with healthy, normal weight males and stated that the studies with a power of 0.8 or 0.9 should have 17 to 23 participants, which is sufficient to detect 500kJ difference (187). Therefore, using a randomized repeated measures design, the sample size of 25 participants would be sufficient to detect statistical significance in satiety and food intake All procedures in this study has been approved by the University Research Ethics Board of Mount Saint Vincent University (ethics protocol # 2019-114).

2.3.4 Materials and instruments

2.3.5 Flour ingredients

Laird lentil flour was provided by Avena Foods Limited (formerly Best Cooking Pulses, BCP©). Avena Foods Limited production of lentil flour is in Manitoba, Canada (108). The mean particle size distribution for lentil flour is 3.8-92.5 μm. Wheat flour used during the experiment was from Master's Hand All Purpose Wheat Flour produced by Ardent Mills ULC in Ontario, Canada. The mean particle size distribution for the Master's Hand flour is 17.9-157.5μm with a volume-weighted mean particle size of 83.5μm. Lentil flours of varying particle sizes are available from the processors and flours having a particle size of <80μm have been designated as superfine flours by the CIGI (102). Since the wheat flours and lentil flours used in the formulation of snack bars in the current study have a mean particle size around and less than 80μm respectively, both the flours are considered to be in the superfine category. The main purpose was to use the flours with similar particle size in the snack bar formulation so that the physiological effects can be attributed to the differences in the nutritional composition of the flours and not the difference in the particle size.

Table 5. List of ingredients in the snack bars

Dough (per one bar)	**Weight (g)**	**Source**
Laird lentil flour	30 in LF bars, 0 in WF bars, 15 in MLW-F bars	BCP
Master's Hand wheat all purpose flour	30 in WF bars, 0 in LF bars, 15 in MLW-F bars	Costco
Honey	17	Sobeys
Cinnamon	0.70	Sobeys
Salt	0.01	Sobeys
2% Farmers Milk	5	Sobeys
Baking Soda	0.02	Sobeys
Filling (per ~ 10 bars)	**Weight (g)**	**Source**
Rogers Sugar	30	Sobeys
Dried apples	15	Pete's Foods
Honey	5	Sobeys
Corn starch	5	Sobeys
Cinnamon	0.01	Sobeys

All types of snack bars (LF, WF and MLW-F) contained the same ingredients except the source and amount of the flour as indicated in Table 5.

2.3.6 Treatment preparation

Three snack bar treatment formulations were prepared: 100% lentil flour (LF), 50% lentil flour and 50% wheat flour (MLW-F), and 100% wheat flour (WF) snack bars. An industrial kitchen was used to formulate the snack bars. All ingredients were weighed on a digital scale. The list of ingredients and their origin are indicated in Table 5.

The LF treatment dough was prepared for one serving by combining 30g of lentil flour with 17g of honey, 0.2g of baking soda, pinch of salt, 0.70g of cinnamon and 5ml of 2% milk. The MLW-F treatment dough was prepared for one serving by combining 15g of lentil flour and 15g of wheat flour with 17g honey, 0.2g of baking soda, 0.01g of salt, 0.70g of cinnamon and 5ml of 2% milk. For the WF treatment dough for one serving, 30g of wheat flour was combined with 17g of honey, 0.2g of baking soda, pinch of salt, 0.70g of cinnamon and 5ml of 2% milk. Each dough was created individually with exact measurements as described in Table 5. For the dried apple filling (for ~ 10 bars), 15g of dried apples were chopped finely and added to 30g sugar, 5g of honey, 0.01g of cinnamon, and 5g corn starch into a frying pan. Boiled water was added to dissolve sugar and then the mixture was brought to a boil. The filling was cooked to a sticky consistency and set aside for cooling. The snack bar was prepared by rolling out the prepared dough to half an inch rectangle and placing 5g of the dried apple filling on the centre of the dough with both the sides folded to create a granola bar appearance (Figure 1). Overall, a total of 120 snack bars were prepared (40 snack bars per treatment). Snack bars were steamed in a small steamer with half a cup of water for 5 minutes. Snack bars were then transferred to a baking sheet and baked in a convection oven at 350ºF for 12 min. For all the snack bars, each snack bar ingredient was measured individually and timed during the steaming and baking process to ensure consistency. Based on the Table of Reference Amounts for Food set by Health

Canada in 2016, the reference amount for grain-based bars with filling or partial or full coating is 40g; however, the serving size can be between 50% and 200% of the reference amount (i.e., more than 20g and less than 80 g) (192). The treatment bars were cooked and had a weight of 58.5 g meeting the above-mentioned requirements. The snack bars were cooled and then stored in the freezer. The snack bars were coded with a line pattern to indicate the type of a treatment and the containers were color-coded and labelled. Prior to the study session, snack bars were placed in a refrigerator 24 hours before session and then warmed in microwave for 15 seconds and served to participants.

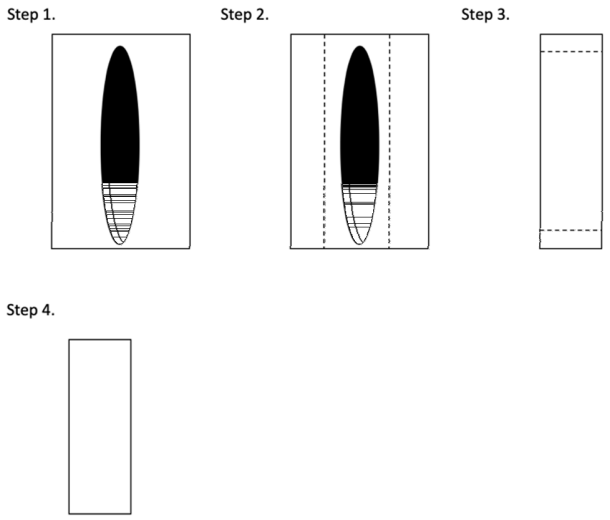

Figure 1. Snack bar dough folding

*Rectangles- granola bar dough, ovals- granola bar filling, dashed lines- folding inward of dough

2.3.7 Snack bar nutrition content

All nutritional content was analyzed using the Canadian Nutrient File (Health Canada) for all snack bar formulations and the output is summarized in Table 6. All bars contained the same ingredients, the only difference between the snack bars being the flour. All three treatments

were an isocaloric (Table 6). The snack bar treatments with a descending content of fibre and protein in order are; LF, MLW-F, and WF.

Table 6. Macronutrient composition of the treatments (per serving)

Treatment	Per serving Kcal	Carbohydrates (g)	Fibre (g)	Protein (g)	Fats (g)
C	0	0	0	0	0
LF	184.5	38.2	4.1	9.4	0.4
MLW-F	184.3	39.8	2.7	6.7	0.6
WF	184.2	41.3	1.4	3.9	0.8

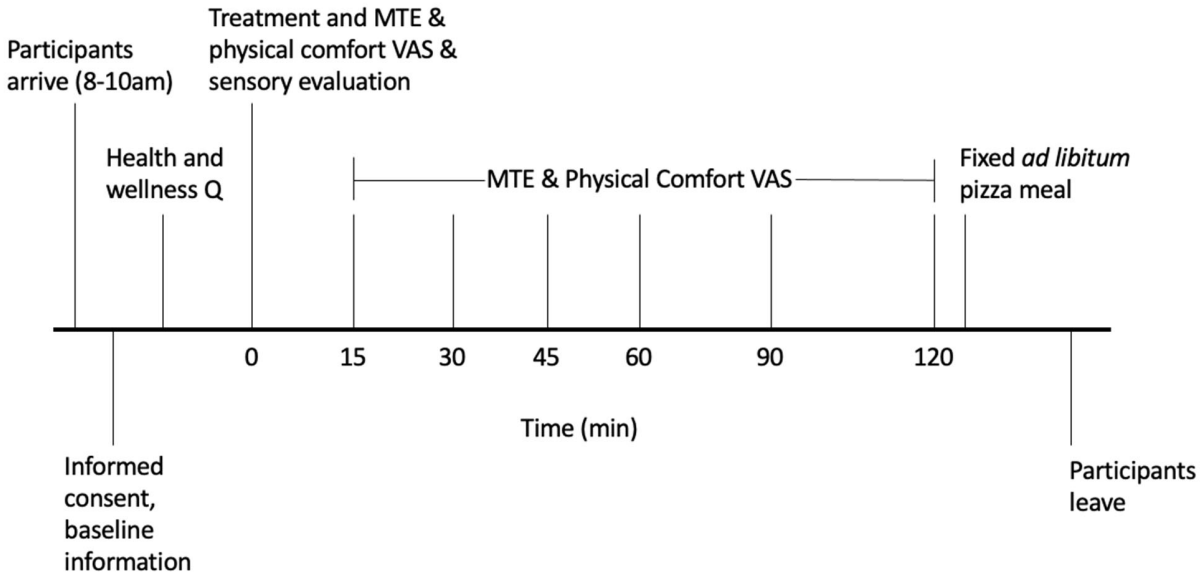

Figure 2. Session timeline.

2.3.8 Pizza meal

Dr. Oetker mini pizzas were provided to participants. The pizzas that were provided had a uniform density crust to ensure a consistent energy density. During the screening process, participants choose one of the following Dr.Oetker standardized pizzas; three cheese, pepperoni, or deluxe. Pizzas were cooked as per instructions indicated on the frozen pizza box. During the data collection Dr.Oetker pizzas products changed. After the new product was released, participants had a choice of three cheese or pepperoni. New and old pizza boxes of the products were kept for calculation of calories for food intake. Calories were calculated from grams per serving as indicated on the nutritional information from the pizza boxes.

2.3.9 Food intake at 120 minutes

fixed pizza meal with water was pre and post weighed in grams. Participants consumed one type of pizza of their choice: three-cheese, pepperoni, or deluxe. Each participant received the same variety of pizza at all four sessions, based on their initial preference determined at the screening session. All pizza were cooked in a convection oven and followed the instructions given on the box. Pizza was cooked, quartered, weighed, recorded, and served to participants. Pizza was served every eight minutes; participants were offered a new tray of quartered mini pizzas with 350 g water until they refused. Leftover pizza and water were weighed and recorded.

During the data collection Dr.Oetker changed their mini pizzas products from rounded

2.3.10 Fasting blood glucose

To measure fasting blood glucose as the indication of overnight fasting, a research assistant collected capillary blood from the participants. A Hemocue© blood glucose analyzer (HemoCue AB, Ängelholm, Sweden) had calibration checked for each session and used to analyze participants' blood glucose levels at the beginning of each session. A research assistant used disposable normal blood flow lancelets and Hemocue© cuvettes to collect the blood samples. An initial blood sample was discarded to removed alcohol and interstitial fluid and the second blood sample was collected. If blood glucose level was more than 6.0mmol/L, participants were asked to reschedule for another day.

2.3.11 Sensory, appetite and food intake evaluations

Participants were given a 100mm VAS motivation to eat and physical comfort questionnaire prior to consumption of the treatment (0 minutes). Participants then were asked to consume a random order of one of the four treatments, C (350g water), LF, MLW-F, or WF snack bars, over four laboratory sessions. Participants were served either water control, or a snack bar with 350g of water; participants were asked to consume the whole treatment. All treatments were provided through a steel cubical with white lighting. During the treatment consumption, participants were asked to fill out a sensory analysis questionnaire containing 9-point hedonic scale and 100mm VAS with anchoring words from 0-12 minutes. Postprandially, participants were asked to fill out a 100mm VAS questionnaire about the motivation to eat and physical comfort every 15, 30, 45, 60, 90, 120 minutes and food intake was measured at 120

minutes. The participants were asked to place an "X" on a 100mm VAS line for: i) Desire to eat (How strong is your desire to eat?), ii) Hunger (How hungry do you feel?), iii) Fullness (How full do you feel?), and iv) Prospective food consumption (How much food do you think you can eat?) with opposing word anchors (46). Physical comfort questionnaire contained the following questions on i) Nausea (Do you feel nauseous?), ii) Stomach pain (Does your stomach hurt?), iii) Overall wellness (How well do you feel?), iv) Gas (Do you feel like you have gas?), and v) Diarrhea (Do you feel like you have diarrhea?) All participants were asked not to talk during the consumption of the treatment and pizza meal during the session. The average score for subjective appetite was calculated using the formula: average appetite score =[desire to eat + hunger + (100 – fullness) + prospective consumption]/4. Food palatability assessment used VAS and hedonic scales. Participants were asked to answer questions on pleasantness, sweetness, taste, texture, flavour, aftertaste, chewiness, lentil flavour, and prospective purchasing.

2.3.12 Data Analysis

A two-way repeated measures analysis of variance (ANOVA) using a PROC MIXED procedure was used to determine the effect of time, treatment and a time by treatment interaction for the outcomes measured with the motivation to eat and physical comfort questionnaires (2,4,5,24). In addition, a one-way ANOVA using a PROC MIXED procedure was applied to determine the effect of a treatment on pizza meal at 120 minutes with pizza meal, and for the effect of treatment on tAAC for subjective appetite measures (2,4,5,24) A two-way repeated measures ANOVA was used to analyze time and time by treatment interaction for motivation to eat and physical comfort. When a significant effect of a treatment was observed, the differences between the pairs of the treatments were analyzed using a Tukey-Kramer post hoc test. The significant difference p-value was set at $p<0.05$. Results are reported in means and

standard deviations (SD). Participants annual physical activity logs were calculated to hours per week and converted into metabolic equivalence (METS) per week. Pearson's correlation coefficient was calculated to determine the relationship between the measures of physical activity, subjective appetite factors and FI.

The analysis followed other studies and Health Canada draft guidance document for satiety health claims on food (45). The VAS questionnaires were measured using a millimeter ruler to measure appetite scores and double-checked by a second research assistant. All statical analysis was conducted through SAS© 9.4 software (Cary, NC, U.S.A.). Motivation to eat questionnaire scores (desires to eat, hunger, fullness, and prospective consumption) were calculated as a difference from the baseline. The total area above the curve (tAAC) for average appetite, desire to eat (DTE), hunger, fullness, and prospective consumption scores was calculated over 120 minutes.

3.0.0 Results

3.1.0 Participants

The study recruited participants who are non-smokers between the ages of 19 and 35 with a BMI between 18 and 24.9 kg/m^2. Twenty-five participants completed all four sessions (Figure 3). The demographic of the participants averaged 25 ± 4.7 years old and had an average BMI of 23.39 ± 1.98. All other characteristics of the participants are summarized in Table 7. On average, participants ranked their previous subjective health and wellness no more or no less than their usual routine. Participants average previous food intake, physical activity, and stress levels on a 100 mm VAS averaged, 49.57 ± 13.46, 44.74± 15.05, and 49.52±16.0, respectively. Moreover, at the beginning of each session, participants blood glucose levels reflected those of normal 10+ hours glucose fasting levels (<6 mmol/L).

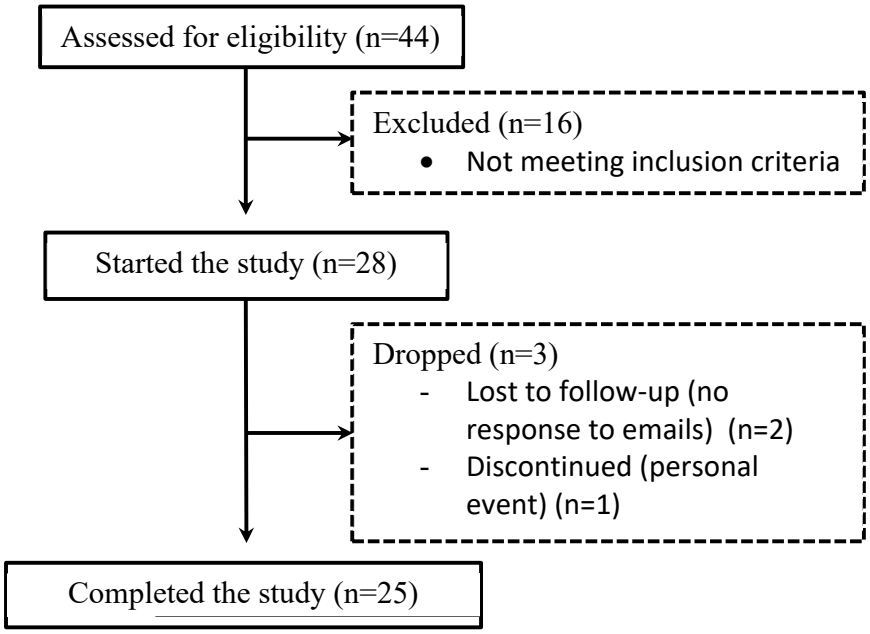

Figure 3. Recruitment CONSORT diagram

Table 7. The characteristics of participants

Characteristics	
	Mean ± SD
Age (years)	25 ± 4.7
Weight (kg)	71.0 ± 6.7
Height (cm)	173.3 ± 7.3
BMI (kg/m^2)	23.4 ± 1.98
Previous Food Intake (mm)	49.6 ± 13.5
Physical Activity (mm)	44.7± 15.1
Stress (mm)	49.5±16.0
Baseline Blood Glucose (mmol/L)	5.2 ±0.6
Restraint Score (max 18)	12.1±7.0
TFQ Factor I (Behavioural Restraint) (max 20)	7.4±2.4
TFQ Factor II (Behaviour & weight) (max 16)	4.5±3.0
TFQ Factor III (Hunger & behaviour ramifications) (max 36)	5.0±4.7
Total Three Factor Q (max 72)	16.8±1.2

Means ± SD, n=25. Values for previous food intake, physical activity, stress and blood glucose are cumulative averages and standard deviations from each session. Scoring of TFEQ Factors I, II, III followed Stunkard, A. J. & Messick, S. (1985) scoring guidelines.

3.2.0 Motivation to eat

All motivation to eat questionnaire scores for desires to eat (DTE), fullness, hunger and prospective consumption, were calculated as the difference from the baseline (0 min) at each time point (15, 30, 45, 60, 90, and 120 minutes).

3.2.1 Desire to eat

DTE scores fell immediately post consumption of treatments and steadily increased over 120 minutes (Figure 4). LF, MLW-F and WF suppressed subjective DTE compared to the control (water). There was no effect of a treatment at baseline (0 min; $p=0.1$) on DTE. There was an effect of treatment ($p <0.0001$), time ($p<0.0001$) and a treatment by time interaction ($p<0.0001$) for DTE delta scores. Additionally, there was an effect of session ($p<0.002$). The average DTE delta score over 120 min was lowest after LF (-12.3±25.0 mm) and highest after C (5.49±17.9 mm). The intake of MLW-F and LF treatments resulted in intermediate average DTE delta scores over 120 min: 0.61±13.4 mm and –1.33±21.4mm, respectively. There were significant differences between LF and C ($p<0.0001$), and LF and MLW-F ($p<0.0001$), and LF and WF ($p=0.0007$) for average delta DTE over 120 minutes. There were no significance differences between MLW-F and C, and WF and C, and MLW-F and WF for average DTE delta scores over 120 minutes. There was a significant difference between LF and C at 15, 30, 45, 60, 90 and 120 min ($p<0.05$) for average delta DTE scores (Figure 4). There were no significances between C and MLW-F, C and WF, and LF and MLW-F for 15, 30, 45, 60, 90 and 120 min. There were significant differences between LF and MLW-F at 45, 90, and 120 min ($p<0.05$). There were significant differences between LF and WF at 90 and 120 min ($p<0.05$) for delta DTE scores.

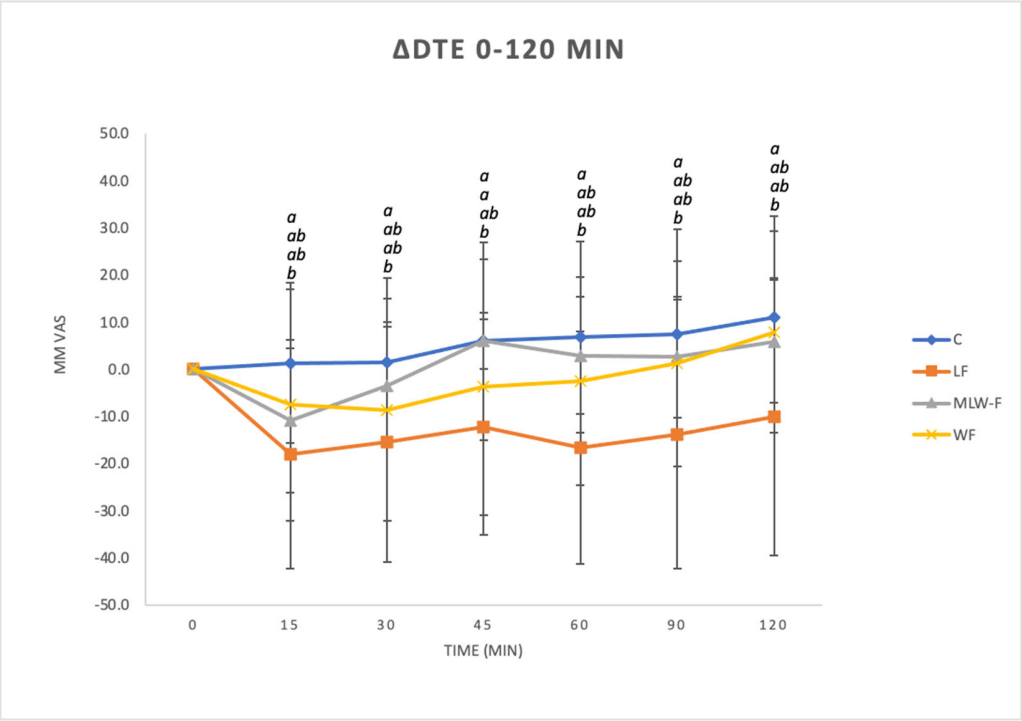

Figure 4. ΔDTE over 2 hours. Means ± SD, n=25. Two-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between treatments (P<0.05). DTE VAS scale: 0- very weak; 100- very strong.

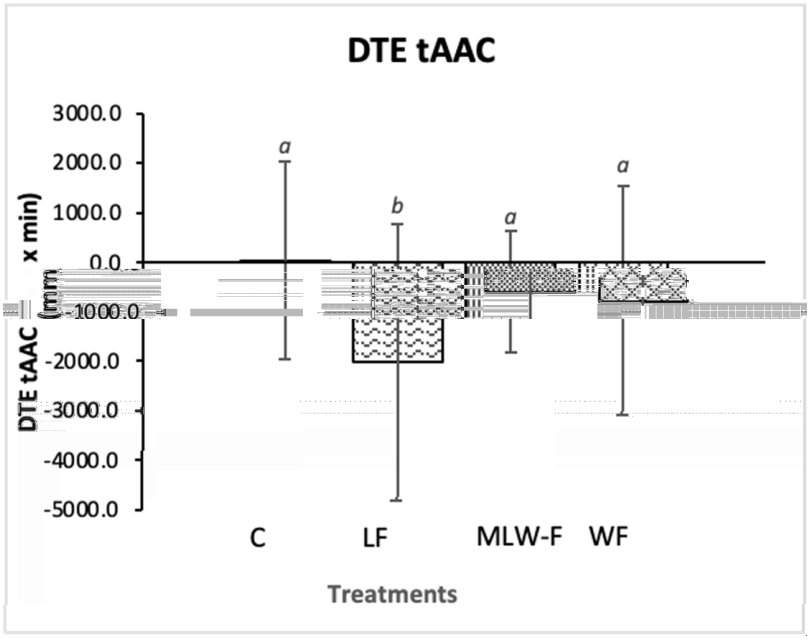

Figure 5. DTE tAAC over 120 min. Means ± SD, n=25. One-way RM ANOVA with Tukey-Kramer post-hoc test. The values with different superscripts are statistically different (P<0.05).

There was an effect of treatment ($p<0.0001$) for DTE tAAC (Figure 5). The tAAC for LF was lower (-2028.6±-2797.56 mm×min) compared to control (36.2±2002.3 mm×min) ($p<0.0001$). The treatments with MLW-F, and WF resulted in intermediate tAAC for desire to eat: -591.6± 1237.23 mm×min, and –781.2±2326.3 mm×min, respectively. There were significant differences between LF and MLW-F ($p<0.001$), and LF and WF ($p=0.05$). There were no significant differences between control and MLW-F ($p= 0.71$), and control and WF ($p=0.47$). There was no difference between MLW-F and WF ($p=0.97$) (Figure 5)

3.2.2 Hunger

Hunger scores fell immediately post consumption of treatments and steadily increased over 120 minutes (Figure 6). LF, MLW-F and WF suppressed subjective hunger compared to the control. There was no difference between the treatments at baseline (0 min; $p=0.1$). There was an effect of treatment ($p<0.0001$), time ($p<0.0001$), session ($p=0.04$) and, a treatment by time interaction ($p<0.0001$) on hunger over 120 min. The average hunger score over 120 min was lowest after LF (-13.4±29.3 mm) and highest after C (2.3±26.1 mm). The intake of MLW-F and LF treatments resulted in the intermediate delta hunger scores over 120 min: -3.4±21.7 mm and –4.3±26.5mm, respectively. There were significant differences between LF and C ($p<0.0001$), and LF and MLW-F ($p<0.005$), and LF and WF ($p<0.004$) for average delta hunger over 120 minutes. There were no significance differences between MLW-F and C ($p=1$), and WF and C ($p=0.13$), and MLW-F and WF ($p=1$) for average delta hunger over 120 minutes. There was a significant difference between LF and control at 15,30, 45, 60, 90 and 120 ($p<0.05$) (Figure 6). There was a trend between C and MLW-F, C and WF, and LF and MLW-F for 15, 30, 45, 60, 90

and 120 minutes (p=0.05). There was a significant difference between LF and MLW-F (p<0.05) for delta hunger scores at 45 minutes (Figure 6).

Figure 6. Δ Hunger over 2 hours. Means ± SD, n=25. Two-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between treatments (P<0.05). Hunger VAS scale: 0- not hungry; 100- very hungry.

There was an effect of a treatment on hunger tAAC over 120 min (p=0.0002) (Figure 7). There was no effect of session (p=0.09). The tAAC was lowest after LF (-627.9±1885 mm×min)

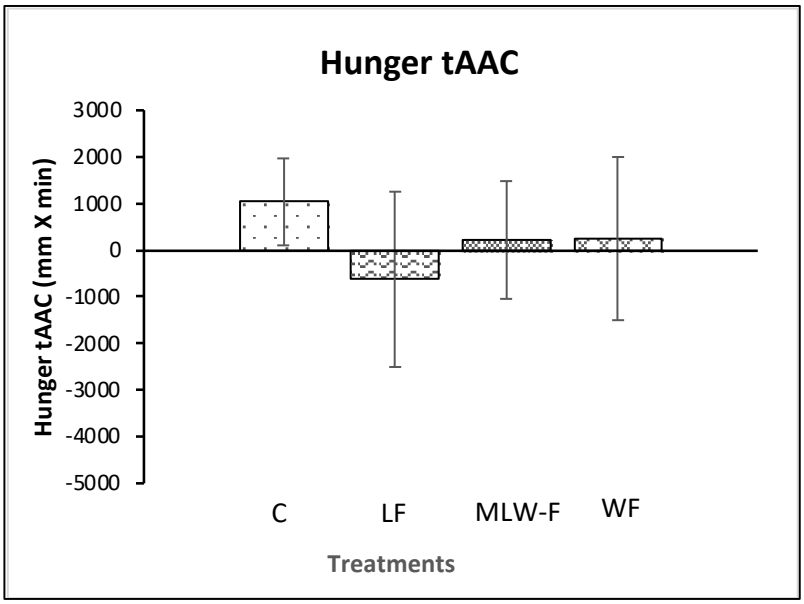

Figure 7. Hunger tAAC over 120 min. Means ± SD, n=25. One-way RM ANOVA with Tukey-Kramer post-hoc test. The values with different superscripts are statistically different (P<0.05).

3.2.3 Fullness

Fullness scores increased immediately post consumption of treatments and steadily decreased over 120 minutes (Figure 8). There was no difference between the treatments at baseline (0 min; p=1). There was an effect of time (p<0.0001), treatment (p=0.0002), a time by treatment interaction (p=0.003), and session (p<0.0001) on average fullness (Figure 8). The average fullness score over 120 was highest after LF (8.19± 22mm) and lowest after C (-4.24±17 mm). The intake of MLW-F and LF treatments resulted in intermediate scores: 6.1±15 mm and 3.7±17mm, respectively. There were significant differences between LF and C (p=0.0002), C and MLW-F (p=0.002), and C and WF(p=0.01) for average fullness over 120 minutes. There were no significant difference between LF and MLW-F (p=0.91), LF and WF (p=0.66), and MLW-F and WF (p=0.95) for average fullness over 120 minutes. The fullness delta scores between C and LF were significant at 15, 30, 60, and 90 minutes (p<0.05). There were no

significant differences between C and WF, LF and MLF-F, LF and WF, and MLW-F and W at 15, 30, 45, 60, 90 and 120 minutes for fullness scores ($p>0.05$) (Figure 8). There was a significant difference between MLW-F and C at 30 minutes. There was no effect of a treatment ($p=0.21$) or session (p- 0.98) on fullness tAUC (Figure 9).

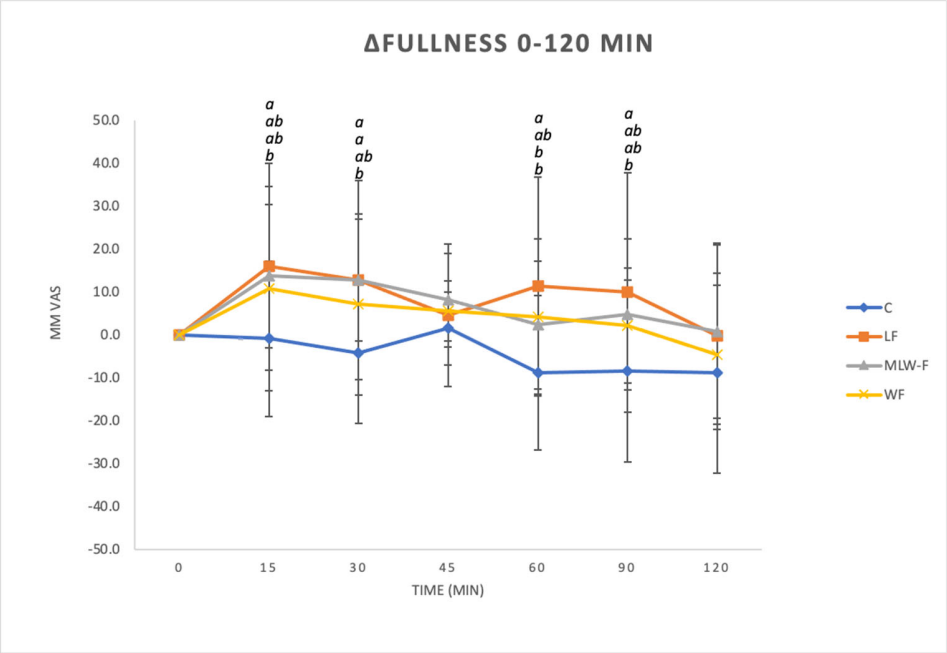

Figure 8. Δ Fullness over 2 hours. Means ± SD, n=25. Two-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between treatments ($P<0.05$). Fullness VAS scale: 0- not full; 100- very full.

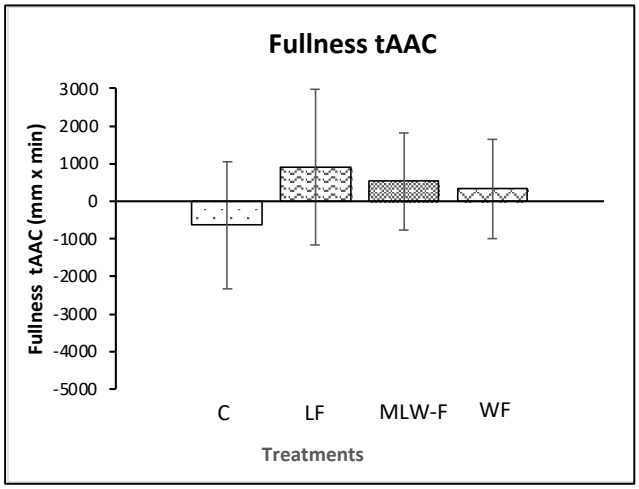

Figure 9. Hunger tAAC over 120 min. Means ± SD, n=25. One-way RM ANOVA with Tukey-Kramer post-hoc test.

3.2.4 Prospective food consumption

Prospective food consumption scores fell immediately after the treatments and steadily increased over 120 minutes (Figure 10). There was no difference between the treatments at baseline (0 min; p=0.1). There was no effect of treatment (p=0.52) for average prospective consumption, but there was an effect of time (p<0.0001), treatment by time interaction (p=0.005) and session (p=0.0005).

There was an effect of a treatment (p=0.01) but no effect of session (p=0.31) on prospective food consumption tAAC. The tAAC was lowest after LF (-519.2±2110 mm×min) and highest after C (449.85±1062 mm×min).

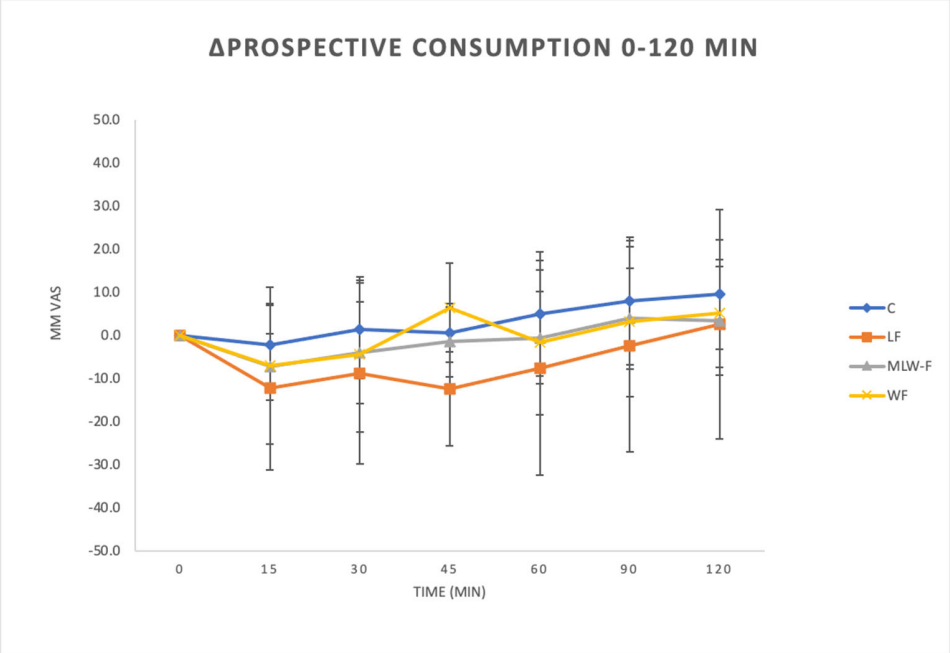

Figure 10. Δ Prospective food consumption over 2 hours. Means ± SD, n=25. Two-way RM ANOVA followed by Tukey-Kramer post-hoc test. Prospective food consumption VAS scale: 0- nothing at all; 100- a large amount.

The treatments with MLW-F and WF resulted in the intermediate tAAC scores: 24±869.2 mm×min and 29±1421 mm×min, respectively. There was a significant difference between LF

and C (p=0.007) but not between C and MLW-F (p=0.28), C and WF (p=0.27), LF and MLW-F (p=0.33), LF and WF (p=0.08), and MLW-F and WF (p=0.88) (Figure 11).

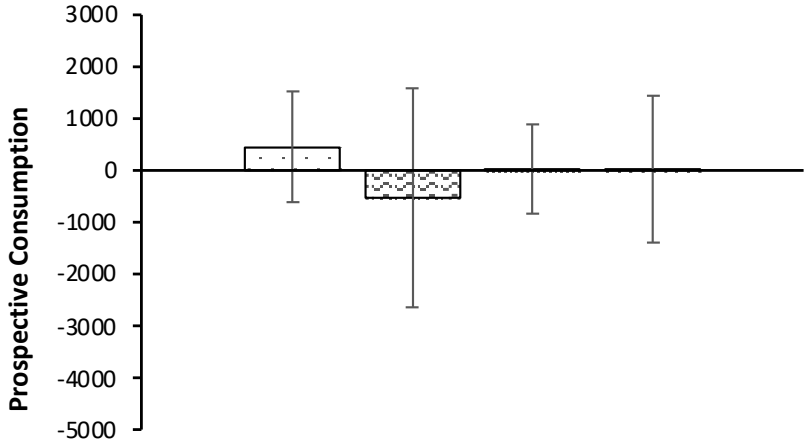

Figure 11. Prospective food consumption tAAC over 120 min. Means ± SD, n=25. One-way RM ANOVA with Tukey-Kramer post-hoc test. The values with different superscripts are statistically different (P<0.05).

3.2.5 Average Appetite

Average appetite scores were calculated using the formula: average appetite = [desire to eat + hunger + (100-fullness)+prospective consumption]/4. The subjective appetite decreased after the treatment and steadily increased between 15 and 120 minutes (Figure 12). There was no difference between the treatments at baseline (0 min; p=0.1). There was an effect of a treatment (p<0.0001), time (p<0.0001), and a treatment by time interaction (p<0.0001) on average appetite. There was an effect of session on average appetite scores (p=0.01). The average appetite delta score over 120 was lowest after LF (-6.68±22.41 mm) and highest after C (6.88±11.53 mm). The intake of MLW-F and WF treatments resulted in intermediate average

appetite delta scores over 120 min: -0.41±11.15 mm and –0.77±16.19 mm, respectively. Although there was significant difference between LF and C ($p<0.0001$), C and MLW-F ($p= 0.0006$), C and WF ($p=0.044$), LF and MLW-F ($p=0.025$), and LF and WF ($p=0.005$) over 120 min, only C and LF scores were significantly different at 15, 30, 45, 60, 90 and 120 min ($p<0.05$), and the scores between C and MLW-F at 60 min ($p<0.05$) (Figure 12). There was no significant difference between MLW-F and WF ($p=0.94$) average appetite scores over 120 minutes.

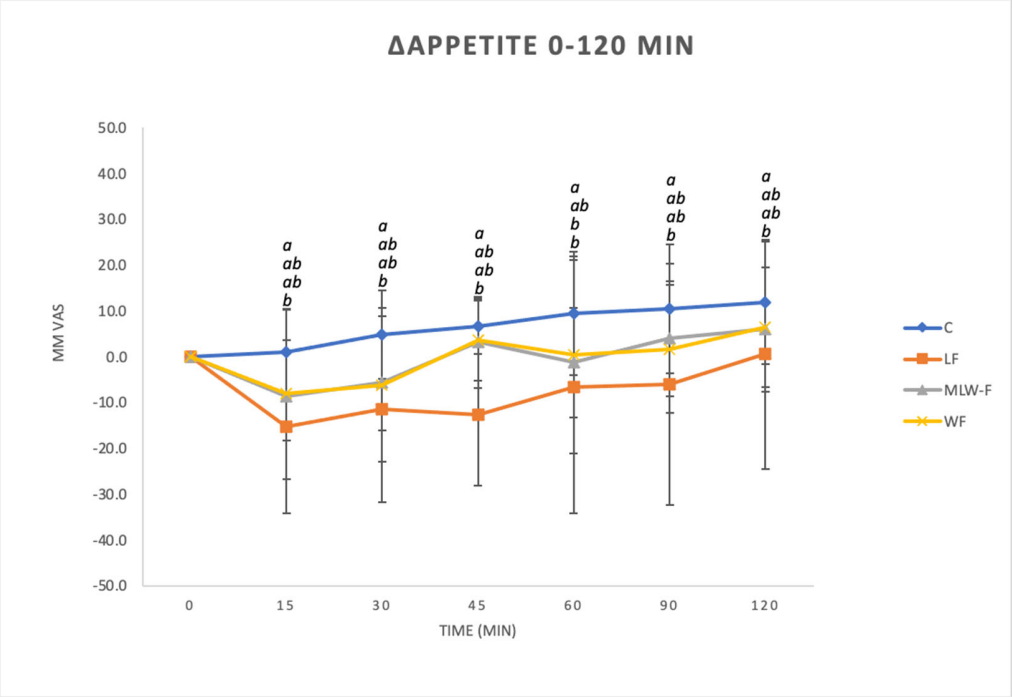

Figure 12. Δ Average appetite over 2 hours. Means ± SD, n=25. Two-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between treatments ($P<0.05$).

There was an effect of a treatment (p=0.003) but no effect of session (p=0.21) on average appetite tAAC. The appetite tAAC was lowest after LF intake (-1438.63±2414.2 mm×min) and highest after C (102.0±1286.99 mm×min). The treatments with MLW-F and WF resulted in intermediate values: -634.01±1237.9 mm×min and -752.8 mm×min, respectively. There were significant differences between LF and control (p=0.0001), LF and WF (p=0.04), and MLW-F and control (p=0.03) (Figure 12). There was no difference between C and WF (p=0.2), LF and MLW-F (P=0.2), and MLW-F and WF (p=0.86) (Figure 13).

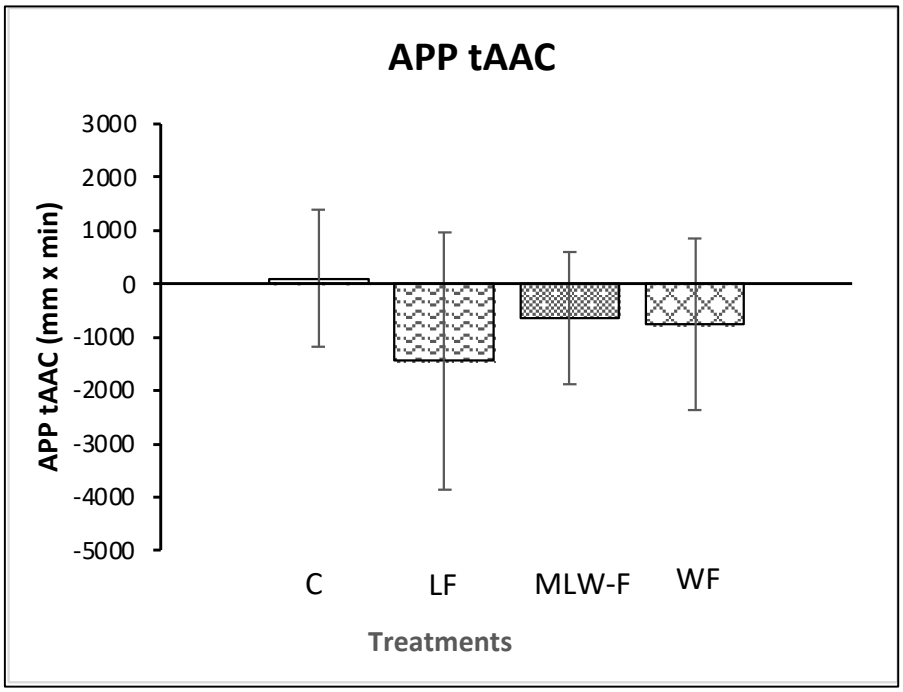

Figure 13. Average appetite tAAC over 120 min. Means ± SD, n=25.
One-way RM ANOVA with Tukey-Kramer post-hoc test.
The values with different superscripts are statistically different (P<0.05).

3.3.0 Ad libitum food and water intake

There was no effect of a treatment on food intake at 120 min (p=0.13) (Figure 14). There was a significant effect of session on food intake (p=0.02) with a higher intake at session 3 than in session 4 (p=0.038). There was no effect of a treatment on cumulative food intake (kcal of a treatment plus kcal of meal at 120 min (p=0.81) (Figure 15). There was a significant effect of session on cumulative food intake (p=0.018).

There was no effect of a treatment (p=0.35) or session (p=0.13) on water intake with the pizza meal at 120 min (Figure 16). There was no effect of a treatment (p=0.35) or session (p=0.13) on cumulative water intake over two hours (350 g water with a treatment plus water intake with pizza meat at 120 min) (Figure 17).

There was no effect of a treatment (p=0.97) or session (p=0.12) on caloric compensation (Figure 18).

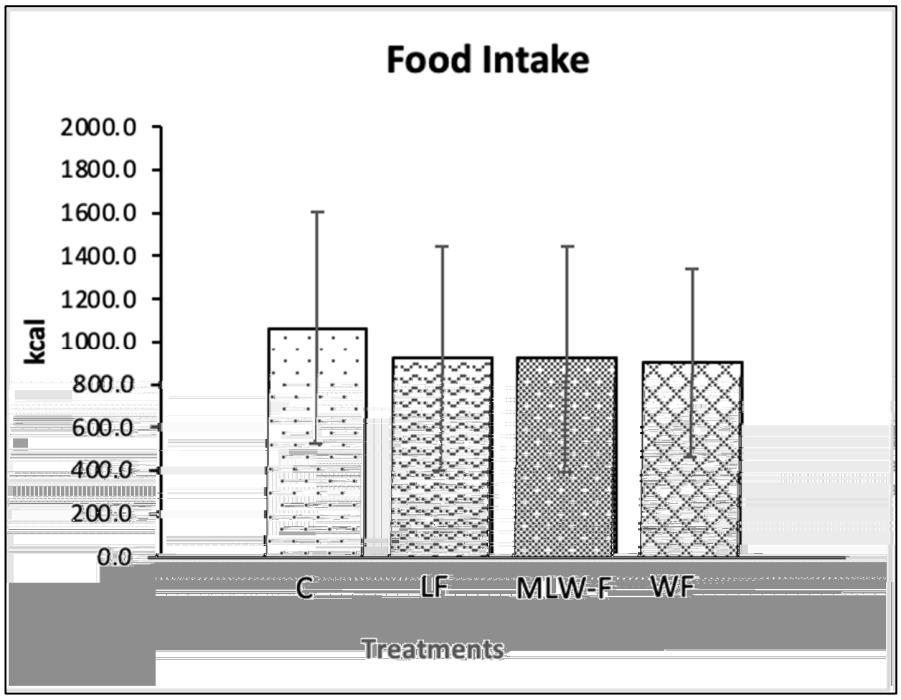

Figure 14. food intake at 120 min. Means ± SD, n=25. One-way RM ANOVA.

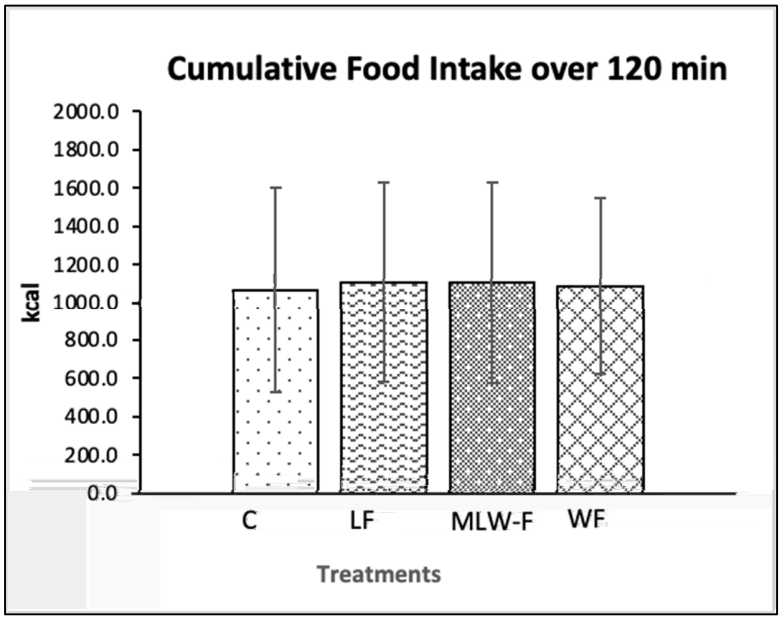

Figure 15. Cumulative food intake over two hours. Means ± SD, n=25. One-way RM ANOVA.

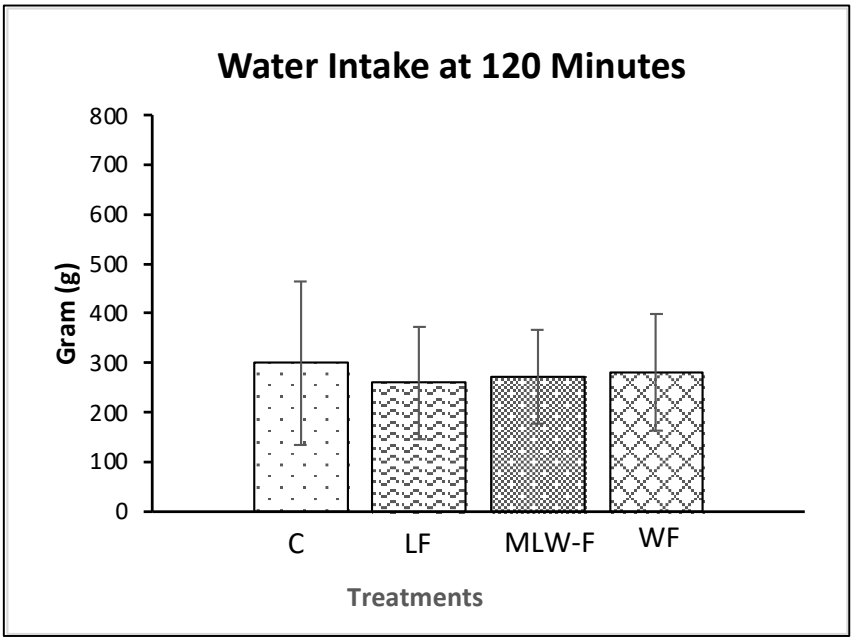

Figure 16. water intake at 120 min. Means ± SD, n=25. One-way RM ANOVA.

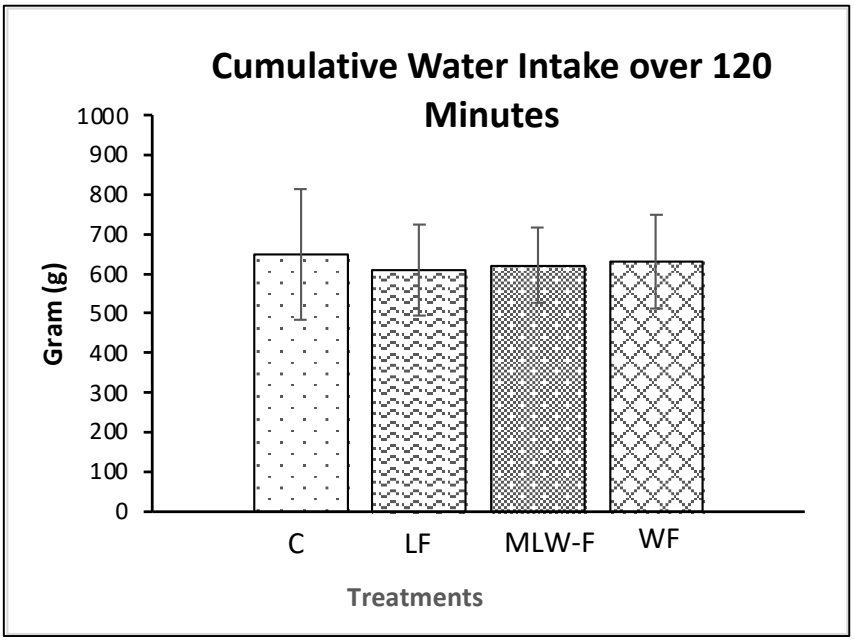

Figure 17. Cumulative water intake over two hours. Means ± SD, n=25. One-way RM ANOVA.

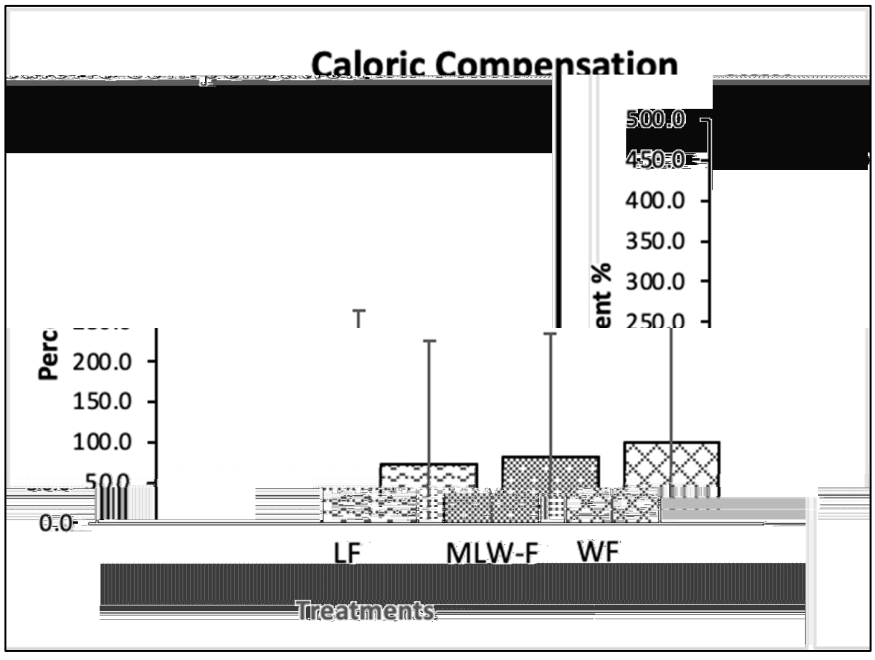

Figure 18. Caloric compensation. Means ± SD, n=25. One-way RM ANOVA.

3.4.0 Physical comfort

There was an effect of time on nausea ($p<0.0003$), stomach pain ($p<0.0001$), overall feeling ($p<0.0001$), gas ($p<0.0001$), and diarrhea ($p<0.0001$) (Figures 19-23). There was no effect of a treatment on nausea ($p=0.37$), stomach pain ($p=0.19$), gas ($p=0.56$), and diarrhea ($p=0.28$). The was a significant effect of a treatment on overall feeling ($p=0.02$). The overall feeling over 120 min was perceived better after the treatment with LF compared to the treatment with MLF-F ($p=0.017$). There were differences in feeling perception between control and LF (<0.0001), control and WF ($p<0.0001$), LF and MLW-F ($p <0.0001$), and MLW-F and WF ($p <0.0001$) at 45 minutes. There was an of a session on all physical comfort components; including for nausea ($p=0.02$), stomach pain ($p=0.01$), overall feeling ($p=0.0007$), gas ($p=0.0007$), and diarrhea ($p=0.0001$); however, there was no effect of session by treatment interaction for all physical comfort components ($p>0.05$). All gastrointestinal discomfort parameters such as nausea, gas, stomach pain and diarrhea remained at the very low level (less than 10mm on VAS) over the whole duration of the sessions.

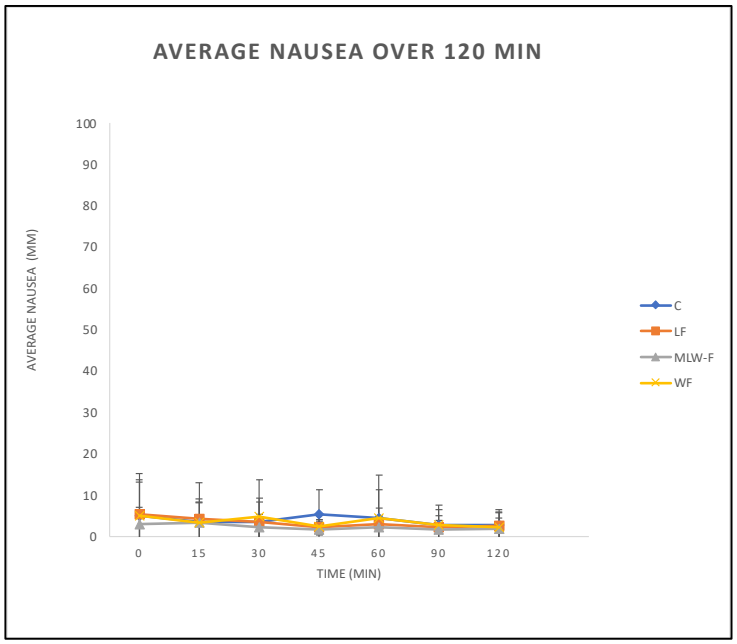

Figure 19. Perception of nausea over two hours. Means ± SD, n=25. Two-way RM ANOVA. VAS Scale: 0- not at all; 100- very much.

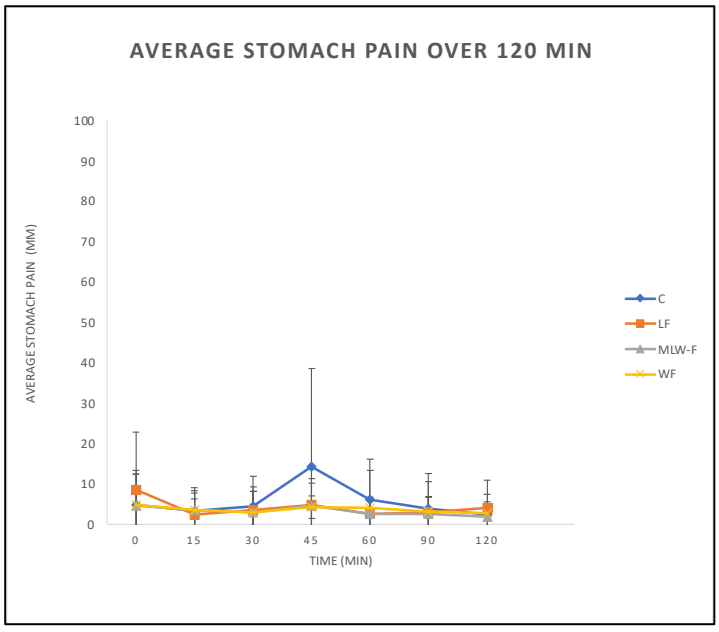

Figure 20. Perception of stomach pain over two hours. Means ± SD, n=25. Two-way RM ANOVA. VAS Scale: 0- not at all; 100- very much.

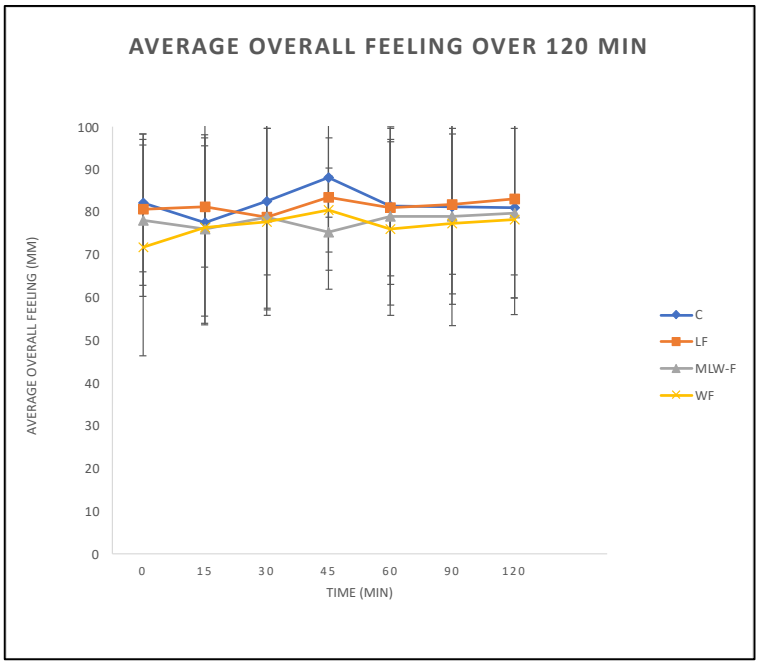

Figure 21. Subjective overall feeling over two hours. Means ± SD, n=25. Two-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between the treatments ($P<0.05$). VAS Scale: 0 - not well; 100 - very well.

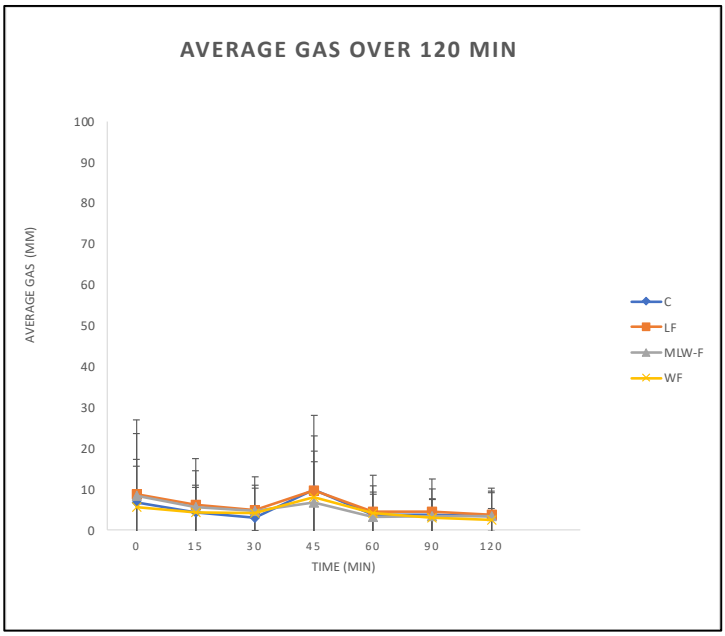

Figure 22. Perception of gas over two hours. Means ± SD, n=25. Two-way RM ANOVA. VAS Scale: 0- not at all; 100- very much.

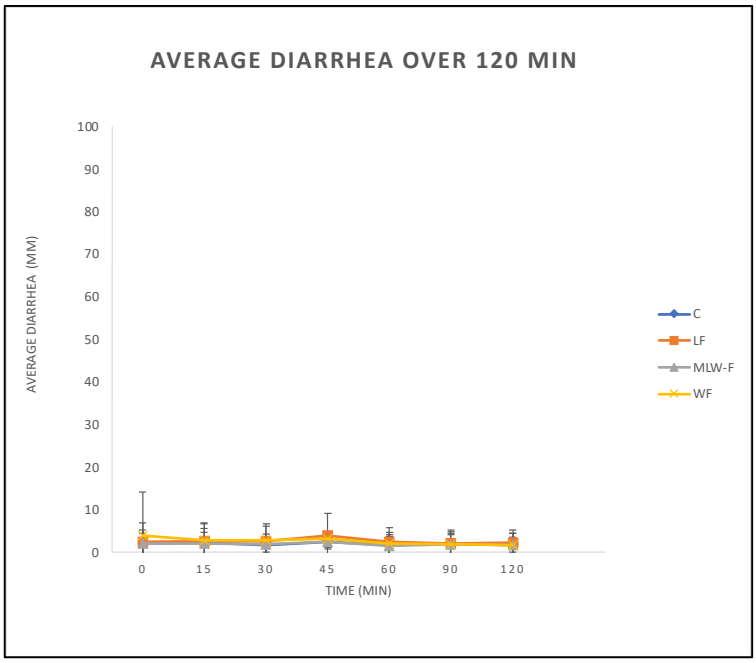

Figure 23. Perception of diarrhea over two hours. Means ± SD, n=25. Two-way RM ANOVA. VAS Scale: 0- not at all; 100- very much.

3.5.0 Food sensory characteristics

There was no effect of a treatment on perceived pleasantness ($p=0.93$), taste ($p=0.19$), texture ($p=0.64$), aftertaste ($p=0.13$) and perspective purchasing ($p=0.83$). There was an effect of a treatment on perceived sweetness, chewiness, and lentil flavour ($p<0.0001$). The sensory attributes of the treatments are illustrated below in Figures 24-32. There was no effect of session on all sensory properties: pleasantness, sweetness, taste, texture, flavour, aftertaste, chewiness, lentil flavour, and prospective purchasing ($p>0.05$).

The perceived sweetness and chewiness were different between C and LF, C and MLW-F, C and WF ($p<0.0001$). The chewiness was barely detected for C (<5%) compared to snack bars (>70%). Similarly, the sweetness of C was very low (6.9%) compared to snack bars (58-64%).

There were significant differences between LF and MLW-F (p=0.05), and LF and WF (p=0.038) with the LF treatment resulting in the highest perception of lentil flavour.

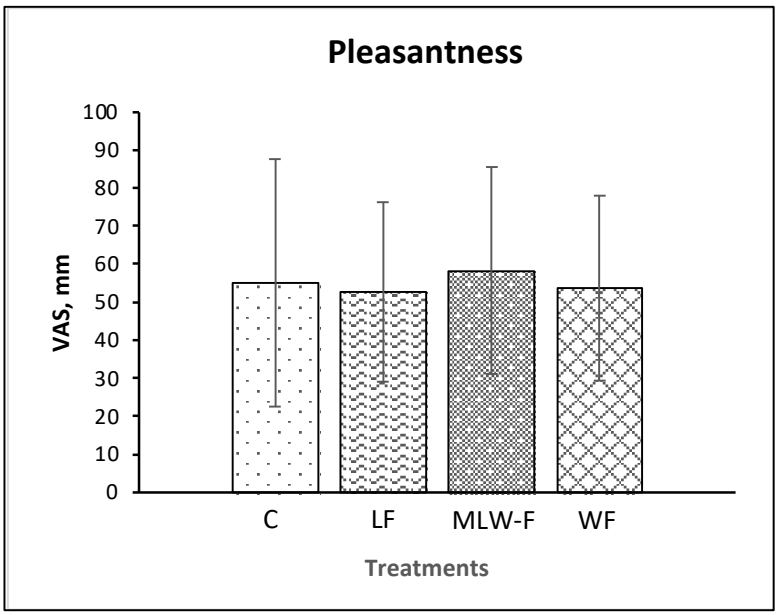

Figure 24. The pleasantness of the treatments. Means ± SD, n=25.
One-way RM ANOVA. VAS Scale: 0 - not at all pleasant; 100- very pleasant.

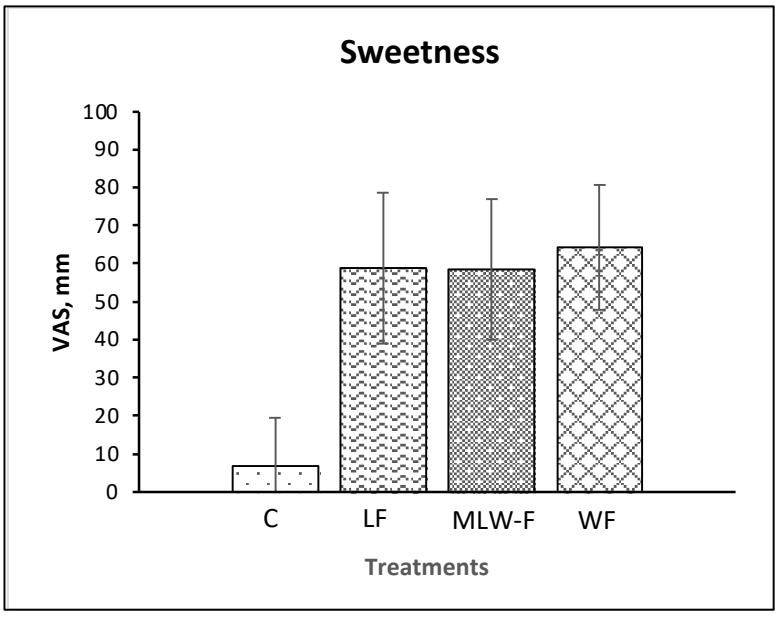

Figure 25. The sweetness of the treatments. Means ± SD, n=25.
One-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between the treatments (P<0.05).

VAS Scale: 0 - not sweet at all; 100 - very sweet.

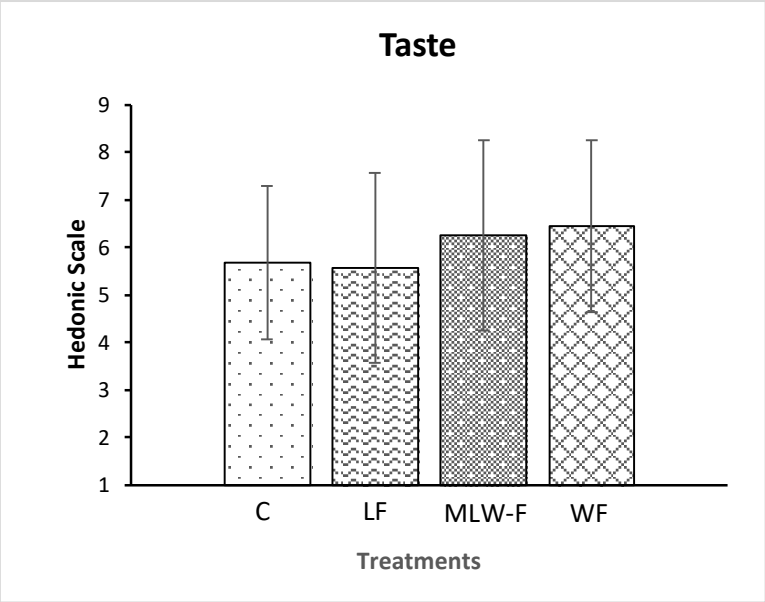

Figure 26. The taste of the treatments. Means ± SD, n=25.
One-way RM ANOVA. 9-point hedonic scale: 1- dislike extremely; 9- like extremely.

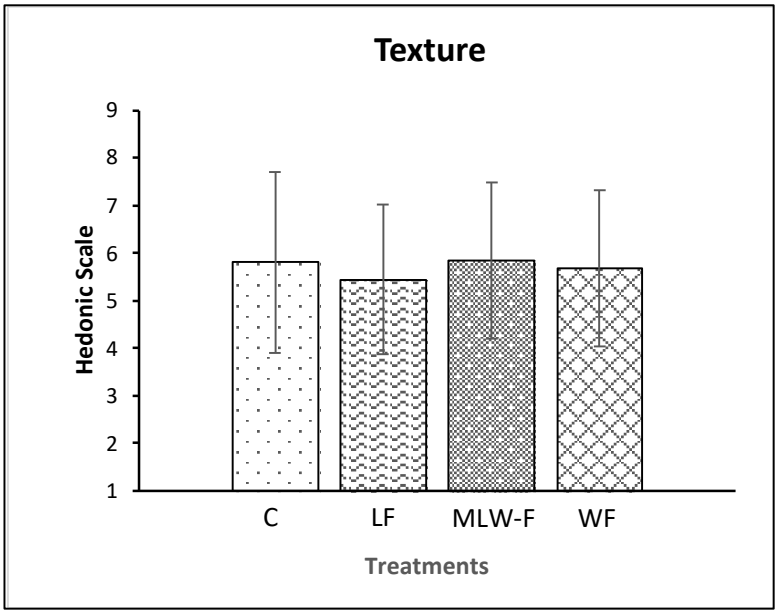

Figure 27. The texture of the treatments. Means ± SD, n=25.
One-way RM ANOVA. 9-point hedonic scale: 1- dislike extremely; 9- like extremely.

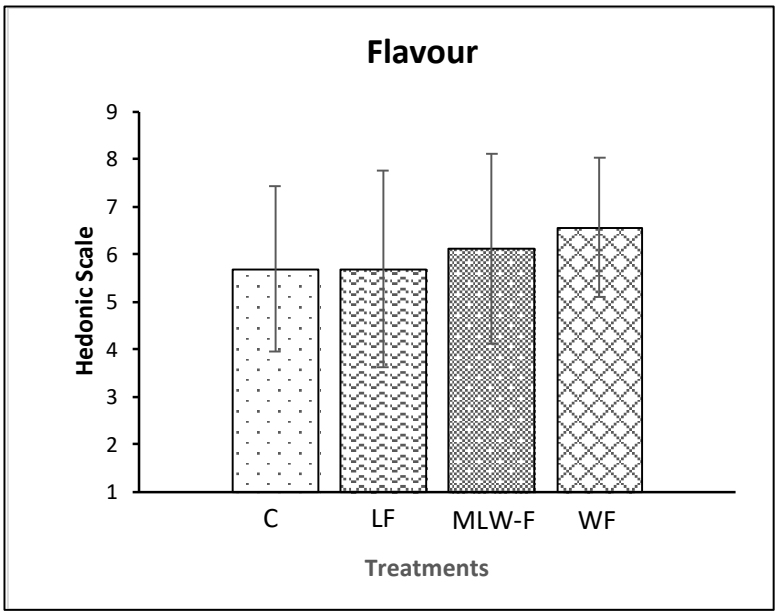

Figure 28. The flavour of the treatments. Means ± SD, n=25.
One-way RM ANOVA. 9-point hedonic scale: 1- dislike extremely; 9- like extremely.

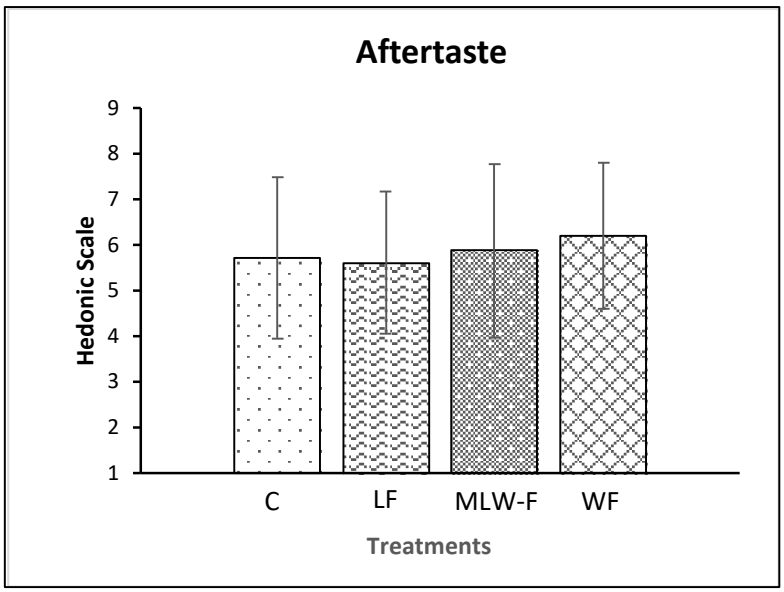

Figure 29. The aftertaste of the treatments. Means ± SD, n=25.
One-way RM ANOVA. 9-point hedonic scale: 1- dislike extremely; 9- like extremely.

Figure 30. The chewiness of the treatments. Means ± SD, n=25.
One-way RM ANOVA followed by Tukey-Kramer post-hoc test. Different superscripts represent significant differences between the treatments ($P<0.05$).
VAS Scale: 0 - not chewy; 100 - very chewy.

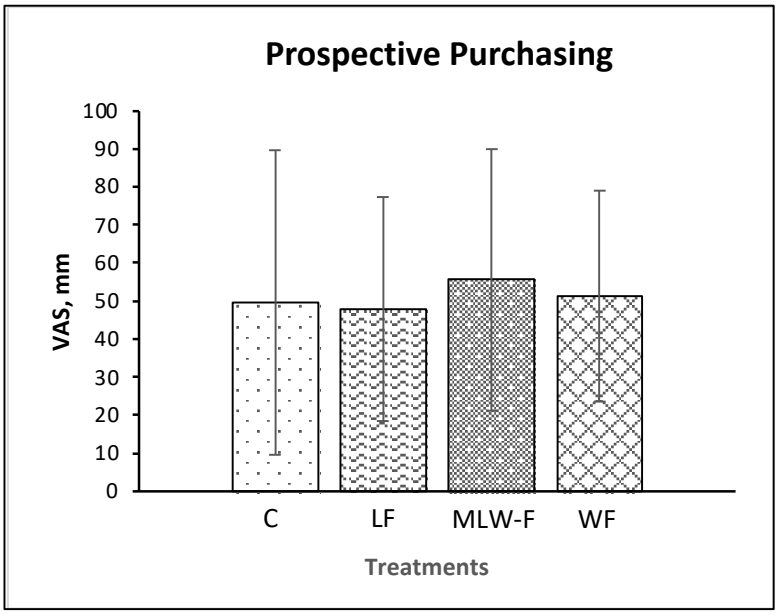

Figure 32. The prospective purchasing. Means ± SD, n=25.
One-way RM ANOVA. VAS Scale: 0 - would not purchase, 100 - would purchase.

3.6.0 Relationship between the variables

The average physical activity (PA) for the participants was 7.0 ± 6.3 hours per week and equal to 10.4 ± 5.2 metabolic equivalents (MET) per week. There was no relationship between tAAC for average appetite and PA hours/week, and tAAC for average appetite and PA METS. There was no relationship between PA hour/week and DTE (0 min), or PA METS and DTE (time 0 min). There was an inverse relationship between PA METS and FI (= -0.39, p=0.05). There was no relationship between PA hours/week and FI. There was no relationship between DTE (0 min) and FI, and tAAC for average appetite score and FI. Table 8 summarizes the results of correlation analysis.

Table 8. Relationship between the variables

Measure 1	Measure 2	Pearson's	P-value
PA (Hours/Week)	Mean average appetite (AA) score over 120 min	-0.25	0.22
	AA tAAC	0.29	0.14
	DTE score at 0 min	-0.14	0.50
	FI (kcal) at 120 min	-0.09	0.65
PA (METS)	AA	0.03	0.87
	AA tAAC	0.014	0.94
	DTE score at 0 min	-0.026	0.90
	FI at 120 min	**-0.39**	**0.05**
FI (kcal) at 120 min	AA	0.016	0.94
	AA tAAC	0.14	0.49
	DTE score at 0 min	0.05	0.8

4.0.0 Discussion

Lentil flour incorporated into functional food products has promising effects on satiety and short-term food intake. Currently, Canadians do not include pulses (lentils) regularly in their diet (2,8) despite having an abundance of production (1). Not only are pulses easily accessible, but there have been studies on regular consumption of pulses which have reported links to improvement in weight management (8), glycemic control (3–5), and other various health benefits that aid in the prevention of obesity (8), CVD (6,10,12,14), hypertension (16), and DM (174). Thus, Canada's Food Guide currently recommends including more plant-based proteins (41).

There is a potential for the Canadian food market to incorporate more pulse flour into snacks products. This study sought to create a functional food product that would impact satiety and short-term food intake from the partial and total replacement of wheat flour with lentil flour. To date, there have not been any studies published that have assessed the impacts of partial and total replacement of wheat flour with lentil flour of similar particle size in the snack bars and their effects on satiety and short-term food intake. The recruitment goal was to include 30 male participants with a normal BMI. However, 25 male participants with a normal BMI completed the study which met the study requirements. Part of the study was completed during the COVID-19 pandemic, resulting in difficulties in recruiting participants and collecting data due to result of laboratory closures and restrictions placed by Mount Saint Vincent University to abide by the public health regulations (188).

This study formulated three snack bar treatments, which were 100% lentil flour (LF), 50% lentil and 50% wheat flour (MLW-F) and 100% wheat flour (WF). The snack bars were formulated in the Appetite Lab at MSVU (Drs. Luhovyy and Kathirvel) and recommendations

from a previous Masters thesis by Andrew Hamilton (2018) on snack bar formulations and their effects on satiety, food intake and postprandial glycemia (193). The questionnaires and measurements in this study followed the guidelines from Health Canada's Draft Guidance Document - Satiety Health Claims on Food (46).

In this current study, all snack bar ingredients were individually weighted, steamed, and cooked with precision and consistency for a final weight of 58.5 g. Adjustments were made to ensure bars' weight were consistent with the conventional snack bars found in the Canadian market. An unofficial survey of snack bars available on the market found an inconsistency of their weight. Many snack bars on the Canadian food product marketplace are wheat or oat-based snack bars, (i.e., Nutrigrain ®, Quaker ®, Fibre One ®, CLIF bars®). It is uncommon for a snack bar in the conventional marketplace to contain lentil flour. Lentil flour is not common in the Canadian food product market, therefore, this may have affected the participants' acceptance of LF snack bars, although there was no difference in taste and pleasantness between the bars.

Other studies reported higher VAS ratings for taste, texture, pleasantness and prospective purchasing for their WF conventional snack products compared to their LF products (24,48). Majority of the studies with lentil flour have only replaced 20-30% of wheat flour, as effects of higher replacement results in issues in physiochemical structures in dough and palatability acceptance of food products. In the present study, the participants correctly identified lentil flavour in LF treatment; this however did not affect the taste and pleasantness of LF treatment, which was not statistically different from MLW-F and WF treatments. Unlike Clark et al (2019) who found the differences between green lentil muffin and wheat muffins for taste, texture, and pleasantness, this study did not find a difference between the snack bars formulated with lentil flour, wheat flour or their mixture for pleasantness, taste, texture, aftertaste and flavour. The

sensory properties for the formulated snack bars were in neutral or low-acceptance rate on VAS or between “neither like nor dislike” and “like slightly” on 9-point hedonic scale. On the other hand, a study conducted in Pakistan with 10 participants who testing the biscuits prepared with lentil flour reported higher hedonic perception (49). However, it is not clear whether the blinding and randomization were used in the sensory analysis and whether the participants were aware of the products they test. In another study, Erikson and Slavin compared the smoothies prepared with either 0.5 cup of cooked red lentil or 0.5 cup of ice cream (30). Not surprisingly, the smoothie with lentil resulted in inferior sensory characteristics compared to the smoothie in which red lentil was replaced with the same amount of ice cream (30). The treatment formulation used in the present study was using the same amount of apples and cinnamon in all three bars to partially mask the flavour of lentil and provide uniform sensory characteristics to the snack bars formulated with lentil flour, wheat flour or the mixture. Similarly, in Ryland et al (2010) study, the snack bars, including the bars formulated with lentil, were favoured with fruit resulting in similar sensory characteristics (48). Although in the present study, the participants could correctly detect a higher lentil flavour in LF bars compare to all other treatments, they could not discriminate between MLW-F and WF bars. This indicate that the dose of 30 g lentil flour is associated with a strong lentil flavour while the dose of 15 g of lentil can be successfully masked. Nevertheless, the detection of lentil flavour in LF bars did not lead to any inferior perception of the flavour or other sensory characteristics compared to the other treatments. The lack of the difference in texture perception between the snack bars may be explained by the similar particle size of lentil and wheat flours.

Prospective purchasing of the snack bars high

sugar, apples and honey was the same in all bars. As already discussed in the literature review, the lentil flour contains more resistant starch in its starch fraction. Lentil flour contains more sugar compared to wheat flour (Table 3); however, the content of sucrose or other glycemic sugars in lentil flour are low. Other sugars in lentil flour are represented by oligosaccharides of the raffinose family that can be classified as resistant carbohydrate and contribute to the observed effect on subjective appetite. As seen in Appendix 1, the protein content of LF bars is 5.5 g higher compared to WF bars. Therefore, the appetite-suppressing effect observed for LF bars can be due to the synergistic effects of macronutrients including protein, dietary fibre fractions, resistant starch and oligosaccharides. Also, the potential effect of some minor lentil bioactive components should not be excluded.

This study supports the findings from Holt et al. (1995) who demonstrated that the protein and fibre of foods were positively correlated with satiety index (31). Although there was appetite suppression with LF snack bars, it is difficult to separate the impacts of fibre and protein. It is possible that combination factors of fibre and protein impacted participants satiety sensations.

Results in this study on physical comfort are comparable to Wong et al. (2009) study examining the effect of pulses on food intake and satiety (2). Similarly, there were no significant difference in any of the aspects for the physical comfort questionnaire. LF naturally contains a higher concentrations of oligosaccharides (54), which may contribute to perception of meteorism, diarrhea, stomach pain and nausea postprandially. However, in the present study, the subjective perception of gastro-intestinal discomfort factors were well below 10% (Figures 19-20 and 22-23) while the feeling of wellness was high above 75% over two hours (Figure 21).

Participants were given an pizza meal at 120 minutes. Although there was a significant difference in subjective appetite between the treatments, there was no effect of a treatment on food intake at 120 minutes. This impact did not transpire to food intake. Overall, these findings on food intake are similar to other appetite studies where satiety is enhanced by pulse food products but does not transpire to food intake suppression (4,24,25,27). In the present study, there was no effect of treatment on food intake or cumulative food intake. Pizza intake for control, LF, MLW-F and WF were 1064.8 kcal, 921.3 kcal, 920.8 kcal, 901.0 kcal, respectively. A similar trend is observed for caloric compensation, which resulted in no significant difference between the snack bars. The caloric compensation for LF, MLW-F and WF was 77%, 83%, and 108%, respectively.

While the difference in FI between control and LF (144 kcal), control and MLW-F (144 kcal), and between control and WF (164 kcal) could be possibly detected with a larger sample size, the differences between LF and WF (20 kcal), LF and MLW-F (0.5 kcal) and MLW-F and WF (20 kcal) are not meaningful to be explored further.

Cumulative food intake was calculated as the sum of the energy consumed with the treatment and with the pizza meal. The cumulative food intake after control, LF, MLW-F and WF treatment was 1065 kcal, 1106 kcal, 1105 kcal, and 1085 kcal, respectively, and the effect of a treatment on food intake was not statistically significant (P=0.8). Surprisingly, the control did not result in a higher food intake at 120 min compared to the snack bar treatments. The control and the WF treatments resulted in similar cumulative energy intake. Likewise, the cumulative intake after LF and MLW-F treatments was similar. The lack of the difference in cumulative food intake between the water control and all three types of the snack bars draws the

important conclusion that the intake of these snack bars will not be associated with the excessive short-term energy intake.

Water intake over two hours was not significantly different between the treatments. The overall average for water intake for control, LF, MLW-F, WF was 300 g, 259.8 g, 271.7 g, and 282.0 g, respectively. There was no effect of treatment on water intake or cumulative water intake over two hours. The cumulative water intake for control, LF, MLW-F and WF treatment was 650.1 g, 609.8 g, 621.7 g, and 632.0 g, respectively. The data on water intake suggests that the treatments did not affect the thirst of the participants during the meal.

The participants in this study retrospectively recorded an average of 60 minutes per day (7 ± 6.5 hours/ week) of PA. This participant pool in this study was more active compared to the average population that currently does not meet the Canadian physical activity guidelines (22). To date, the recommendation for PA for the Canadian population is to accumulate 150 minutes per week (39,177). The participants in this study exceeded the recommendations. Habitual activity has an impact on body composition (23). Body composition can impact the feelings of satiety and food intake (155). Additionally, the intensity of exercise, acutely, can affect subjective appetite and food intake (156,157); however, the relationship between PA and subjective appetite are weak (117).

A correlational analysis was conducted to assess the predictability of appetite and intensity of habitual PA measured in hours per week and metabolic equivalents (METS). All participants PA was calculated into hours per week and converted into METs, and were averaged. The average METS for the participants in this study were 10.4 ± 5.2 METS. Overall, the correlation analysis reported no relationship between PA hr/week and appetite scores. There was a similar pattern with PA METs and appetite score. A study by King et al. (2009) reported

that the exercise sessions designed to expend 2500 kcal per week for 12 weeks led to an increase in fasting hunger and in average hunger across the day (153). However, the study was conducted with the cohort of participants assigned to the same intense level of physical activity. In the present study, there was no significant relationship between subjective appetite and PA, possibly due to the highly variable MET values among participants. Although there was no relationship between PA hour/week and food intake, there was an inverse relationship between PA METS and food intake (= -0.39, p=0.05). This observation may reflect a part of the so-called curvilinear relationship first observed in 1956 by Mayer et al. in industrial workers (195). When energy intake declined as activity levels increased from inactive to moderate and then increased again towards the upper end of the PA spectrum (196).

Although the study was designed to investigate the effect of partial of full replacement of wheat flour with lentil flour of the same particle size in young healthy males on satiety and short-term food intake, there are several limitations that need to be addressed. This study was designed following the recommendations from Health Canada on substantiating satiety health claims on food (46). According to the section 5.3.5 of this document, the evidence of an effect on satiety should be demonstrated for the serving of the food as consumed (46). This is achieved by comparing the effects of foods with the similar serving sizes on satiety and short-term food intake. However, it remains unknown what would be a minimum dose of lentil flour that can trigger satiety and food intake. To answer this question, a study should be designed using an

food intake approach (i.e., the snack bars to be consumed up to the feeling of fullness). Another limitation of the study is that it was conducted using healthy young adult males. It would be important to investigate the same effects in other cohorts, including females, individuals in other age groups and health status (e.g., overweight and obese individuals).

Finally, the study did not involve the investigation of physiological and biochemical parameters such as gastric emptying rate, blood glucose and insulin, and other biomarkers that could explain the metabolic changes in response to the ingestion of lentil- and wheat-based solid food products.

5.0.0 Summary

- The ingestion of snack bars formulated with lentil flour or wheat flour, or equal amount of lentil and wheat flour led to suppression of subjective appetite over two hours compared to the water control in young healthy males.
- The ingestion of snack bars formulated with lentil flour provided a stronger suppression of subjective appetite over two hours compared to the snack bars formulated with wheat flour. Since the particle sizes of lentil flour and wheat flour were similar and the amount of other ingredients in both types of snack bars was identical, the observed effect of the snack bars formulated with lentil flour on subjective appetite is driven by the nutritional composition of lentil flour.
- The ingestion of snack bars formulated with wheat flour or lentil flour, or their combination does not lead to suppression of food intake at 120 min compared to the water control.
- There was no difference between the snack bars formulated with wheat flour or lentil flour, or their combination on food intake at 120 min.
- The intake of snack bars formulated with wheat flour or lentil flour, or their combination did not lead to increased cumulative energy intake over two hours compared to the water control.

- The full or partial replacement of wheat flour with lentil flour of the same particle size did not result in adverse sensory perception. The pleasantness, taste, flavour and aftertaste of all solid treatments were similar.
- The partial replacement (15 g) of wheat flour with the lentil flour in the snack bars formulated with fruit filling does not result in the detection of lentil flavour.
- The full replacement (30 g) of wheat flour with the lentil flour in the snack bars formulated with fruit filling led to detection of lentil flavour by study participants, although it did not result in the adverse hedonic perception of the flavour.
- The ingestion of snack bars formulated with lentil flour did not lead to the gastrointestinal discomfort. The ingestion of all types of snack bars led to the subjective perception of wellness.

6.0.0 Conclusion

The replacement of wheat flour with lentil flour of the same particle size in snack bars provides effective and practical way to control appetite and avoid excessive energy intake in young healthy adults. The formulation of the snack bars with lentil flour provides a convenient food product with acceptable sensory characteristics and a high level of physical comfort. The effect of lentil flour on appetite in comparison to wheat flour is determined by the nutritional composition of lentil flour, not by the difference in particle size. The physiological determinants of the effect of lentil flour on appetite need to be further investigated.

7.0.0 Further implementations

The incorporation of lentil flour has the potential to create functional products for appetite control in Canada and internationally. Although the present study used a fixed size of snack bars to determine their effect on satiety and short-term food intake, future studies should determine the satiating effect of lentil and wheat flours. Future research should be expanded to other groups of population, including females, children and adolescents, and senior adults. Considering the role lentil plays in blood glucose control, it will be important to assess the effect of food products formulated with lentil flour on biomarkers associated with the control of satiety, energy intake and blood glucose. Further studies should investigate the effect of lentil flour on blood glucose control in people with normoglycemia and people with impaired blood glucose, and in overweight and obese individuals.

Further additions to the study should take into consideration a range of levels of physical activity of human participants. A striation of high, medium, and low activity participants and its impacts on satiety and food intake should be explored.

Finally, the effects of the regular consumption of food products formulated with lentil flour should be further explored in long-term randomized clinical trials. The assessment of body composition, resting metabolic rate and other physiological and biochemical markers associated with satiety, food intake control and risk factors associated with chronic metabolic diseases will help to understand the underlying mechanisms involved in the response to the regular intake of lentil and lentil ingredients.

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Appendix A: Nutrient composition of the treatments

Proximate	**LF**	**MLW-F**	**WF**	**Control (Water)**
Grams (g)	58.5	58.5	58.5	350
Calories (kcal)	184.5	184.3	184.2	0
Carbohydrates (g)	38.2	39.8	41.3	0
Protein (g)	9.4	6.7	3.9	0
Fats (g)	0.4	0.6	0.8	0
Dietary Fibre (g)	4.1	2.7	1.4	0
Sugars (g)	20.2	19.4	18.6	0
Retinol (mcg)	3.5	3.5	3.5	0
Beta-carotene (mcg)	24.8	20.7	16.6	0
Vitamin B1 (mg)	0.5	0.5	0.6	0
Vitamin B2 (mg)	0.3	0.3	0.3	0
Vitamin B3 (mg)	2.8	3.3	3.8	0
Vitamin B6 (mg)	0.6	0.5	0.4	0

Appendix B:

Informed Consent Form

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

Investigators:

Dr. Bohdan Luhovyy, Principal investigator
Department of Applied Human Nutrition, Mount Saint Vincent University
Phone:
Email:
Ms. Morgan King, Study Coordinator
Department of Applied Human Nutrition
Phone:
Email:

Introduction

You have been invited to participate in the research study named above. This consent form provides information regarding the study. Before you decide if you want to volunteer, please read the information carefully and understand the purpose of the study, its risks and benefits, and activities that you will be asked to do. We will provide all information before authorization to participate. We will keep you informed about any new information that may influence your willingness to continue to participate.

?

Pulses are edible seed from pods or legume plants such as lentils. Lentils are high in fibre, protein, and nutrients that have nutritional benefits. Lentils have been shown to reduce risks of lifestyle diseases such as cardiovascular disease and Type II diabetes. We hope this study will encourage further development of pulse flours in functional food products to become commercially available to Canadians.

The experiment will be conducted through the Department of Applied Human Nutrition at Mount Saint Vincent University. You will be asked a set of screening questions each time before u A ll be asked to attend at Mount saint Vincent University.

This study will not cost you anything. You will

summer during vacation over four weeks. We will arrange the days that will be most convenient for you.

We ask you to arrive at MSVU between 8:45-10am after an overnight fast. After arrival, a blood finger prick will be taken for eligibility. Then you will be asked to complete a questionnaire about physical activity, food intake, and sleep. After you will be asked to complete a second questionnaire about appetite and physical comfort. Once the questionnaires are completed, you will be asked to consume one of three different products that are snack bars. While you are consuming the snack bar you will be given a questionnaire asking about the taste, texture, and smell of the product. After eating the snack bars, you will be asked every 15 minutes a questionnaire about appetite, and physical comfort for the next 2 hours. After 2 hours you will then be asked to consume a pizza meal until you are comfortably full.

Approximate Time schedule

Time	Activity
8:45	Arrive at lab
8:50	Blood finger prick and Complete questionnaire on food intake, activities, sleep appetite and physical comfort.
9:00 am	Eat the Treatment (0 min)
9:15-11:00 am	Fill out VAS Questionnaires at 15, 30, 45, 60, 90, 120 minutes
11:00- 11:20am	Consume pizza until comfortably full
11:20am	The session is complete

Confidentiality

The study will protect your privacy. The recordings and the collection of the data will remain anonymous and will be stored on a password protected file on the computer or in a lock and key filing cabinet for hard copies. Each participant will be issued an ID to protect your privacy. If you choose to withdraw from the study, all data will be removed an all hard copies will be destroyed.

Risks

There are very little risk related to this study. Our research team will make sure all foods served to you are freshly prepared so you will have very little chance of food poisoning. Research Coordinator holds a food safety certificate and has previous work experience handling food and food products.

Benefits

There are no direct benefits from volunteering for the study. However, information gained from this study might help develop a better understanding of lentil flour on satiety and foods intake. This will also inform the future projects to create new food products with using lentil flour. It may also support other studies to understand the role of pulse flour and its effects on satiety and food intake in a healthy population.

Results of the study

Results of the study may be provided through a Mount Saint Vincent Thesis defence, dissertation and other local Nova Scotia conferences. Participants may also request a summary of the results of the study.

Voluntary participation

This is a voluntary participation study, as a participant you have the right to change your mind whether to participate in the study. You also have the right to withdraw at any time during the duration of the study. Cash $10 provided from each session completed will be received regardless of whether you fully complete all four sessions.

Questions and Further information

If you have any questions or would like further information concerning the research project please do not hesitate to contact our Research Coordinator Ms. Morgan King at xxx-xxx-xxxxor at email.com

Consent

I have read and I understand the information provide in this form. I understand the demands and nature of the study. I have had the opportunity to ask questions and I understand that my participation is voluntary, and I am free to withdraw at any time, without giving a reason. I understand that I will be given a copy of this consent form. I have received a copy of the information and authorization form for future reference. I voluntarily agree to take part in this 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) _____

Participant's Signature _____ Date_____

Research Coordinators Signature _____ Date _____

Financial Incentive

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.

Name (Print) _____
Participants Signature _____
Date _____

Appendix C:

Eligibility Questionnaire

:

How old are you? _____

Weight:_____ 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?

_____
_____
_____

Are you a regular consumer of 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: _____

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
_____

To be completed by Staff:

Assigned ID #:_____

Appendix D:

Health and Activity Questionnaire

:

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 usual stress? (E.g. exams, reports, work deadlines, personal, etc…)
Yes___ No___

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

Yes___ No ___

To be completed by Staff:

Blood glucose reading: _____mmol/L

ID:_____

Treatment (code): _____ Session (visit #): _____

Date (dd/month/yr): _____ Time: _____

Appendix E:

Baseline Information

:

Name: _____

Address: _____

_____

Phone #: _____

E-mail: _____

ID #: _____

Appendix F:

.

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

Appendix G:

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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 H:

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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

:

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 at all _____ VERY sweet

:

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

:

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

:

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

:

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

:

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

:

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 lentil flavour _____ STRONG lentil flavour

:

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 feelings.

Would not purchase _____ Would purchase

Appendix I: Recruitment Poster