Development and characterization of peptides with antidiabetic activities from oat protein

by

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Abstract

Type 2 diabetes mellitus (T2DM) occurs when the cells in the body are unable to respond to the effect of insulin, resulting in a state of hyperglycemia that could involve other health complications. The prevalence of this disease generates a great concern worldwide and suggests a move towards prevention through diet and lifestyle modifications. For instance, oats consumption has been related to blood lipid and glucose regulation, mostly from its fiber and phenolic compounds. Recently, oat protein content started gaining importance due to its functional capacities and glucose regulatory effects; however, research is still limited. Exploring oat protein health benefits could leverage the Canadian oat production process to obtain value-added products since western provinces are known to be the major oat producers in the country. Therefore, this research aimed to generate antidiabetic peptides from oat protein to inhibit α-amylase, α-glucosidase, and dipeptidyl peptidase (DPP)-IV enzymes.

In this study, oat protein hydrolysates were prepared by alcalase and flavourzyme treatment and then fractionated based on their different molecular weight and hydrophobicity. Enzyme inhibition assays *in vitro* indicated that the relatively hydrophobic fraction with a molecular weight of 1-5 kDa inhibited enzymes that regulate glucose digestion, absorption, and metabolism activities. Identification of oat peptides from the most effective sequence was made using LC-MS/MS. The analysis disclosed the presence of two 8 amino acid sequences from the most effective fractions, identified from 12S oat globulin (GDVVALPA and DVVALPAG) and new de novo sequences rich in amino acids like proline, leucine, valine, phenylalanine, and glutamine. The results suggest that proline plays a crucial inhibitory role and may favor hydrophobic interactions and hydrogen

bonding at these enzymes' active site. Hydrophobic characteristics combined with the presence of amino acids like proline, valine, and leucine, especially the enclosed Leu-Pro sequence found in potent DPP-IV inhibitors, might have conferred their antidiabetic effect. α-Amylase, α-glucosidase, and DPP4 inhibitors are used as targets in the development of antidiabetic drugs. Thus, the ability to generate peptides with antidiabetic activities from oat represents a strategy to develop natural healthy products or functional foods for T2DM prevention and management.

Keywords: oat protein; antidiabetic peptides; α-amylase inhibition; DPP-IV inhibition; type 2 diabetes.

Preface

The research project, of which this thesis is a part, is an original work by Lourdes Ramirez Fuentes under the supervision of Dr. Lingyun Chen. Chapter 2 of this thesis is submitted as L. Ramirez Fuentes, C. Richard, and L. Chen to the Journal of Functional Foods. Dr. Lingyun Chen is the corresponding author.

Dedication

This dissertation is dedicated to my loving family for their support, motivation, and unconditional love.

Acknowledgements

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there for me. I want to thank my parents for teaching me and encouraging me to be a better person, reach for my goals, and be persistent. Thank you for all your love.

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List of Abbreviations

ATP	Adenosine triphosphate
TCA	Tricarboxylic acid
GTP	Guanosine triphosphate
PDH	Pyruvate dehydrogenase
CoA	Coenzyme A
NADH	Reduced nicotinamide adenine dinucleotide
FADH	Reduced flavin adenine dinucleotide
T2DM	Type 2 diabetes mellitus
IGT	Impaired glucose tolerance
FPG	Fasting plasma glucose
OGTT	Oral glucose tolerance test
HDL	High-density lipoproteins
LDL	Low-density lipoproteins
DPP-IV	Dipeptidyl peptidase-IV
GLP-1	Glucagon-like peptide-1
CCK	Cholecystokinin
GIP	Glucose-dependent insulinotropic peptide
ACE	Angiotensin-converting-enzyme
RP-HPLC	Reverse Phase-Hight Pressure Liquid Chromatography
ACN	Acetonitrile
AH	Alcalase hydrolysate
AFH	Alcalase-flavourzyme hydrolysate

GCPR	G-protein-coupled receptors
ANOVA	Analysis of variance

Chapter 1- Literature Review

1.1 Glucose metabolism

1.1.1 Food as a source of energy for the body

To perform different functions and metabolic reactions, the human body requires energy obtained from the oxidation of carbohydrates, fats, and proteins present in our food. This oxidative process aims to obtain adenosine triphosphate (ATP), a fundamental piece in the production and expenditure of energy for the reactions happening in the body (Hall, 2016b). Macronutrients are broken down through different pathways into smaller units such as glucose, glycerol, fatty acids, and amino acids to produce pyruvate and acetyl CoA, and in consequence: ATP (Whitney & Rolfes, 2013). The body cells mainly use glucose for their correct functioning, especially the nervous system and red blood cells. Our body stores glucose in the form of glycogen in muscles and the liver; the excess glucose is then stored as fatty acids in the adipose tissue. However, when stores of glucose are low the process of gluconeogenesis starts through the conversion of amino acids and glycerol into pyruvate, and therefore, making glucose (Hall, 2016b). Fatty acids cannot make glucose since they are transformed into acetyl CoA, and the latter can immediately produce fat (Whitney & Rolfes, 2013). In general, the human body is constantly finding different pathways to obtain energy from consumed nutrients.

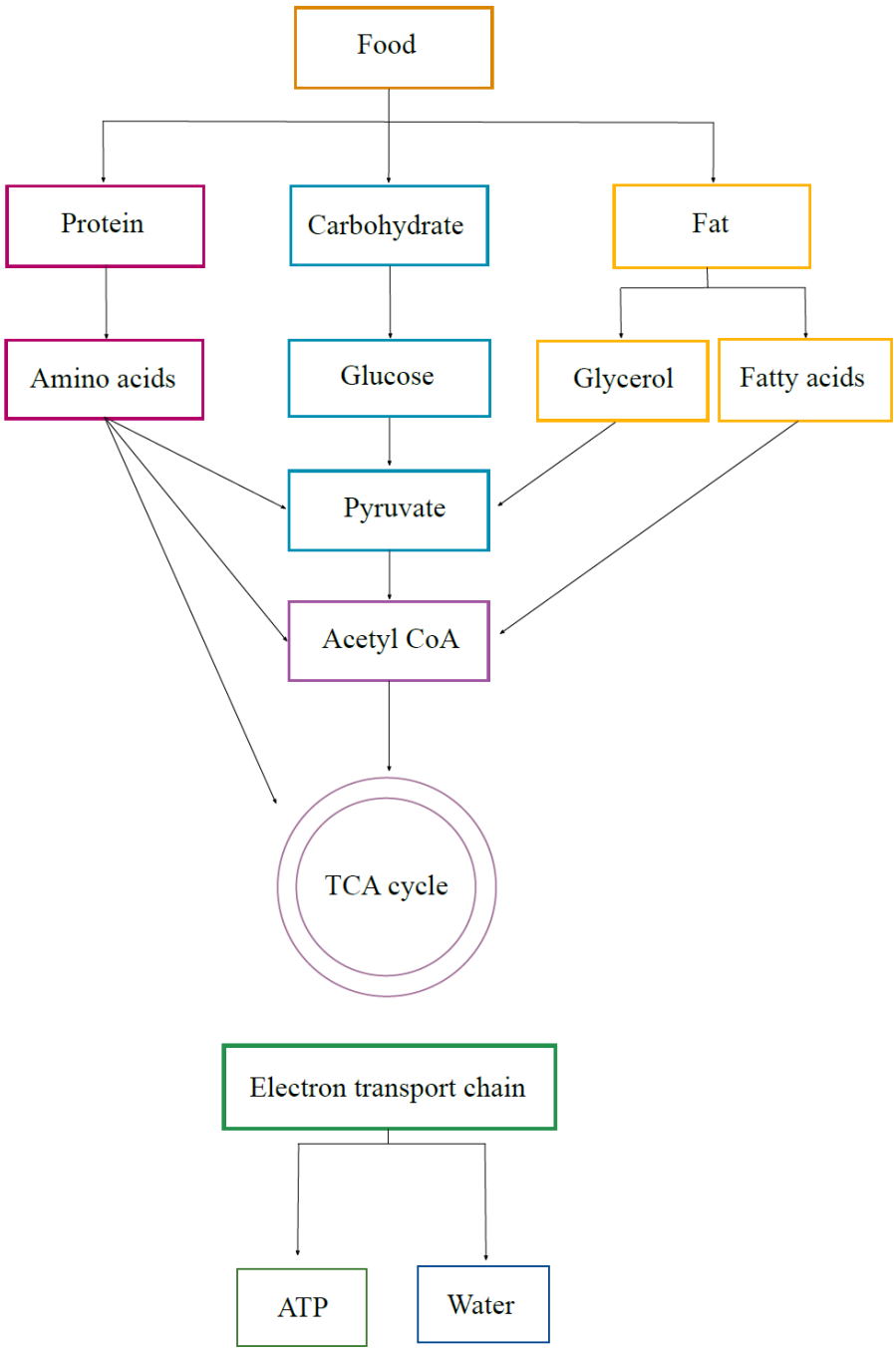


Figure 1. 1 Energy-yielding pathway.

Adapted from Whitney & Rolfes, 2013.

1.1.2 Glycolysis and the tricarboxylic acid cycle

Even though glycolysis is one of the first pathways that glucose takes on for energy production, its ATP yield is not as high as other metabolic pathways. However, the formation of pyruvate in this pathway is an essential step for further energy yield. Glycolysis is a cytoplasmic pathway where the enzyme hexokinase phosphorylates glucose (6-carbon compound), and it continues through several reversible and irreversible reactions where the original glucose splits into 3-carbon compounds for the formation of 2 pyruvate and 2 ATP molecules (Kumari, 2018; Whitney & Rolfes, 2013). Subsequently, the pyruvate obtained can either enter the tricarboxylic acid cycle (TCA cycle) for further ATP generation or produce lactate through lactic acid fermentation (Nelson & Cox, 2005).

When entering the TCA cycle, the pyruvate oxidates to produce H_2O and CO_2. First, the pyruvate dehydrogenase (PDH) complex is in charge of a decarboxylation reaction that results in a 2-carbon molecule bonded to Coenzyme A's molecule also known as acetyl-CoA (Nelson & Cox, 2005). The TCA cycle has eight steps from which energy is preserved in the electron carriers like NADH and $FADH_2$, and every time that the cycle is completed, three molecules of NADH, one $FADH_2$, one nucleoside triphosphate (GTP or ATP), and two CO_2 are produced (Nelson & Cox, 2005).

1.1.3 Carbohydrate digestion and blood glucose regulation

The intake of foods containing carbohydrates as a component will lead to three main molecules after digestion: glucose, fructose, and galactose. This breakdown process is completed by enzymes that are present in different parts of the digestive tract. For

instance, when saliva mixes with our food, α-amylase starts the digestion of starch and amylopectin into maltose (disaccharide) and smaller polymers of glucose (Berdanier, Dwyer, & Heber, 2013). When food moves down to the stomach, α-amylase inactivates due to the gastric juices' acid pH. The digested matter from the stomach that enters the duodenum is further digested by pancreatic juices. In this step, pancreatic α-amylase fully breaks down carbohydrates into disaccharides; later, intestinal epithelial enzymes, like α-glucosidase, cut disaccharides into monosaccharides (Hall, 2016b). The latter are readily available for blood stream absorption.

The absorbed glucose pool of less than 20 g generally produces a small disruption in blood glucose due to homeostasis mechanisms, where the removal rate matches glucose absorption (Marks, 2005). As glucose circulates through the body, uptake in different tissues begin, triggering the release of insulin from the pancreatic β-cells; consequently, stimulation of glucose uptake in the tissues increases (Mendes, Koetzner, & Chen, 2018). As mentioned previously, liver and muscle cells can store glucose in the form of glycogen, and the excess glucose is converted into fat. After a period of fasting or when glucose levels fall below normal, glucose needs to be replaced by that originated from glycogen break down (Marks, 2005). The former process is determined by rising glucagon hormone and fatty acids in the blood (Marks, 2005; Whitney & Rolfes, 2013). Overall, these mechanisms aim to maintain normal blood glucose levels by either signaling cells for glucose uptake through insulin secretion and glycogen formation (glycogenesis) or through glucose synthesis (gluconeogenesis) by glucagon action (Whitney & Rolfes, 2013).

1.2 Prevalence of type 2 diabetes mellitus (T2DM)

According to the International Diabetes Federation (IDF), 463 million people aged 20-79 live with diabetes, representing 9.3% prevalence that will increase to 10.2% and 10.9% for 2030 and 2045, respectively (International Diabetes Federation, 2019). Countries with the most affected number of adults with this disease are China, India, United States, Pakistan, and Brazil, with an increased prevalence in urban areas, since rural areas are related to lower levels of overweight and obesity; moreover, regional distribution reports the highest prevalence for impaired glucose tolerance (IGT of 12.3%) for the North American and Caribbean region (International Diabetes Federation, 2019). It has been reported that 29% of Canadians live with diabetes or prediabetes nowadays, and it is likely to increase to 33% by 2025 (Yaghoubi et al., 2019). Furthermore, the increased prevalence of this condition has represented for Canada an estimated health care costs of $15.36 billion for new cases given since 2011 (Bilandzic & Rosella, 2017). All the direct and indirect costs related to diabetes represent a burden for the world's health care systems that could be relieved through prevention and early detection of the disease to avoid further complications.

1.2.1 Pathophysiology of T2DM

Type 2 DM is characterized by a resistance to insulin; this means that fat, muscle, and liver cells are no longer responding to the effects of insulin, resulting in poor uptake of glucose by the tissues and higher concentrations of blood glucose (hyperglycemia) (Seifert, 2019). As a response to the low sensitivity to insulin effects, the pancreatic β-cells secrete a higher amount of insulin to exert an uptake response; however, in

further stages of T2DM pancreatic function is depleted and not enough insulin is secreted (Hall, 2016a). Consequently, more severe symptoms of hyperglycemia and other metabolic abnormalities such as non-regulated lipolysis will contribute to high levels of free fatty acids in plasma (Poretsky, 2010). All this together will lead to specific symptoms that should be taken care of to avoid complications.

Common symptoms presented in T2DM are increased thirst, hunger, urination, ketones in the urine (which shows muscle and fat breakdown by-product), fatigue, blurred vision, persistent infections, and slow healing wounds (Mayo Clinic, 2011). A water and electrolyte imbalance mainly cause these symptoms due to the high amounts of glucose in the blood (Bagchi & Sreejayan, 2012), and as a consequence of the lack of glucose that the cells need to produce energy. In the aim of obtaining energy from sources other than glucose and together with the decreased levels of insulin, patients with T2DM present high levels of cholesterol and other lipids (Hall, 2016a), which leads to developing micro and macrovascular complications, atherosclerosis, hypertension, retinopathy, coronary heart disease, neuropathies, and stroke (Serrano-Ríos & Gutiérrez-Fuentes, 2010), to mention some.

1.2.2 Risk factors and diagnosis for type 2 DM

Glucose metabolic disorders and diabetes development is caused by multiple factors; for instance, genetics, ethnicity, and environment play an important role in this process (Family, Ripsin, Kang, & Urban, 2009). Some modifiable risk factors are sedentarism, a poor diet, smoking, overuse of alcoholic drinks, stress, depression, anxiety, obesity, hypertension, elevated plasma triglycerides, low HDL levels; other non-modifiable risk factors are medical conditions such as polycystic ovary syndrome

(increased ovarian androgen), Cushing's syndrome (excess glucocorticoids), and acromegaly (excess growth hormone) (Alberti, Zimmet, & Shaw, 2006; Hall, 2016a; Hu et al., 2001; Naicker et al., 2018). Moreover, the use of certain antibiotics during infancy have shown a decreasing effect on anti-obesogenic gut bacteria and, as a result, an increased obesity propensity in further life stages (Penders et al., 2006). Also, the use of endocrine-disrupting chemicals, including pesticides, plasticizers, and other industrial pollutants, are correlated to an increased prevalence of metabolic diseases (Casals-Casas & Desvergne, 2011).

Several biochemical tests and etiological factors contribute to the diagnosis of T2DM. According to the American Diabetes Association, presenting classic symptoms of high blood sugar levels and the use of one the following tests are enough for the diagnosis: A1C (glycosylated haemoglobin) test, which represents the average glucose levels of the past 2 to 3 months; fasting plasma glucose or FPG test measures blood sugar levels after 8 hours fasting before breakfast; OGTT or oral glucose tolerance test monitors blood sugar levels 2 hours before and after the consumption of a glucose solution to observe how the body processes sugar; and the casual plasma glucose test, which can be obtained at any time of the day when presenting severe T2DM symptoms (ADA, 2014).

Table 1.1 Diagnosis Tests Values for Diabetes

Test	Value
A1C	6.5% or higher
Fasting Plasma Glucose (FPG)	126 mg/dl or higher
Oral Glucose Tolerance Test (OGTT)	200 mg/dl or higher
Casual Plasma Glucose Test	200 mg/dl or higher

Source: ADA, 2014

1.2.3 Management and pharmacological treatments

Lifestyle modifications to regulate blood glucose concentrations are the main target to reduce symptoms, the risk for complications, and improve people's life quality with type T2DM (Bagchi & Sreejayan, 2012). It is recommended to maintain healthy body weight by changing food habits and assessing non-dietary factors, like smoking or sedentarism (Hu et al., 2001). Pharmacological treatment is added when lifestyle changes fail to reverse insulin resistance (Hall, 2016a). Some of the most well-established drugs are biguanides (Metformin), sulfonylureas, α-glucosidase inhibitors (Acarbose), thiazolidinediones, insulins and amylin analogues, although new drugs like dipeptidyl peptidase-IV inhibitors (Sitagliptin) and glucagon-like peptide-1 receptor agonists have emerged as glucose-lowering therapies (Bagchi & Sreejayan, 2012). This treatment aims to control glycemia through different mechanisms, such as decreasing glycogen breakdown and gluconeogenesis, enhancing insulin sensitivity and secretion, retarding carbohydrate digestion and absorption, delaying gastric emptying, stimulating satiety, inhibiting enzymes that decrease insulin action, and acting as insulin replacements (Bagchi & Sreejayan, 2012; Olokoba, Obateru, &

Olokoba, 2012; Seifert, 2019). Although these therapies are effective for T2DM management, they also produce substantial side effects; for example, sulfonylureas are related to body weight gain and hypoglycemia, metformin and α-glucosidase inhibitors might cause nausea, diarrhea, and flatulence, some other drugs like thiazolidinediones have been associated to cardiovascular events, bone loss and fractures in women (Bagchi & Sreejayan, 2012; Olokoba et al., 2012). Therefore, it is important to notice that drug tolerability, psychological concerns, polytherapy, and cost are some of the factors implied in treatment adherence (García-Pérez et al., 2013).

Modifying eating patterns through healthy eating recommendations is one of the most relevant approaches to improve impaired glucose tolerance, assuring the ingestion of a balanced meal. The U.S. Dietary Guidelines Advisory Committee stated that diets high in plant-based foods promote a healthy and positive environmental impact, compared to an animal-based diet (USDA, 2015). Canada's dietary Guidelines suggest following a healthy diet based on vegetables, fruits, whole grains, and "protein foods' coming preferably from plants, such as legumes, nuts, seeds, tofu, and fortified soy beverages (Health Canada, 2019). Likewise, these sources should contemplate the protein requirement for adults of 0.83 g proteins/kg per day according to the WHO (World Health Organization, 2007). These guidelines also support plant-based protein products in need of soil, water, and air conservation through food loss and waste reduction due to the latter's implications on greenhouse emissions in Canada (Environment and Climate Change Canada, 2019; Health Canada, 2019). Plant proteins have also shown great potential in the creation of bioplastics used for food packaging due to their fast degradability; in addition, plant protein gelling and

emulsifying capacity makes them suitable materials for delivery systems for bioactive compounds (Perez-Puyana, Felix, Romero, & Guerrero, 2016; Shao & Tang, 2016; Wu et al., 2011).

Currently, research has been focusing on ways to prevent and treat diabetes in a more sustainable and natural way and expecting fewer side effects by using active compounds found in our food. In addition, the use of nutraceuticals and herbal supplements has been reported as part of T2DM management in different cultures and regions around the world (Bagchi & Sreejayan, 2012). Some of these studied complementary treatments are based on foods like bitter melon, cinnamon, green tea, prickly pear cactus, and components such as chromium, selenium, magnesium, ginseng, alpha-lipoic acid, vitamin D, and β-glucan (Bagchi & Sreejayan, 2012; Ley, Hamdy, Mohan, & Hu, 2014). Moreover, the study of dietary proteins and their different sources have been associated with glucoregulatory activities (Comerford & Pasin, 2016). Plant products, for instance, have been considered a cost-effective source of proteins with healthy properties to improve the diet (Aggarwal & Drewnowski, 2019); considering that plant-based food matrices are richer than animal sources in terms of fiber, vitamin E, unsaturated fatty acids, and phytochemicals (Ahnen, Jonnalagadda, & Slavin, 2019). These compounds are known by their antioxidant capacity, insulin-like and insulin secretion activities, gastrointestinal absorption delaying, and satiating effects, which contribute to improving glucose concentrations in blood, insulin sensitivity, LDL cholesterol, triglyceride levels, and weight management overall (Ahnen et al., 2019; Bagchi & Sreejayan, 2012; Yibchok-Anun et al., 2006).

Protein consumption has also been related to satiety effects when intake goes above 0.8 g/kg/day (de Carvalho, Pizato, Botelho, Dutra, & Gonçalves, 2020), although this goal might be challenging to reach through a plant-based diet, plant protein hydrolysates and peptides from plant sources like rice bran, common beans, and soybean have shown to promote satiety signals, at a dosage level of up to 2 mg/mL, to increase the time response of incretins like GLP-1, through inhibition of its degrading enzyme: dipeptidyl peptidase-IV (González-Montoya, Hernández-Ledesma, Mora-Escobedo, & Martínez-Villaluenga, 2018; Hatanaka et al., 2012; Oseguera Toledo, Gonzalez de Mejia, Sivaguru, & Amaya-Llano, 2016). Other studies focusing on satiety hormones for obesity and overweigh management have found that increasing doses of oat β-glucan (4 to 6 g) increased peptide Y-Y plasma levels for appetite regulation (Beck, Tapsell, Batterham, Tosh, & Huang, 2009). Moreover, a daily oat β-glucan intake has also shown to improve total cholesterol, postprandial glucose and insulin response in hypercholesterolaemic subjects (Biörklund, van Rees, Mensink, & Önning, 2005). Foods like oats have demonstrated to be a good source of bioactive compounds with antidiabetic effects due to their high fiber contents and their protein quality; therefore, this work has focused on the study of oat peptides and their mechanisms for glucose regulation as a strategy for T2DM management.

1.3 Bioactive peptides and their biological properties

Bioactive peptides are components of protein foods. These peptides are likely to exert biological activities that contribute to a healthy state due to a specific amino acid sequence obtained after proteins hydrolysate through food processing or natural

digestion (Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008). Its use as part of therapy could provide a more natural and targeted way to manage certain medical conditions and possibly a reduction in the side effects that common pharmacological treatments cause to the patients. The use of bioactive peptides could be promoted as a preventive strategy for chronic illnesses. Their continuous use could have long-term benefits to reduce the burden of having an illness and its treatment costs.

There are multiple sources of bioactive peptides, including animal, plant, insect, fungal, amphibian, among other sources, that have displayed a wide range of healthful activities. For instance, milk, whey, soy, oat, pea peptides have demonstrated blood pressure lowering effects through inhibition of angiotensin-converting-enzyme (ACE) and/or increase of vasodilating agents like nitric oxide. Moreover, peptides cholesterol-reducing properties have shown their action through modulation of endogenous cholesterol production and faecal excretion (Cicero, Fogacci, & Colletti, 2017). Other studies have explained bioactive peptides' anticancer effect and antioxidant activity at a cellular and genetic level, which greatly contributes to the understanding of intricate mechanisms for cell-induced apoptosis and metastasis process in cancer treatments (Dia & Mejia, 2010). Some other functions involve anti-inflammatory and antioxidant activities for atherosclerosis and lipid oxidation prevention targeting cardiovascular disease (Kannan, Hettiarachchy, & Marshall, 2011), which in developed countries is a main cause of mortality. The coexistence of other diseases that play a role as risk factors to developing metabolic syndrome has also generated interest in the antidiabetic activity of peptides, as their effects have shown to modulate glucose impairment through multiple pathways.

Hence, contributing to the management strategies for T2DM, in addition to diminishing side effects and costs of treatment. However, research in this area is still limited for plant-based proteins and especially for oats, which has shown to be an excellent source of bioactive compounds.

1.3.1 Peptides for T2DM management

Antidiabetic peptides offer the improvement of postprandial glycemia by either lowering starch digestive enzymes, stimulating insulin secretion, or appetite regulation (Patil, Mandal, Tomar, & Anand, 2015). Some of these activities are achieved through mechanisms like α-amylase, α-glucosidase, and DPP-IV inhibition; stimulation of insulin and incretin secretion; glucose uptake improvement; and lowering glucose production (Oseguera-Toledo, González de Mejía, Reynoso-Camacho, Cardador-Martínez, & Amaya-Llano, 2014). For instance, animal sources like bovine milk and camel milk were compared for their DPP-IV inhibition activity through trypsin hydrolysis, which resulted in distinct amino acid sequences according to the source and to trypsin cleavage selectivity in both kinds of milk (Nongonierma, Paolella, Mudgil, Maqsood, & Fitzgerald, 2017). It was observed that the presence of Pro in the sequence of the peptides contributed to the DPP-IV inhibitory effect. (Nabeno et al., 2013). Collagen, rich in Pro contents, was also identified as a good source of animal protein to generate DPP-IV inhibitory peptides (Huang, Hung, Jao, Tung, & Hsu, 2014; Jin, Yan, Yu, & Qi, 2015; Lafarga, O'connor, & Hayes, 2014).

α-Amylase and α-glucosidase inhibitory peptides have also been found in the sequences of multiple food protein sources. For instance, a novel peptide (KLPGF) from egg albumin displayed both α-amylase and α-glucosidase inhibitory activity,

with IC_{50} values of 120.0 ± 4.0 μmol l^{-1} and 59.5 ± 5.7 μmol l^{-1}, respectively. However, another egg albumin peptide sequence (RVPSLM) showed a higher inhibition rate with an IC_{50} of 23.07 μmol l^{-1} (Z. Yu, Yin, Zhao, Liu, & Chen, 2012; Z. Yu et al., 2011), suggesting that some peptide sequences and amino acid contents are important to produce an antidiabetic effect. Other animal-based peptides from sardine (IC_{50}= 3.7 mg/mL) and dairy proteins (IC_{50}=4.5 mg/mL) have also shown α-glucosidase inhibitory capacity (Lacroix & Li-Chan, 2013; Matsui, Oki, & Osajima, 1999). Peptide structures from these inhibitors possessed both hydrophilic and hydrophobic amino acids, and it is hypothesized that α-glucosidase inhibition is better if the peptide is a three to six amino acid sequence with a "serine, threonine, tyrosine, lysine, or arginine at the N-terminal and a proline residue closer to the C-terminal" (Ibrahim, Bester, Neitz, & Gaspar, 2018a).

Plant proteins have been suggested as sources of antidiabetic peptides; however, reported peptides are mostly derived from animal sources. Research based on plant proteins is only available for some products like soybean, common bean, rice, amaranth, and some oilseeds, suggesting that further investigation is required to unlock the potential of plant protein sources for bioactive peptide production. Recent studies showed that peptides prepared from buckwheat, barley, and oats possess DPP-IV and α-glucosidase inhibitory activities through tryptic digestion (F. Wang, Yu, Zhang, Zhang, & Fan, 2015a; F. Wang et al., 2018a). This demonstrates the potentiality of proteins from cereals to produce bioactive peptides for T2DM management; nevertheless, more research is needed to understand the mechanisms of action better and the role that peptide sequences play in the inhibitory interactions.

1.3.2 α-Amylase inhibitors

α-Amylases are a group of enzymes present in saliva and intestinal secretions that hydrolyze (α1→4) linkages from glycogen and starch from our food (Nelson & Cox, 2005). As previously mentioned, pancreatic amylase digest complex carbohydrates into disaccharides and oligosaccharides (**Figure 1.3**), which are not ready for intestinal absorption (Lebovitz, 1997), yet they are substrates for other enzymes like α-glucosidases. This enzyme is secreted by humans and most living species (Osain, 2018). For instance, while we produce α-amylase for digestion of carbohydrates, cereals and legumes develop α-amylase for starch hydrolysis during germination stages for seedling growth (Andriotis et al., 2016). Alongside, they generate α-amylase inhibitors as a defense mechanism against insect pests, viruses, bacteria, and fungi, thus resisting to predation during their growth and storage stages (Bashary et al., 2019; Franco, Rigden, Melo, & Grossi-de-Sá, 2002). Pancreatic α-amylase consists of three domains formed by a 496 amino acid single polypeptide chain of 56 kDa; the main catalytic domain is constituted by a $(\beta\backslash\alpha)_8$ barrel structure, and this is where a "V-shaped depression" accommodates the active site of the enzyme (Brayer, Luo, & Withers, 1995; Nahoum et al., 2000). The enzymatic inhibition of α-amylase in humans would prevent further breakdown of their hydrolysis products by α-glucosidases. Consequently, glucose absorption into the bloodstream would decrease as its absorption process requires previous digestion of starch into oligo-, di-, and finally monosaccharides (Ibrahim, Bester, Neitz, & Gaspar, 2018b). This activity would beneficially reduce high blood glucose levels in patients with T2DM.

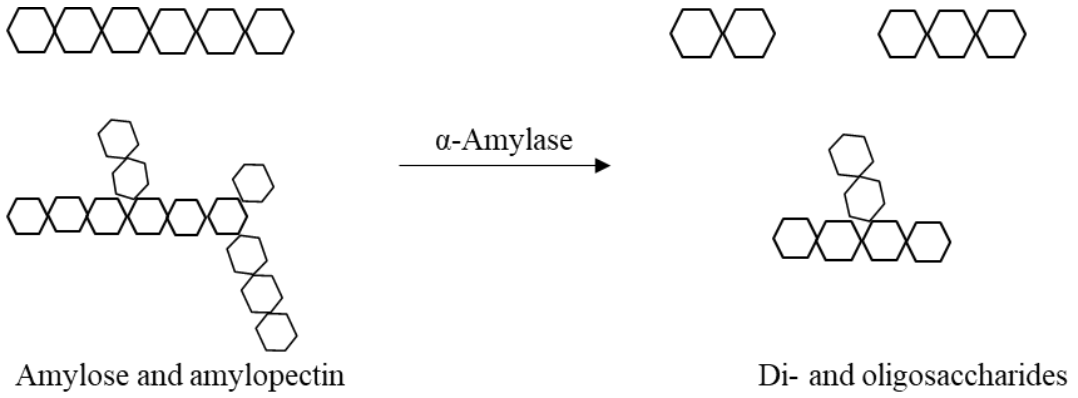


Figure 1. 2 α-Amylase hydrolysis of amylose and amylopectin occurring in starch. Glucose units are represented as hexagons. Modified from Hii, Tan, Ling, & Ariff, 2012.

Increased interest in developing antidiabetic peptides has allowed the study of different sources as generators of α-amylase inhibitors. Kinetic analyses in some foods have demonstrated that α-amylase inhibitors act as non-competitive compounds as they can inactivate α-amylase through conformational changes when binding at a different site other than the active site, before or after forming a complex with the substrate (Arise, 2016; Powers & Whitaker, 1978). Isolated peptides from seaweed identified as Gly-Gly-Ser-Lys and Glu-Leu-Ser presented a non-competitive inhibition mode and could bind to the allosteric site of the enzyme to trigger its conformational changes and therefore, impeding the union with its substrate (Admassu, Gasmalla, Yang, & Zhao, 2018). The synthesized macrocyclic peptides have shown to bind competitively to the enzyme with great potency. These peptides present dynamism to develop intra- and intermolecular hydrophobic interactions within the enzyme to inhibit its activity (Goldbach et al., 2019).

The α-amylase inhibitors have mainly been developed from non-proteinaceous sources from a diversity of plants such as *Urtica dioica*, *Juglans regia*, *Adiantum caudatum*, *Celosia argentea*, *Salvia aurita*, among others. The list of proteinaceous sources to generate α-amylase inhibitors is still limited. So far, the reported peptide structures go from 2 to 20 amino acids long (Awosika & Aluko, 2019; Hao et al., 2009). Some authors suggest that peptide fractions under 3 kDa display better bioactivity (Admassu et al., 2018). For instance, products such as common beans (*Phaseolus vulgaris* L.) had an increased α-amylase inhibition by 44% compared to their non-germinated version after 24 h germination (de Souza Rocha et al., 2015). Moreover, fractions from <1 kDa showed higher inhibition for α-amylase; the obtained peptides showed interaction with the active site of amylase, specifically with Asn197, Glu233, and Asp300 residues in FFL and QQEG sequences (Oseguera-Toledo, Gonzalez de Mejia, & Amaya-Llano, 2015). It is proposed that inhibitory activity may be influenced by the number of interactions between the enzyme and terminal groups derived from shorter peptides, together with the presence of amino acids like histidine, methionine, and proline (Ngoh & Gan, 2016). These interactions are mostly compared to the amylolytic inhibitory effects of acarbose. The latter is a 645.6 kDa molecule that resembles an oligosaccharide with a reversible competitive type of inhibition, acting at the brush border of the small intestine to slow down glucose absorption; moreover, its activity also influences the secretion of GIP and GLP-1 (Campbell, White, & Campbell, 1996). Even at lower inhibition rates when compared to acarbose, proteinaceous inhibitors from plant sources show α-amylase inhibitory activity. It is of interest for both the scientific community and industry to

develop more natural inhibitors with less side effects such as the ones caused by acarbose. Study of peptides from plants for generation of α-amylase is still limited; thus, this gives a great opportunity to explore plant protein sources to generate α-amylase inhibitory peptides for applications in food or health related areas.

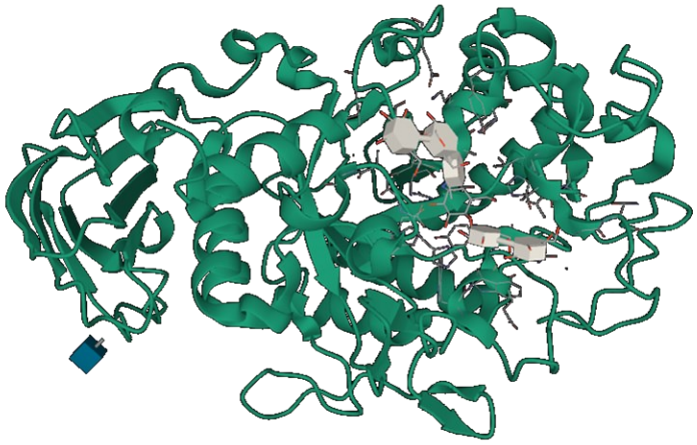


Figure 1. 3 Structure of the N298S variant of human pancreatic alpha-amylase complexed with acarbose. Image of 1xh0, (Maurus et al., 2005), Mol* (D. Sehnal, A.S. Rose, J. Kovca, S.K. Burley, S. Velankar (2018) Mol*: Towards a common library and tools for web molecular graphics MolVA/EuroVis Proceedings. doi:10.2312/molva.20181103), and RCSB PDB.

1.3.3 α-Glucosidase inhibitors

α-Glucosidases are enzymes of the glycoside hydrolases group, which cleave disaccharides into monosaccharides for intestinal absorption. They are found in the small intestinal brush border acting on α (1-4) glycosidic bonds (Osain, 2018). Current inhibitors such as acarbose and voglibose are competitive inhibitors that bind to the active site of the enzyme. Consequently, they avoid the cleavage of oligosaccharides into simple, absorbable monosaccharides (Lebovitz, Irl Hirsch, & Vassello, 1997),

thus improving postprandial blood glucose levels for T2DM treatment. Some of the reported side effects of these inhibitors, due to their mechanism of action, are flatulence, pain, and gastrointestinal disorders (Ibrahim et al., 2018b); therefore, research of peptides with α-glucosidase inhibiting bioactivity have gained great interest as these peptides from food protein sources may have fewer side effects.

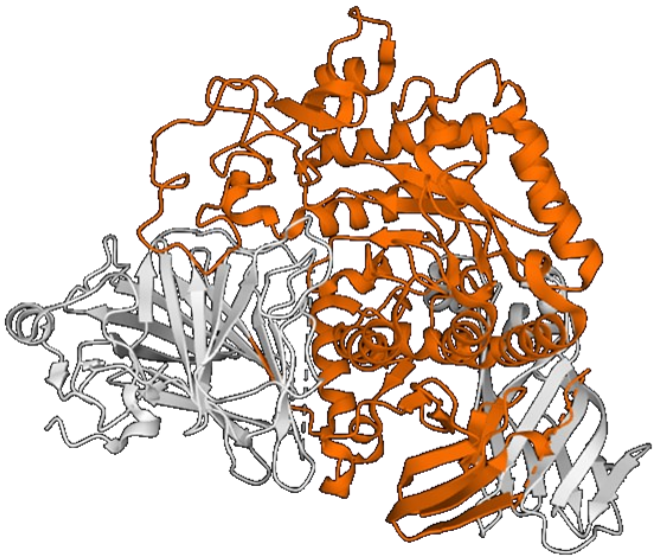


Figure 1. 4 Crystal structure of human lysosomal acid-alpha-glucosidase, GAA, in complex with acarbose. Image of 5nn8, (Roig-Zamboni et al., 2017), Mol* (D. Sehnal, A.S. Rose, J. Kovca, S.K. Burley, S. Velankar (2018) Mol*: Towards a common library and tools for web molecular graphics MolVA/EuroVis Proceedings. doi:10.2312/molva.20181103), and RCSB PDB.

Plant sources and extracts from microorganisms are showed to produce compounds called iminosugars that mimic carbohydrates and can function as α-glucosidase inhibitors (Fauser & Jonikas, 2018). Likewise, non-saccharide inhibitors like peptides can exert the same function with fewer unwanted side effects; however, more research

is needed in this area to recognize potential peptide-based inhibitors of α-glucosidase (Ghani, 2020). For instance, plant sources of protein like common beans, soybeans, and walnuts have been reported to contain α-glucosidase inhibitors (de Souza Rocha et al., 2015; González-Montoya et al., 2018; Oseguera-Toledo et al., 2015; Yibchok-Anun et al., 2006). Peptides derived from above mentioned sources were obtained through enzymatic hydrolysis using pepsin and pancreatin to simulate the gastrointestinal tract conditions or by using food grade enzymes like alcalase and bromelain. In addition, the combination of multiple enzymes could increase the degree of hydrolysis to generate a diversity of peptide sequences with biological activity. Still, research on peptides from plant sources to target α-glucosidase inhibition is limited.

Studies show that hydrolysates from germinated soybean by pepsin and pancreatin digestion could inhibit two types of α-glucosidases (maltase and sucrase) with the IC_{50} of 3.73 and 2.90 mg/mL, respectively, compared to the positive control acarbose (IC_{50} 0.07, and 0.03 mg/mL, respectively) (González-Montoya et al., 2018). Some others have found peptides with strong α-glucosidase inhibition effect derived from alcalase hydrolysis of *Cannabis sativa* L. seeds with an IC_{50} of 0.024 mg/mL; in addition, the generated peptides were rich in hydrophobic and branched amino acids like Pro, Leu, Arg, and Met that are considered to be the potential contributors of the bioactivity (Ren et al., 2016). Other amino acids that can contribute to the α-glucosidase inhibitory activity include Ser, Thr, Tyr, Lys, or Arg at the N-terminus of the peptide chain, usually of three to six amino acids long, and Met or Ala at the C-terminus with a Pro close to the same end (Ibrahim et al., 2018b). From different α-

glucosidase inhibition studies, it was stated that hydrophobicity and charge were not requirements for this activity; instead, length and amino acid content of the sequences seem to be more important, leading to the recommendation for studies to better understand the inhibition mechanisms of α-glucosidase by peptides (Ibrahim et al., 2018b).

It is important that bioactive peptides can resist gastric digestion to reach their enzyme target. According to Lebovitz, Irl Hirsch, & Vassello (1997), most of the ingested carbohydrates are absorbed in the jejunum, especially in the upper segment rather than distal jejunum or ileum; thus, peptides must remain active until reaching the glucosidases activity site. One of the most potent α-glucosidase inhibitors found was a peptide with four amino acid residues Ser-Thr-Tyr-Val. After gastrointestinal digestion in silico, using BIOPEP tools, the peptide was cleaved into Ser-Thr-Tyr, which still showed important interactions through hydrogen bonding with the enzyme's active site (Singh & Kaur, 2016). Study observations have proposed that residues enclosing hydroxyl groups at the N-terminus of the peptide chain as an essential factor for the inhibitory effect of the enzyme (Ibrahim et al., 2018b). Research on peptide structures and their interactions with α-glucosidase have demonstrated that plant-derived foods are a good source for assessing potential components for glucose regulatory activity even after digestion. Moreover, some of them have shown a comparable inhibitory effect to that obtained for acarbose, which gives a good opportunity to develop protein and peptide products for patients with T2DM. However, regardless of the plant proteins potential and the trends towards a more sustainable diet, additional studies are required to discover the biological

activity of a wide variety of plant proteins to explain better their impact on the prevention and treatment of chronic illnesses.

1.3.4 DPP-IV inhibitors

Insulin is a hormone secreted by the islets of Langerhans (β-cells), which stimulate glucose uptake by peripheral cells, including hepatocytes in the liver and muscles; therefore, lowering plasma glucose concentrations. Initially, when glucose reaches the duodenum and jejunum, the so-known incretins: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are released in plasma (Poretsky, 2010). These gut-derived hormones or incretins produce a decreased gastric emptying, induce insulin secretion and inhibit glucagon production according to the ingested amount of carbohydrates (Goldenberg & Face, n.d.; Lebovitz et al., 1997). Their insulin secretion activity is given through signals by binding specific receptors. GIP interacts with G-protein-coupled receptors (GCPR), and GLP-1 acts on GLP-1 receptors, each of them expressed in multiple tissues engaging β-cells, adipose tissue, central and peripheral nervous systems, and organs such as the kidney, heart, lungs, and the GI tract (Drucker & Nauck, 2006). Subsequently, receptors' response to incretins triggers an intracellular cAMP process, increasing calcium entering the cell and allowing insulin exocytosis from the β-cells (Drucker & Nauck, 2006; Poretsky, 2010). Unfortunately, incretins half-life is rather short due to its inactivation by DPP-IV enzymes, which metabolize more than 50% of the secreted incretins before entering blood circulation (Nauck, 2009). DPP-IV is extensively expressed by endothelial and epithelial cells in different parts of the body like in the brush-border cells in the intestine, kidney, liver, and blood plasma (Mentlein, 1999). Therefore, it

causes a rapid and extensive inactivation of incretins (Gough, 2016). Hence, by inhibiting DPP-IV, the action time of incretins is prolonged, and insulin secretion is stimulated for a longer period, providing a beneficial effect for glucose uptake mechanisms in cells and tissues. Additionally, GLP-1 inhibiting glucagon secretion function as well as gastric emptying and motility generates satiety signals resulting in lower food intake. (Holst, 2003)

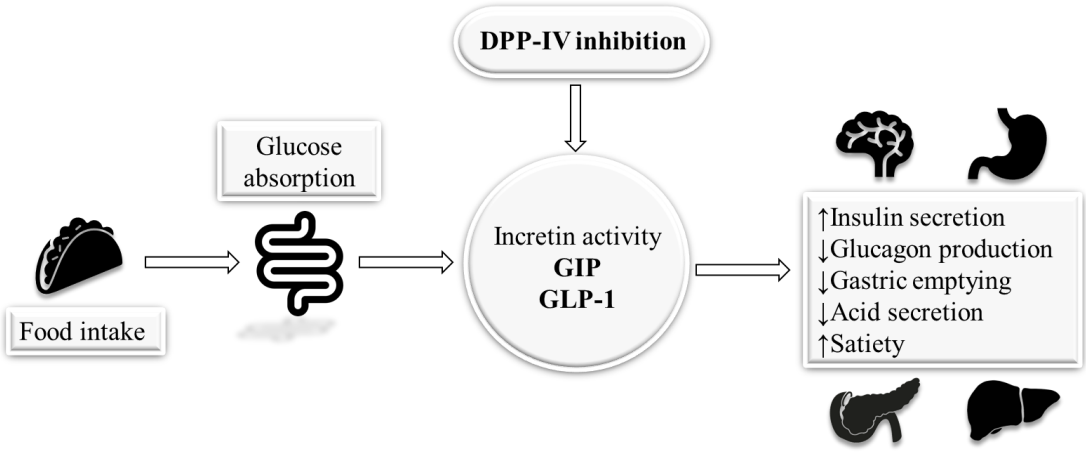


Figure 1. 5 Prolonged incretin activity through DPP-IV inhibition.

DPP-IV is a serine exopeptidase from the prolyl oligo-peptidase family; this means that it preferentially cleaves substrates containing proline or alanine at the second position of the peptide chains (Gorrell, 2005; Nakajima, Ito, & Yoshimoto, 2008; Nongonierma & FitzGerald, 2019). This 110 kDa glycoprotein dimer comprises two main domains (an α/β-hydrolase and an eight-blade β propeller domain) in each monomer, enclosing a cavity where unfolded or partially unfolded peptides reach the active site of the enzyme (Gorrell, 2005). The catalytic site of the enzyme is composed of Ser630, Asp709, and His740 residues; moreover, conformational aspects for

alignment of substrates based on glutamate residues interaction through salt bridges provides space at the hydrophobic active site for only two amino acids with small side chains (Pro, Ala, Gly). This explains why incretins GIP and GLP-1 are the main substrates of DPP-IV, as both contain Ala at the second position of their structures (Drucker & Nauck, 2006). Therefore, when generating peptides for DPP-IV inhibition, the presence of proline or alanine as part of their aminoacidic sequence will give a more effective inhibitory activity.

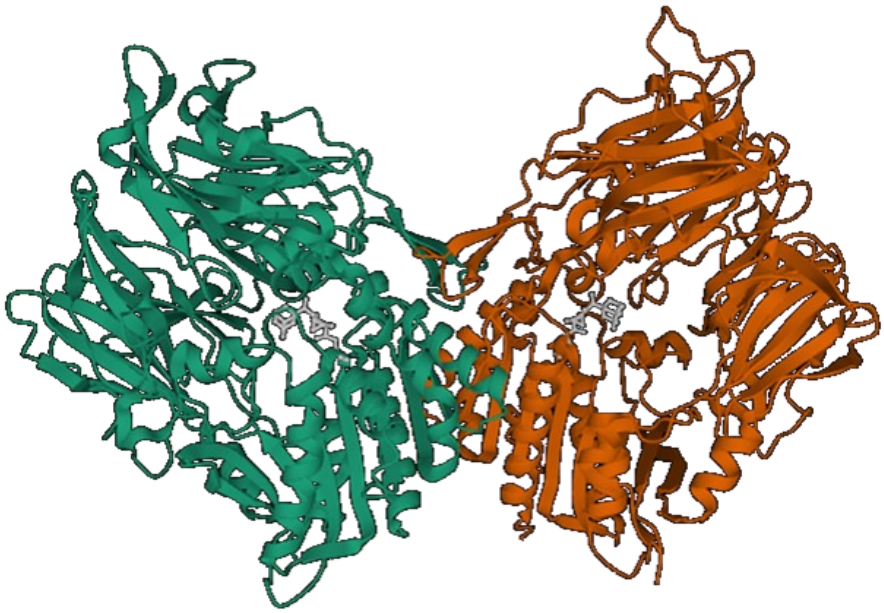


Figure 1.6 DPP-IV structure (monomers shown in different colors) in complex with a vildagliptin analogue inhibitor. Image of 5lls, (Berger et al., 2018), Mol* (D. Sehnal, A.S. Rose, J. Kovca, S.K. Burley, S. Velankar (2018) Mol*: Towards a common library and tools for web molecular graphics MolVA/EuroVis Proceedings. doi:10.2312/molva.20181103), and RCSB PDB.

As DPP-IV inhibitors gain more interest for diabetes management, research on peptides coming from various animal and plant sources are being studied for this purpose; for example, bovine milk gastro intestinal digestion *in silico*, generated effective peptides like Leu-Pro-Leu-Pro-Leu and Leu-Pro-Leu from β-casein (Nongonierma & Fitzgerald, 2014); another study showed that *in vitro* peptic hydrolysis of bovine α-lactalbumin produced a potent inhibition activity with an IC_{50} of 0.036 mg/mL (Lacroix & Li-Chan, 2013). Studies carried out *in vitro* and *in vivo*, using Atlantic salmon skin gelatin, provided peptides like Gly-Pro-Ala-Glu and Gly-Pro-Gly-Ala with positive effects on DPP-IV inhibition, GLP-1 regulation, insulin secretion, and glucagon production (Hsieh, Wang, Hung, Chen, & Hsu, 2015; Li-Chan, Hunag, Jao, Ho, & Hsu, 2012). Furthermore, porcine and deer skin hydrolysates using Flavourzyme and gastric enzymes, respectively, resulting in peptide sequences enclosing a proline as the penultimate N-terminal residue with potential anti-diabetic effects (Huang et al., 2014; Jin et al., 2015). The effectiveness of animal source peptides may lie in the high content of collagen, which is a rich source of Pro (Lafarga et al., 2014). Moreover, estimated molecular weights of 200 to 800 Da were preferred when using porcine skin hydrolysates in STZ-induced diabetic rats, although an *in silico* analysis suggested that greater inhibition of DPP-IV was obtained if peptide sequences were large and medium lengths (Huang et al., 2014; Jin et al., 2015).

Comparable inhibitory activity to that from animal sources was obtained by plant-based proteins from common beans, soybean, amaranth, oilseeds (flaxseed, rapeseed, sunflower, and sesame), rice bran, black tea, oat, buckwheat, and highland barley, by using gastrointestinal enzymes, Umamizyme G, Bioprase SP, subtilisin,

and bromelain (Admassu et al., 2018; González-Montoya et al., 2018; Han, Maycock, Murray, & Boesch, 2019; Jorge et al., 2015; Y. Lu et al., 2019; Oseguera-Toledo et al., 2015; Oseguera Toledo et al., 2016; Velarde-Salcedo et al., 2013; F. Wang, Yu, Zhang, Zhang, & Fan, 2015b). Some of these food hydrolysates have the capacity of inhibiting other enzymes other than DPP-IV like α-amylase, α-glucosidase, and angiotensin-converting enzyme (ACE), the latter participates in the regulation of blood pressure. Interestingly, plant peptides from beans and oats have shown to be potent generators of DPP-IV inhibitors showing IC_{50} values of less than 0.2 mg/mL alongside sources like bovine milk and fish (Mojica, Luna-Vital, & González de Mejía, 2017; Nongonierma & FitzGerald, 2019; F. Wang et al., 2015a). Studies have reported the generation of plant peptide sequences that go from 2 to 23 amino acids long containing Pro at the second position of the N-terminus in some cases. In addition, Ala, Asp, Gln, Leu, Phe, Val, Trp, and Gly were observed on repeated occasions. Although the function of hydrophobic residues has not been clearly explained, it is suggested that their role has to do with establishing interactions with the active site of the enzyme for cleavage; furthermore, a positive correlation was found between inhibitory potency and the hydrophobicity of the first two amino acids at the N-terminus (Nongonierma & FitzGerald, 2019). Studies made on rice bran demonstrated that a diversity of dipeptide combinations with Pro at the second position generated good inhibition rates; however, the combination Pro-Ile did not present an inhibitory effect (Hatanaka et al., 2012), reinforcing the importance of proline's position for peptide bioactivity. This kind of findings might indicate that

regardless of the peptide source, characteristics like amino acid positions within the sequence and its hydrophobic nature play a crucial role in DPP-IV inhibition.

Peptides displaying the most effective inhibitory activity also varied in their molecular weight. For instance, rice bran and black tea effective peptides were less or equal to 1 kDa, less than 3 kDa for oat globulins, 5 to 10 kDa for soybean and amaranth, and peptides greater than 10 kDa for common beans (de Souza Rocha et al., 2015; González-Montoya et al., 2018; Hatanaka et al., 2012; Y. Lu et al., 2019; Velarde-Salcedo et al., 2013; F. Wang et al., 2015b). It is known that DPP-IV has a great range of substrates that bind the active site, such as neuropeptide Y, peptide YY, GLP-1, and GIP, among others (Ahrén, 2007). The latter, for instance, is formed by a total of 42 amino acids and GLP-1 is formed by 36 or 37 amino acids, so the length of DPP-IV natural substrates vary their molecular weight too. A known low molecular weight inhibitor of 3 amino acids length, Diprotin A (Ile-Pro-Ile), showed to bind the active site through six hydrogen bonds and hydrophobic interactions (Chakraborty, Hsu, & Agoramoorthy, 2014); therefore, reported fractions are in accordance with a molecular weight range of peptides that easily interact with DPP-IV. The above findings signal the need for additional studies to understand more about the length of peptides and the specific amino acids contributing to the DPP-IV inhibitory effect from different plant protein sources. Previous authors did not explain why these factors may play a role in DPP-IV inhibition.

1.3.5 Other mechanisms for glucose regulation

As mentioned previously, when GIP and GLP-1 incretins bind to their specific receptor at the β-cell, they trigger intracellular signaling for the release of insulin.

However, the activity of certain amino acids such as Leu, Ile, Arg, and Ala have also shown to participate in signaling the release of insulin from the β-cells through the depolarization of the cell membrane and activation of the calcium channels (Newsholme, Brennan, & Bender, 2006). Several clinical trials have shown that ingestion of L-glutamine, L-leucine, whey protein isolates, and casein hydrolysates positively affects GLP-1 levels, insulin, and glucagon responses. It is speculated that some of these amino acids and peptides within isolates and hydrolysates possess an insulinotropic effect which stimulates the activity of the calcium channels for insulin release, or that can stimulate L-cells (Goudarzi & Madadlou, 2013; Greenfield et al., 2009; Kalogeropoulou, LaFave, Schweim, Gannon, & Nuttall, 2008; Manders et al., 2006; Samocha-Bonet et al., 2011). The latter is in charge of producing incretins; however, the exact mechanistic approach is not yet elucidated.

Insulin secretion leads to the activation of pathways for glucose tissue uptake through the cellular membrane translocation of glucose transporters known as GLUTs (Lammi, Zanoni, & Arnoldi, 2015). These transporters are expressed in different body tissues, and they mediate the passage of different substrates. Therefore, energy uptake is possible mainly through the translocation of glucose transporters present in skeletal and heart muscle, adipose tissue, and liver (Choudhuri & Chanderbhan, 2016). For instance, peptide L-leucyl-L-isoleucine and the amino acid L-isoleucine found in whey protein increased GLUT-4 transporters translocation into the plasmatic membrane of skeletal muscle in Wistar rats (Morato et al., 2013). Other studies have found GLUT1 and GLUT4 translocating activity from soy glycinin peptides in HepG2 cells (Lammi et al., 2015). Also, induced diabetic mice studies showed that aglycin, a

3742.3 Da soy peptide, resists enzymatic digestion and crosses the intestinal wall into the systemic circulation to increase cell surface translocation of GLUT4 transporters (Dun et al., 2007; J. Lu et al., 2012). This means that sensitivity in tissues for uptake of circulating blood glucose can be improved, thus lowering glucose plasma levels.

Another glucose regulating mechanism is food intake. Appetite regulation through protein ingestion has shown to induce satiation and satiety responses. These anorexigenic signals produced by GLP-1, peptide YY, cholecystokinin (CCK), and leptin are triggered by food components such as fat, proteins, and carbohydrates (Abot, Cani, & Knauf, 2018). For example, the amino acid glutamate sends gastric and intestinal motility signals and stimulates pancreatic secretions that generate a response from appetite-related hormones that will then, send hypothalamic responses to decrease energy intake and therefore, regulate glucose homeostasis (Abot, Lucas, et al., 2018; Blundell & Bellisle, 2013). Studies showed that ingestion of a high protein meal prompts the release of PYY and GLP-1 if the meal is combined with carbohydrates (Moran & Dailey, 2011; Smeets, Soenen, Luscombe-Marsh, Ueland, & Westerterp-Plantenga, 2008). In addition, it has been observed that administration of glucose and peptide hydrolysates together stimulate insulin secretion even if protein sources vary (Calbet & Maclean, 2002). Understanding the underlying mechanisms is important to know how peptide intake can regulate long or short time appetite responses to manage blood glucose levels.

Over time, extensive research has developed on bioactive peptides and their potential effects to manage a wide range of conditions, among which T2DM has started to gain great interest. Overall, we can observe various protein sources and

diversity of peptides sequences generated by different hydrolysis processes. Although an important number of animal protein-based peptides have shown antidiabetic effects, plant protein peptides demonstrated to be a potential source of α-amylase, α-glucosidase, and DPP-IV enzymes; this provides an alternative for a more natural and sustainable therapy for glucose regulation. This regulation is given by competitive and non-competitive inhibition of the enzyme targets; in addition, *in silico* docking studies have provided great insight on the possible interactions given between the allosteric or active site of the enzyme and the generated peptide sequences. For instance, α-amylase inhibition requires shorter peptides to bind through hydrophobic interactions with the enzyme, as well as the presence of amino acids like His, Met, and Pro; α-glucosidase inhibitory activity relies more on the length and amino acid content like Ser, Thr, Tyr at the N-terminus and Met or Ala at the C-terminus; finally, DPP-IV inhibition depends upon the presence of Pro and Ala at the second position of the peptide structure to engage the active site of the enzyme through hydrophobic interactions and hydrogen bonding. It is observed that in all enzymatic interactions, the presence of Pro has a crucial role. Identification of peptide structures in plant products that can contribute to biological activity for health applications remains to be addressed, thus the importance of going beyond the already known non-proteinaceous components in some foods like fiber or phenolic compounds.

Moreover, enzymes for hydrolyzing and generating bioactive peptides from a wide variety of plant proteins is a factor to explore, as their cleavage specificity influences the obtained sequences, affecting their bioactivity. Although there are many *in vitro* studies focusing on the obtention of bioactive peptides and

understanding of their mechanisms, *in vivo* studies or clinical trials remain limited. Hence, collaborative efforts to investigate the surrounding mechanisms of bioactive peptides in more complex organisms and even humans will lead to fully comprehend the underlying process behind their glucose regulatory effect and improve the current prevention and treatment approach along with life quality of patients with T2DM.

1.4 Oats

1.4.1 General characteristics of the crop and current research on oats

Oat is one of the most abundant cereals produced worldwide, although most of its production, nearly 75% of its usage, is still for animal feed (Mäkinen, Sozer, Ercili-Cura, & Poutanen, 2016). However, human consumption has gradually increased as the demand for plant-based, non-dairy, and gluten-free products arise, besides the potential health benefits that oat can provide due to its soluble fiber, unsaturated fatty acids, and polyphenols contents (Prairie Oat Growers Association, 2021). This crop tends to have a better adaptation to cool and humid weather and acidic and alkaline soils; such conditions have given Russia, Canada, the U.S., the European Union, and Australia the 77% of oat grains worldly supply. (Mäkinen et al., 2016; Murphy & Hoffman, 1992; Strychar, 2011). Oat production in Canada has remained constant as oat milling capacity increases too; for instance, Manitoba, Saskatchewan, and Alberta (Canadian Prairies) are one of the main oat production and breeding provinces, which makes Canada one of the largest exporters of oat (60% of global exports) (Strychar, 2011; Yan, Fetch, Frégeau-Reid, Rossnagel, & Ames, 2011). Traditional oat use for human consumption has been as a breakfast cereal and as an added ingredient in the

form of oatmeal, oat bran, oat flours and flakes (Strychar, 2011). The development of new applications of oat and value-added products is required to drive oat production and take advantage of all the benefits that different fractions of this cereal can provide.

There are two main varieties of oats: *Avena sativa* L. and *Avena nuda* L., from which *A. nuda* or naked oats has been considered to have high nutritional value due to its rich content in essential amino acids (Arendt & Zannini, 2013; Zwer, 2015). The understanding of oat health properties greatly impacts its use for human food products and other industrial applications. As a fiber-rich food, oat has become of great interest in the study of β-glucan and its effects on postprandial glucose and insulin levels. Conducted meta-analysis has shown that β-glucan has a positive response when lowering triglycerides, LDL cholesterol and total cholesterol (Tiwari & Cummins, 2011). Furthermore, it showed a delaying effect on gastric emptying, which reduces digestion and absorption of nutrients, inducing satiety, reducing caloric intake, and impacting obesity management (Beck et al., 2009; Bozbulut & Sanlier, 2019). This response is attributed to the increased viscosity that fiber provides due to its water holding capacity and gel formation, thus contributing to the delayed gastric emptying and low absorption of carbohydrates and decreasing bile acids and cholesterol absorption and reabsorption (Lazaridou & Biliaderis, 2007). These mechanisms help control hyperinsulinemia, which is related to the impairment of intracellular signaling for regulation of the vascular system, thus normalizing vasoconstriction, blood pressure and cardiovascular functions (Rains & Maki, 2013). Fibers like β-glucans are highly fermentable by the gastrointestinal microbiota, which generates short chain fatty acids (SCFAs). The latter are absorbed in its majority by the enterocytes to

produce energy or act as secondary messengers to express hormones like GLP-1 or modulate cholesterol synthesis pathways to promote the excretion of bile acids (Alexander, Swanson, Fahey, & Garleb, 2019). Therefore, as a source of prebiotics, consumption of fibre will provide antidiabetic and antihypercholesterolemic effects through nondigestible carbohydrate fermentation. Animal model studies evaluated the antihypertensive and cardioprotective role of oat β-glucan, following the 3 to 4 g recommended daily intake provided by 100 g of rolled oats per day (Raj, Ames, Joseph Thandapilly, Yu, & Netticadan, 2020)

Other bioactive compounds found in oats are avenanthramides (Avns), which are polyphenols that have shown antioxidant, anti-inflammatory, antidiabetic, antiatherosclerotic, and anticancer effects (Bazzano, He, Ogden, Loria, & Whelton, 2003; Ji, Lay, Chung, Fu, & Peterson, 2003; Kim et al., 2013; P. Wang et al., 2015). It has been demonstrated that antioxidant activity and antidiabetic activity, through the inhibition of the α-amylase enzyme, increased after germination of oat grains due to activation of enzymes and nutrients like phenolics, dietary fiber, magnesium, or zinc, needed for the seed to sprout (Khang, Vasiljevic, & Xuan, 2016) Also, the use of oat roots for extraction of phytochemicals proved positive bioactivity through, free radical scavenging of 87% and hemolytic assays that showed less than 10% lysis; moreover, methanol extract showed 23% inhibition of α-amylase (Idrees et al., 2017). Therefore, other oat plant components could represent a safe source of antioxidant and antidiabetic compounds. Furthermore, oat derived ingredients are also used for cosmetic purposes as skin and hair conditioners, antioxidants, absorbents, abrasives, and other agents (Becker et al., 2019). Oat based products have a prebiotic effect on

skin microbial diversity as it improves dryness and itchiness through its hydrating effect (Futterer, Rush, Meyer, & Crider, 2019). The use of colloidal oatmeal has proved to be an effective and safe treatment for atopic dermatitis due to its anti-inflammatory properties (Becker et al., 2019; Fowler & Silverberg, 2008).

1.4.2 Oat as a source of plant proteins

Plant proteins have gained great interest nowadays as they represent a more sustainable dietary source of high-value nutrients. Oat is popularly consumed as a morning breakfast cereal in the form of flakes or used as flour for food preparation; nevertheless, industrial-scale production of oat protein has recently emerged due to the impact that this crop has in terms of land, water, and energy use, for the creation of plant-based ingredients and other products that could have health benefits (Mäkinen et al., 2016). Oats are a good source of proteins as they represent 12.4 to 24.5% of the total macronutrients, and the main fraction is constituted by globulins (80%) which are salt soluble proteins (Yue et al., 2021). As the globulin fraction increases, the prolamin contents decrease, and opposite to cereals like wheat, rye, and barley, the content of prolamins (10-15%) in oat does not represent a problem for celiac patients (Butt, Tahir-Nadeem, Khan, Shabir, & Butt, 2008). Currently, oat processing has focused on the extraction of β-glucan; however, other remaining components like flours are a good source of proteins and other valuable nutrients, yet these are used as animal feed. Hence, oat protein from milling by-product stream is still waiting for exploration of its full potential.

1.4.3 Nutritional/ amino acid composition of oat protein, protein compositions, structures, functional properties

As mentioned previously, oats contain a large range of nutritional components, as observed in Table1.2. This crop has higher energy, protein content, calcium, thiamin, vitamin E, and total mono and polyunsaturated fatty acids compared to wheat, rice, barley, and rye (Tosh & Miller, 2015; U.S. Department of Agriculture (USDA), 2019). Globulins are the major storage protein in this crop (70-80%), prolamins (avenins) account for 4-14 %, albumins for 1-12%, and glutelins account for less than 10% of the protein content; furthermore, three main fractions from globulins have been identified as 3S, 7S, and 12S (Mäkinen et al., 2016). The latter consists of two subunits (53-58 kDa) held by a disulfide-bond; these subunits have acidic and basic characteristics similar to those found in soy 11S globulins (Burgess, Shewry, Matlashewski, Altosaar, & Miflin, 1983a; Mäkinen et al., 2016). FT-IR helped to determine the structure of 12S major globulin fraction, where 74% are β-sheet, 19% are α-helices, and 7% are β-turns; moreover, it was shown that β-sheet structures could transition to random coils when acid or alkaline conditions are used (Liu et al., 2009a). Protein fractions 7S and 3S molecular weights go around 50-70 kDa and 48-52 kDa, respectively; both are high in glycine, and even if oat 7S fraction is similar to 7S vicilins from legumes; reports showed higher contents of Glx (Glu+Gln) and Arg in oat globulin (Burgess et al., 1983a).

In terms of the amino acid composition, oat protein is abundant in glutamine, glutamic acid, aspartic acid, and leucine (Peterson, 2011). Lysine was reported higher

than other cereals but still a limiting amino acid in oat protein; however, methionine, cysteine, and tryptophan content in oat are above the FAO scoring (Mäkinen et al., 2016; Peterson, 2011). Oat avenins fraction is rich in glutamic acid and proline; globulins are high in basic amino acids like histidine, lysine, and arginine; and albumins are also high in lysine, asparagine, alanine, and aspartic acid (Peterson, 2011). Since it is still considered an incomplete protein, it is recommended an increased dietary intake of the amount of protein (3-8 times) or its combination with other plant- or animal-based proteins that could counteract the lack of some essential amino acids (Gorissen et al., 2018). Overall, this cereal is considered to be one of the cereals with high content of protein, with good nutritional and functional properties thus, making it a versatile ingredient to use in baked, extruded, and vegan products (Mäkinen et al., 2016).

Table 1.2 Nutritional composition of oats

Nutrient (per 100 g)	**Oats**	**Oat Bran**	**Oat Flour**	**Quick Oat Cereal**	**Oat Puffed Cereal**
Energy (kcal)	389.0	246.0	404.0	379.0	376.0
Water (g)	8.2	6.6	8.6	10.8	5.1
Protein (g)	16.9	17.3	14.7	13.2	12.1
Lipid (g)	6.9	7.0	9.1	6.5	6.7
Carbohydrate (g)	66.3	66.2	65.7	67.7	73.2
Dietary fiber (g)	10.6	15.4	6.5	10.1	9.4
Minerals (mg)					
Calcium	54.0	58.0	55.0	52.0	401.0
Iron	4.7	5.4	4.0	4.3	33.2
Magnesium	177.0	235.0	144.0	138.0	114.0

Phosphorus	523.0	734.0	452.0	410.0	481.0
Potassium	429.0	566.0	371.0	362.0	641.0
Sodium	2.0	4.0	19.0	6.0	497.0
Zinc	4.0	3.1	3.2	3.6	16.7
Vitamins (μg)					
Vitamin E	NR	1000.0	700.0	500.0	1300.0
Vitamin K	NR	3.2	3.2	2.0	1.8
Vitamin B1	800.0	1200.0	700.0	500.0	1300.0
Vitamin B2	100.0	200.0	100.0	200.0	100.0
Vitamin B3	100.0	900.0	1500.0	1100.0	21000.0
Vitamin B6	100.0	200.0	100.0	100.0	2400.0
Folate	56.0	52.0	32.0	32.0	1201.0

Modified from Menon et al., 2016; U.S. Department of Agriculture (USDA), 2019.

Table 1.3 Amino acid composition of three Canadian naked oat cultivars (%).

Amino acid	AC Hill	AC Lotta	AC Percy
Threonine	2.88	3.23	2.87
Methionine	1.49	1.38	1.45
Phenylalanine	5.37	5.30	5.28
Histidine	2.47	2.50	2.60
Lysine	3.70	3.84	3.96
Valine	5.62	5.31	5.57
Isoleucine	4.32	4.16	4.18
Leucine	7.90	7.54	7.53
Total Essential	33.75	33.26	33.44
Serine	4.23	4.42	4.24
Glycine	4.46	4.19	4.44
Glutamic acid	23.20	22.31	22.87
Aspartic acid	7.25	7.95	7.38

Proline	6.09	5.28	5.55
Cysteine	4.35	5.50	4.83
Alanine	4.50	4.63	4.60
Tyrosine	4.28	4.05	4.22
Arginine	6.19	6.80	6.77
Total Non-essential	64.55	65.13	64.90

Modified from Zarkadas, Yu, & Burrows, 1995.

In addition, oat protein has demonstrated some good functional properties. Oat protein solubility is usually limited to a slightly acid and neutral pH, which makes it very suitable for various food products; however, to increase the range of applications for oat protein, it is necessary to improve its solubility (Mäkinen et al., 2016; Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009). This and other functional properties can be modified by hydrolysis and by amino acid residue adjustments for improvement of solubility and emulsifying and binding properties, which makes oat protein ideal for meat replacement applications (C. Y. Ma & Harwalkar, 1984). Based on the good functional properties and nutritive value, oat proteins have been developed as food emulsifiers, bakery products, meat substitutes, meal replacement shakes, sports nutrition products, animal feeds, lowering cholesterol products, and more ("Advanced Food Products - Oat Services Ltd," 2017; "PrOatein oat protein | Lantmännen Oats," 2020). Oat protein-gels have shown to be comparable to those of egg proteins, suggesting their potential to be used as a gelling ingredient in various food applications (Nieto-Nieto, Wang, Ozimek, & Chen, 2014). Furthermore, oat protein concentrates have been considered as skim milk protein substitutes in yogurt preparation, as it can improve the product's texture and syneresis; in addition,

consumer's acceptance of this high added value fermented product shows that plant-based enriched foods could be both, tasty and nutritious (Brückner-Gühmann, Banovic, & Drusch, 2019; Brückner-Gühmann, Benthin, & Drusch, 2019).

Cold gelation process has been applied successfully to produce oat protein gels that can protect bioactive compounds like α-amylase, probiotics, and riboflavin to by-pass the stomach as these compounds are labile to the harsh gastrointestinal conditions. The gels are then degraded in the small intestine to release the bioactive compounds to promote their absorption (Yang, Wang, & Chen, 2017). Moreover, oat protein water holding capacity provided the optimal medium for probiotics to survive and reach until the small intestine for released in a controlled manner (Yang et al., 2018). Oat protein isolates and β-glucan conjugate obtained by Maillard reaction were also evaluated for their emulsifying capacity showing stability in oil-in-water emulsions for encapsulation of hydrophobic compounds such as β-carotene (Zhong et al., 2019). Consequently, exploring oat proteins and their functional properties can give a greater vision for their value-added applications in food and natural health products. Recently oat protein isolates were mixed with glycerol to prepare laminated films reinforced with nanofibers with the purpose to create biodegradable films of improved mechanical properties (Habibi Zarabadi, Kadivar, & Keramat, 2018). Such effort is still very limited for oat proteins; however, plant-protein is starting to gain great interest for the obtention of biodegradable films (Sabato et al., 2001; Salgado, Molina Ortiz, Petruccelli, & Mauri, 2010; Xia, Wang, & Chen, 2011).

1.4.4 Oat protein hydrolysis by different enzymes

There are multiple benefits to the obtention of bioactive peptides from plant-based products, and enzymatic hydrolysis is one of the most used methods for this process. This step aims to achieve a certain degree of protein breakdown into smaller chains of peptides according to the enzyme's specificity. Enzymatic hydrolysis modifies protein solubility by degrading the tertiary structure and exposing hydrophobic residues that are usually concealed in the internal protein structure (Kilara & Panyam, 2003). The changes in net charge and hydrophilicity will impact other functional properties like foaming or emulsifying capacity as electrosteric stabilization is important in colloidal systems (Dickinson, 2003; Mäkinen et al., 2016). These functionalities are also dependent on the type of enzyme used, and the degree of hydrolysis obtained (Van Vliet & Walstra, 2017). Allergic reactions produced by food proteins can also be reduced when using enzymatic hydrolysis, in addition, the effect of low allergenicity achieved more efficiently when using non-specific enzymes or a combination of them (Van Vliet & Walstra, 2017). Hydrolysis creates a breakdown of the complete protein sequence that generates the allergic response. This kind of protein hydrolysates are well tolerated and a beneficial option for infants or late adults who are prone to develop food allergies or digestive and nutritional complications.

Protein hydrolysis is becoming relevant in the obtention of peptides with biological activities as antioxidants, antifungals, or hypotensive agents, just to mention some. Oat is a protein source to produce bioactive peptides. The methods to obtain them are based on its hydrolysis; for this purpose, the raw material must undergo a controlled process according to the chosen enzyme. Thus, factors like

temperature and pH play an important role to reach the optimal conditions for the proteins to unfold and the enzymes to perform their function. The type of enzymes will generate an accelerated breakdown of the whole protein structure into smaller molecular weight components or produce free amino acids from oligopeptides, depending on their endopeptidase or exopeptidase function, respectively (Van Vliet & Walstra, 2017). Some of the enzymes found in previous research for oat protein hydrolysis are animal, plant, and microbial proteases used either separately or in combination.

Oat bioactive peptides have been obtained, generally from oat bran and oat groats, through different extraction methods and further hydrolysis with specific enzymes to produce the desired effect tested *in vitro* and *in silico*. For instance, hydrolysis using enzymes like pepsin, subtilisin, thermolysin, trypsin, papain, ficin, and flavourzyme have shown to produce high angiotensin-I-converting enzyme (ACE-I) inhibition (up to 96.5 %); in some cases potentiated by ultrasonic treatment and under high enzyme to substrate ratio conditions for short time hydrolysis, which allowed a competitive inhibition similar to that exerted by a well-known antihypertensive: captopril (Bleakley, Hayes, O' Shea, Gallagher, & Lafarga, 2017; Cheung, Nakayama, Hsu, Samaranayaka, & Li-Chan, 2009; Bei Wang et al., 2015; Zheng et al., 2020). Furthermore, the antioxidant and antiplatelet capacity of oat peptides was demonstrated in peroxyl and hydroxyl radical assays and platelet aggregation assays; where RP-HPLC fractionation enhanced the activity, confirming that hydrophobic residues like Pro, Leu, Ile, Gly, and Met play a key role in this bioactivity (Jodayree, Smith, & Tsopmo, 2011; S. Ma, Zhang, Bao, & Fu, 2020; Vanvi

& Tsopmo, 2016; Walters, Willmore, & Tsopmo, 2020; G. Yu, Wang, Zhang, & Fan, 2016).

Table 1.4 Characteristics of enzymes used in oat bioactive peptides' research.

Enzyme (source)	**Optimal pH & Temp.**	**Mechanism of action**	**Classification**	**Oat bioactivity found***
Pepsin (Porcine stomach mucosa)	2.0-4.0 37 ˚C	Cleaves aminoacyl-proline to amino acid + proline	Aspartic endoprotease	Antioxidant & antihypertensive
Trypsin (Bovine pancreas)	7.5-8.5 37 ˚C	Cleaves peptides after lysine and arginine residues	Serine endoprotease	Antihypertensive & antidiabetic
Thermolysin (*B. thermo-proteolyticus*)	7.0-8.0 70 ˚C	Cleaves bonds on the N-terminal side of hydrophobic residues	Metalloendopro-tease	Antihypertensive
Subtilisin (*B. licheniformis*)	7.0-10.0 25 ˚C	Non-specific protease	Serine endoprotease	Antihypertensive
Papain (*Carica papaya*)	4.0-10.0 80 ˚C	Preference for bulky non-polar side chain like Phe or Val in P2 position	Cysteine/thiol endoprotease	Antihypertensive, antioxidant, & antidiabetic

Ficin (*Ficus glabrata*)	7.0 37 ˚C	Preference for hydrophobic side chains containing Gly, Ser, Glu, Tyr, and Phe.	Cysteine/thiol endoprotease	Antihypertensive & antidiabetic
Flavourzyme (*Aspergillus Oryzae*)	4.0-8.0 30-65 ˚C	Release of free amino acids from C- and N-terminus	Endo/ exoprotease	Antihypertensive, antioxidant, & antidiabetic

Sources: Ward, 2019; *Bioactivities: Bleakley et al., 2017; Cheung et al., 2009; Jodayree et al., 2011; Ma et al., 2020; Vanvi & Tsopmo, 2016; Walters et al., 2020; F. Wang et al., 2015; Zheng et al., 2020.

The use of different proteases promotes the reduction of the original proteins' molecular weight and the release of peptide sequences coming from internal segments near or far from the C- or N-terminus. These enzymes have specific mechanisms of action due to a cleavage preference for certain segments of the peptide chain. Generated peptides from oat enzymatic hydrolysis presented a wide range of lengths that go from 3 to 20 amino acids. Also, some of the most frequent identified amino acids in these peptide sequences were phenylalanine (F), proline (P), Leucine (L), glutamine (Q), glutamic acid (G), isoleucine (I), asparagine (N), lysine (K), and tyrosine (Y). Research has suggested that peptide stability during gastrointestinal digestion and bioactivity will be affected by characteristics like structure, molecular weight, amino acid composition, sequences, charges and hydrophobicity (Bo Wang, Xie, & Li, 2019).

1.4.5 Oat as a protein source to generate anti-diabetic peptides

The impact of oat consumption and its effect on its cardioprotective, lowering cholesterol and glucose effects has been widely studied for oat fiber components like β-glucans (Bozbulut & Sanlier, 2019; Raj et al., 2020; Tiwari & Cummins, 2011). Human studies have shown the benefits of oat consumption as part of a high-fiber diet. Its long-term consumption lowered the postprandial plasma glucose and HbA1C levels in overweight patients with T2DM (Li et al., 2016; X. Ma et al., 2013). The addition of oat bran concentrate in bread products resulted in a lower insulin response than that produced by white bread, suggesting that this kind of products might be a good dietary recommendation for T2DM condition (Pick et al., 1996). Although health benefits have been mainly associated with oat fiber and phenolic compounds, little is known about the effects of peptides and body interactions to act as an antidiabetic nutrient.

Current research on oat peptides and their antidiabetic effect has been mostly performed *in vitro* and *in silico* to understand the possible peptide sequences that can be obtained through enzymatic hydrolysis to inhibit the activity of DPP-IV, α-glucosidase, and α-amylase enzymes; furthermore, studies were done in Caco-2 and NCI-H716 cell models, to investigate the inhibitory effect on cell-secreted enzymes, GLP-1 levels (11.5% increase), and glucose transporters Glut2 and Glut5 (237% and 218% increase, respectively) (Walters et al., 2020; F. Wang et al., 2018b). A study performed in streptozotocin-induced diabetic observed the effect of naked oat alcalase hydrolysates of less than 5 kDa, which showed that high oat peptide doses generated a lower total food intake, as well as hypoglycemic effects that were related to higher

levels of hepatic glycogen thus, it was suggested that oat peptides might work as regulators of glycogen storage in the liver, although the regulating mechanisms are not entirely clear (Zhang & Wang, 2015). Therefore, the study of mechanisms involved in glucose metabolism will provide a better insight into general glucose regulating pathways and how bioactive peptides interact to counteract the effects of T2DM.

Oat peptide research has used multiple types of enzymes like papain, ficin, flavourzyme, alcalase, and trypsin in separate hydrolysis conditions; however, previous research on chickpea protein using continuous hydrolysis with alcalase and flavourzyme showed an enhanced effect on protein functionality and antioxidant capacity (Xu et al., 2020). For this reason, the present study will investigate the effects of continuous hydrolysis in oat peptide sequence obtention and enzymatic inhibition of DPP-IV, α-glucosidase, and α-amylase. Since oat isolates and hydrolysates have shown a positive antidiabetic effect, additional fractionation of peptides into low molecular weight fractions and hydrophobicity might help a better understanding of the peptide's regulating mechanisms.

Peptide sequencing has provided relevant information on the interactions of peptide chains and amino acids regarding enzyme inhibition. In addition, peptide sequencing allows the identification of characteristics that might provide more stability of bioactive peptides after gastrointestinal digestion, as it has been shown that peptides with higher contents of glutamic acid and proline tend to resist better to pepsin and pancreatin action, in contrast, the presence of lysine or arginine residues at the C-terminus makes the peptide prone to trypsin cleavage in the gut (Savoie,

Agudelo, Gauthier, Marin, & Pouliot, 2005; Bo Wang et al., 2019). Some of the peptides found in oats possess 3 to 19 amino acids in their structures, containing a wide range of amino acids. However, in the majority of cases, the recurrent appearance of hydrophobic amino acids such as phenylalanine, proline, leucine, isoleucine, as well as charged amino acids like glutamic acid or lysine could explain their binding behaviour to enzymes like DPP-IV or α-glucosidase (Bleakley et al., 2017; F. Wang et al., 2018b; Zhang & Wang, 2015).

Although inhibition of enzymes like DPP-IV, α-glucosidase, and α-amylase has recently gained interest in diabetes research, the study of oat peptides capacity to inhibit these enzymes remains limited; therefore, exploration on peptide sequences that effectively interact with these enzymes and other glucose regulating routes are needed. Disclosure of oat peptide sequences will allow a better understanding of this popular cereal and will give rise to the future obtention of peptides through specific hydrolyzing methods according to the type of enzyme to be inhibited. Consequently, this will generate a standardized approach for further concentration, purification, and applications development. Oat protein has a great potential for the exploration of characteristics that confer healthy attributes to oats and opens the door to developing beneficial products for human consumption and options to addressing T2DM prevention and management.

1.5 Research hypotheses and objectives

This research aims to study peptide structures for antidiabetic activities from oat protein and further concentrate peptide fractions with desirable structures to maximize their bioactivities.

According to the previous literature review, the following hypotheses were proposed:

1. Peptides with strong antidiabetic activities can be generated from oat protein by enzymatic hydrolysis.
2. Low molecular weight oat peptides with a higher level of hydrophobic amino acids can positively affect their antidiabetic activity.

The overall objective of this research was to generate bioactive peptides with antidiabetic activities from oat proteins for diabetes management.

Therefore, the specific objectives were:

1. To develop oat protein hydrolysis and fractionation processing (e.g., membrane filtration and RP-HPLC fractionation) to generate peptides with antidiabetic activities.
2. To investigate the α-amylase, α-glucosidase, and DPP-IV inhibitory effects of oat protein hydrolysates and their fractions through *in vitro* enzymatic assays.
3. Identify the oat peptide sequences in response to their anti-diabetic activities.

1.6 Significance of this work

This proposed research will provide an optimized fraction processing for the generation of new peptide sequences from oats that can effectively inhibit three of the most studied enzymes involved in hyperglycemia control. Demonstrated dosages effects through enzymatic assays will give rise to further collaboration with nutrition scientists to evaluate other *in vitro*, *in vivo,* and clinical trial responses to assess oat peptide's bioactivity. This research will also explore the potential mode of action of the peptides that contribute to their antidiabetic activities. Exploration on antidiabetic peptides can provide insights into the possibility of creating a more natural option for

the improvement and prevention of diabetes. These peptides could be used in food formulations such as smoothies, protein bars, yogurts, or beverages to create new functional food products to manage type 2 diabetes. Additionally, this research will expand the existing knowledge about oat and oat products as a source of bioactive compounds for healthy food development. This will provide new value-added opportunities for oat to be used in healthy diets and increase human consumption of oats, thus generating additional revenue for oat producers and its processing industry.

Chapter 2- Preparation of antidiabetic peptides from oat protein and identification of their peptide structure in response to their inhibitory effect

2.1 Introduction

The increased prevalence of diabetes around the world represents a significant problem in public health. According to the International Diabetes Federation (2019), 90% of the worldwide population with diabetes have type 2 diabetes mellitus (T2DM). The affected population is prone to develop comorbidities, which means other chronic diseases such as high blood pressure, renal failure, retinopathy, or heart disease (Van Smoorenburg, Hertroijs, Dekkers, Elissen, & Melles, 2019). The diabetic population is generally under treatments requiring more than one medication to control glucose levels and treat any complications. Nowadays, great efforts are being made in research to understand the role of food and nutrition in T2DM management since prevention and remission is feasible for some patients (Hopkins et al., 2020). Current management relies on dietary and lifestyle modifications and the use of anti-diabetogenic drugs such as insulin, biguanides, α-glucosidase inhibitors, dipeptidyl-peptidase (DPP)-IV inhibitors, incretin mimetics, meglitinides, among others (Olokoba et al., 2012). Despite their good antidiabetic effects, medications might be associated with side effects, which in long terms reduces the adherence to the treatment together with other factors like medication costs (García-Pérez et al., 2013).

Currently, the search for food sources that can generate compounds with potential biological activities has become of great interest. Both animal and plant

proteins are sources of the so-called bioactive peptides, which are defined as specific protein fragments that positively impact the functioning or conditions of living beings, thereby improving their health. Among bioactive peptides, those with antidiabetic activities have attracted interest in the last decade. These include peptides that can inhibit α-amylase and α-glucosidase involved in starch hydrolysis and, therefore, regulate glucose absorption into the bloodstream. DPP-IV inhibitors increase glucagon-like peptide (GLP)-1, a hormone involved in glucose metabolism, by stimulating insulin production; thus, they are used as targets in developing antidiabetic drugs. Hence, the ability to generate peptides with antidiabetic activities from food proteins will provide a great opportunity for food and natural health products sectors to develop peptide-based products to create diabetes-friendly foods for the prevention and management of T2DM.

Oat is one of the most abundant cereals cultivated worldwide. Canada is known for being one of the largest producers of oats in its western prairie provinces (Yan et al., 2011). Oat is a good source of both protein and dietary fibre. Oat β-glucan is known for its cholesterol-lowering and glucose regulating effects. Also, oat is the only cereal containing globulin protein, avenalin, as its major protein component; thus, it is more nutritious than most cereals with a greater proportion of essential amino acids like lysine, methionine, cysteine, and tryptophan (Peterson, 2011; Tiefenbacher, 2017). Therefore, oat presents an interesting cereal to develop diabetic-friendly food products. Studies have shown the release of bioactive compounds from oat protein achieved by bacterial and fungal enzymatic hydrolysis, proving their successful antioxidant and ACE-I inhibition activities; however, few studies have

considered investigating proteins from oats as a source of antidiabetic peptides. Preliminary experiments in our lab showed potential antidiabetic activities of oat protein hydrolysates. This positive effect has also been reported recently by Wang et al., 2018. However, oat peptide structures responsible for their antidiabetic activities have not been elucidated as far as we know. The hydrolysis and fractionation processes are yet to be established to concentrate peptides with desirable sequences to maximize intended physiological properties and health benefits. This research aimed to prepare oat protein hydrolysates by alcalase and flavourzyme treatment and fractionate them based on their molecular weight (M_W) and hydrophobicity characteristics to generate potential antidiabetic peptides. This process brings novelty to the study of antidiabetic peptides from oat, since no other study has applied continuous hydrolysis and fractionation based molecular weight and hydrophobicity for glucose regulatory effects from oat protein. The collected fractions were evaluated for their antidiabetic effects including α-amylase, α-glucosidase, and DPP-IV inhibitory activities. The fractions with the highest effects were characterized for the peptide structures and sequences by LC-MS/MS, generating new information about the potential oat peptide sequences that produced a positive antidiabetic effect.

2.2 Materials and Methods

2.2.1 Materials

Naked oat grains (*Avena nuda* L.) were provided by Wedge Farms Ltd., Manitoba, Canada. The oat protein was extracted by the established method in our laboratory (Nieto-Nieto et al., 2014) using alkaline extraction (pH 10) followed by acid

precipitation (pH 5) and collection of the precipitate . The protein content was determined to be 76%, using a combustion nitrogen analyzer (Leco Corporation, St Joseph, MI, USA) and a factor of 5.83 was used for protein conversion. Protease from *Aspergillus Oryzae* (flavourzyme ≥500 U/g; pH 7; 50℃) and from *Bacillus licheniformis* (alcalase ≥5000 U/g; pH 10; 37℃), 3,5-DNSA, sodium hydroxide, potassium sodium tartrate tetrahydrate, pancreatic α-amylase, α-glucosidase from *Saccharomyces cerevisiae* were obtained from Sigma Aldrich (St. Louis, MO, USA). DPP-IV inhibitor screening kit was obtained from Abcam (Abcam, ON, Canada). Other used chemicals were of analytical grade.

2.2.2 Preparation of oat protein hydrolysates

Oat protein hydrolysis was conducted according to the method reported by Orsini Delgado, Tironi, & Añón (2011) with modifications. First, oat protein dispersion (1% w/v) was prepared (pH 10) and stabilized at 37℃ for 1 h before adding alcalase (6.25 U/μL) in a ratio of 8 μL/100 mg protein powder. The reaction was carried out for 4 h with the mixture continuously stirred by a magnetic bar at 37℃ and adjusted to pH 10 every 10 min for the first hour by adding NaOH 0.1 M, and after the hydrolysis treatment, the enzyme was inactivated by heating at 85℃ during 10 min. The suspension was left to cool down at room temperature and then freeze-dried to obtain the powder samples of hydrolysates. Continuous hydrolysis by alcalase and flavourzyme involved an initial alcalase hydrolysis as described above, followed by flavourzyme treatment (0.55 U/μL) using 5 μL/100mg sample at pH 7. The reaction mixture was left stirring for 2 h at 50℃. Finally, the enzyme was inactivated at 85℃ for 15 min, followed by centrifugation at 12 000 g for 20 min, and filtration of the

supernatants. Both alcalase hydrolysate (AH) and alcalase-flavourzyme (AFH) filtered fractions were tested for enzymatic inhibition. M_W distribution of the hydrolysates samples was evaluated using size exclusion chromatography (Agilent 1200 series HPLC system equipped with a TSKgel G3000SW_{XL} column (7.8 × 300 mm, Tosoh Corp., TO, Japan)).

2.2.4 Amino acid composition analysis

The amino acid composition was performed at the Alberta Proteomics and Mass Spectrometry Facility using the Waters AccQ-Tag system (Waters Corp., Milford, Mass.). Hydrolyzed samples were derivatized in borate buffer with Waters AccQ-Fluor reagent at 55℃ for 10 minutes. Chromatographic analysis of the derivatized amino acids was done on an Agilent 1200 series HPLC system (Agilent, Santa Clara, Calif.). Samples were separated by a Waters AccQ-Tag column (3.9 × 150mm) at 37℃ with a three-eluent gradient solvent system (AccQ-Tag eluent, ACN, and water) at a flow rate of 1.5 ml/min and detected at 254 nm using an Agilent G1365D multiple wavelength detector. Asparagine and glutamine were hydrolyzed to their corresponding acids and were quantitated as such.

2.2.5 Reverse Phase- High Performance Liquid Chromatography (RP-HPLC) fractionation.

The ultra-filtration fraction with high activity in antidiabetic assays (M_W 1-5 kDa) was further fractionated based on its hydrophobicity using an Agilent 1200 series HPLC system on reversed-phase column (Zorbax SB-C18 column, 4.6 x 150 mm; 5 μm) with a linear gradient mixture composed of solvent A (0.1% TFA in water) and

solvent B (0.1% TFA in ACN). The gradient elution conditions were: 5% solvent B for 5 min and 5-40% solvent B for 30 min, 40-90 % B for 10 min, and finally 5 min at 90% B. Gradient elution was completed at a flow rate of 0.5 ml/min, 60℃. Peaks were monitored at a UV wavelength of 280 nm and collected as four fractions. After the collection of a suitable volume, samples were freeze-dried and used for evaluation of its antidiabetic properties using the same methods as described in section 2.6.

2.2.6 Antidiabetic properties

2.2.3 Membrane filtration of oat protein hydrolysates

The protein sample hydrolyzed by alcalase-flavourzyme was passed through an ultra/diafiltration system equipped with Centramate Cassettes filtration system (T-series Omega, Pall Life Sciences, Mississauga, ON, Canada) using membranes with M_W cut off values of 5 and 1 kDa. The fractions with M_W distribution of 1-5 kDa and 1 kDa were collected, lyophilized, and stored at 4℃.

2.2.6.1 α-Amylase enzymatic assay

The α-amylase inhibitory effect was evaluated according to Awosika & Aluko (2019) with some modifications. The freeze-dried oat protein hydrolysates or fraction samples were dissolved in a 20 mM sodium phosphate buffer (pH 6.9 with 6.7 mM sodium chloride) and diluted to different concentrations. Then 100 µL aliquots were added into test tubes and incubated with 100 µL of α-amylase solution (1.125 U/mL) for 10 min at room temperature. Next, 100 µL of 1% starch dissolved in the same 20 mM sodium phosphate buffer were added. The reaction was carried out for 10 min,

and then 200 µL of dinitrosalicylic acid (DNSA) color reagent (96 mM 3,5-DNSA, 2M sodium hydroxide and 5.3 M potassium sodium tartrate tetrahydrate) was added to the mixture. Test tubes were placed for 5 min in a boiling water bath to inactivate the enzyme. After cooling to room temperature, 3 mL of double distilled water was added to the solution for the final absorbance reading at 540 nm using a Jenway 6300 spectrophotometer (Cole-Parmer scientific experts, Staffordshire, UK). Acarbose was used as the positive control. Absorbances were corrected with a blank sample, and the inhibition activity was calculated as:

% Inhibition = (Absorbance of the control- Absorbance of the sample/Absorbance of the control) ×100.

2.2.6.2 α-Glucosidase enzymatic assay

The α-glucosidase inhibition effect was assayed according to the method by Kwon, Vattem, & Shetty (2006) with slight modifications. The freeze-dried oat protein hydrolysate or fraction samples were dissolved in 100 mM phosphate buffer (pH 6.9) at different concentrations. A volume of 50 µL sample was mixed with a 100 µL of α-glucosidase solution (0.3 U/mL) and incubated for 10 min in a 96 well plate at room temperature. Upon addition of the 50 µL p-nitrophenyl-α-D-glucopyranoside solution (5mM, as substrate), the sample absorbances at 405 nm were recorded using a multi-mode microplate reader (SpectraMax M3; Molecular Devices, San Jose, CA, USA) for 20 min. The obtained absorbances were corrected using a blank sample and compared to the non inhibited reaction control. Acarbose was used as the positive control. The inhibitory activity was calculated as:

% Inhibition = (Absorbance of the control – Absorbance of the sample/ Absorbance of the control) × 100.

2.2.6.3 DPP-IV enzymatic assay

This assay was assessed by a DPP-IV inhibitor screening kit (ab133081, Abcam, ON, Canada) with minimal modification. The freeze-dried oat protein hydrolysates or fraction samples were dissolved in the assay buffer provided in the kit and diluted to different concentrations (from 100 to 500 μg/mL). The DPP-IV enzyme was diluted at a 1:4 ratio (v/v) by the same assay buffer. The initial activity wells included 40 μL of assay buffer, 10 μL diluted DPP-IV, and 50 μL substrate. Inhibitory activity wells included 10 μL of oat protein hydrolysates or fraction samples, or Sitagliptin (positive control), 30 μL of assay buffer, 10 μL of DPP-IV, and 50 μL of the substrate. The plate was incubated for 10 min at room temperature before starting the reaction by adding the substrate. Then, the plate was covered with a 96 well cover sheet and foil and incubated for 30 min at 37℃. After incubation, the fluorescence readings were recorded using a multi-mode microplate reader (SpectraMax M3; Molecular Devices, San Jose, CA, USA) at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The inhibitory activity was calculated as:

% Inhibition = (Initial activity – Inhibitory activity/ Initial activity) × 100.

2.2.7 De Novo peptide sequencing

The peptide fractions with the highest overall antidiabetic activities were subject to LC-MS/MS analysis on a q-Tof premier mass spectrometer (Waters, Milford, MA) coupled with a nano Acquity UPLC system (Waters, Milford, MA). 5 μL of the peptides were loaded onto a nano trap column (75μm x 20mm, Acclaim PepMapTM

100 nanoViper trap column, Thermo Fisher Scientific) followed by a nano analytical column (75 µm × 150 mm, Acclaim PepMapTM 100 nanoViper column, Thermo Fisher Scientific). Desalting on the peptide trap was achieved by flushing the trap with 1% solvent B (acetonitrile with 0.1% formic acid) and 99% solvent A (water with 0.1% formic acid) at a flow rate of 5 µL/min for 2 -3 minutes. Solvents used were: Solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in acetonitrile at a flow rate of 350 µL/min with the following gradient elution: 1 to 75% solvent B for 40 min, 75-98% solvent B for 10 min, and 98% for 5 min. The mass spectrometer was operated at positive mode with a capillary voltage of 3.2 kV and source temperature of 100°C. Spectra were recorded over the (m/z) range of 175-813 Da in MS mode and 50-1990 Da in MS/MS mode. Instrumental control and data analysis were performed using MassLynx software (Micromass U.K. Ltd., Wythenshawe, Manchester, U.K.). De novo sequencing was done using Peaks X Pro (Bioinformatics Solutions Inc., Ontario, Canada).

2.2.8 Statistical analysis

Experiments were performed in triplicates, and results were presented as mean values ± standard deviation. IBM SPSS version 2.6 (International Business Machines, NY, USA) was used for the statistical analysis of the numerical data. Comparison of samples means was made through one-way analysis of variance (ANOVA) followed by Tukey pos-hoc test at a significance level of 0.05.

2.3 Results and Discussion

2.3.1 Enzymatic hydrolysis of oat protein

The SE-HPLC chromatograms in **Figure 2.1** show the M_W distribution of oat protein and its hydrolysates by alcalase and alcalase-flavourzyme treatment. The major peaks in oat protein were in the range of 30 to 7.2 kDa, which progressively shifted to a more define 6.1 kDa peak when hydrolysed with alcalase. However, obtention of smaller peptides was not efficient with the use of alcalase. Therefore, further hydrolysis was conducted with flavourzyme because it has been shown to produce smaller peptides due to its endo and exopeptidase action (Walters et al., 2020). The peptides M_W was significantly reduced after flavourzyme hydrolysis with major peaks in the range of 5.7 kDa and 1.5 kDa. This suggests the pre digestive effect of alcalase over the protein structure's internal peptide bonds, which favors flavourzyme cleavage of amino acids at the chain terminus to produce lower M_W polypeptide chains. It is possible that initial hydrolysis using alcalase could have been limited in the presence of other compounds in the protein extract such as phenolic compounds and fibers that can bind protein to reduce its hydrolysis (Ahnen et al., 2019).

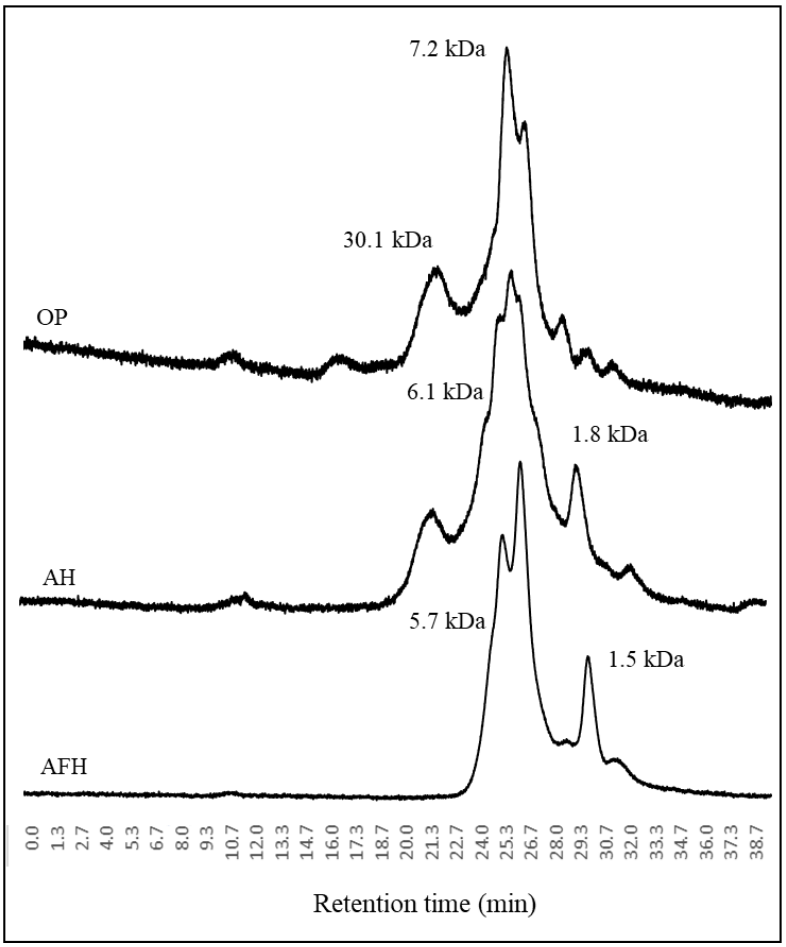


Figure 2.1 Size exclusion chromatogram of oat protein and oat protein alcalase (AH) and alcalase-flavourzyme (AFH) hydrolysates.

2.3.2 Antidiabetic activities of oat hydrolysates.

2.3.2.1 α-Amylase inhibitory assay

α-Amylase inhibition has become an appealing target for diabetes management, as this will delay starch hydrolysis into oligosaccharides and dextrins (de Sales, de Souza, Simeoni, Magalhães, & Silveira, 2012), thus potentially decreasing the amount of substrate for its cleavage into glucose by α-glucosidase enzymes. **Figure 2.2a**

shows the α-amylase inhibitory effect of oat protein hydrolysates by alcalase treatment (AH) at different doses. With increasing hydrolysate concentration from 0.33 to 1.0 mg/mL, the inhibitory rate increased from 18.1 up to 49.5 %. Further increasing AH concentration reduced the α-amylase inhibitory effect. Such a trend was also observed by Powers & Whitaker (1978), where the presence of high concentrations of the inhibitor resulted in its dissociation from α-amylase or conformational changes that modified the inhibitor affinity to the enzyme. Another possible explanation could be attributed to the aggregation of the peptides themselves when increasing the concentrations instead of binding to the enzyme for inhibition. Some of these peptides might be characterized by a net charge creating electrostatic interactions between oppositely charged peptides in addition to increased hydrophobic interactions when concentration increases (Jönsso, Lund, & Barroso da Silva, 2007). The inhibitory effect of AH from oat protein was higher than the inhibition rate reported for seaweed alcalase hydrolysates (~30%) at 1.86 mg/mL (Admassu et al., 2018)

Figure 2.2B shows the α-amylase inhibitory effect of oat peptide fractions from the alcalase-flavourzyme hydrolysate (AFH) with the M_W of 1-5 kDa and ≤1 kDa. Similar inhibition effects of up to 42.6 ± 0.5 % and 56.0 ± 3.7 % were achieved for both fractions, respectively, at a concentration almost six times lower than the one required from AH. This result indicates that the obtention of smaller peptides by combined alcalase and flavourzyme hydrolysis, followed by the hydrolysate filtration to recover the low M_W fraction, effectively concentrated the peptides with α-amylase inhibitory capacity. The 1-5 kDa fraction showed ~33% α-amylase inhibition effect

even at a low concentration of 30 μg/ml. The inhibition rate reached 43% at 100 and 170 μg/ml, but the increase was not significant compared to the 30 μg/ml dose. For the ≤1 kDa fraction, the α-amylase inhibition effect increased from ~7% to 44% and 56% when the concentration increased from 30 to 100 and 170 μg/ml. Further increasing peptide concentration to 330 μg/ml reduced the inhibition rate for both 1-5 kDa and ≤1 kDa fractions. Increasing concentrations for the ≤1 kDa seems to improve the inhibitory effect significantly, while the larger fraction of 1-5 kDa inhibited a more constant inhibitory effect at different concentrations. This could be attributable to the presence of longer peptides that could provide stable interactions with α-amylase. Similar results were obtained for pea protein alcalase hydrolysates where ultrafiltration of the sample into a fraction of M_W 1-3 kDa increased the inhibitory effect. It should be noticed that the 1-5 kDa oat peptide fraction (AFH) exhibited a higher α-amylase inhibitory effect (42.6 ± 1.1%) than that reported for pea protein hydrolysate fraction (<20%) at the same concentration (100 μg/mL) (Awosika & Aluko, 2019).

These findings are consistent with those reported in other studies and support the idea that low M_W peptides have greater bioactivity as more active side chains on amino acid residues can be exposed outside to increase the possibilities of interaction with α-amylase in its catalytic site or subsites (Admassu et al., 2018; Ngoh & Gan, 2016; Oseguera-Toledo et al., 2015). The positive control, Acarbose (82.9± 1.65 to 98.7 ± 1.0 %), displayed a significantly higher α-amylase inhibitory effect even at a low concentration of 30 μg/mL *($p < 0.05$)*, which is expected for a well-known synthetized antidiabetic drug. Nevertheless, around 30% inhibition was also achieved

by oat peptides of low M_W fraction at the same concentration of 30 μg/mL. Therefore, this demonstrates the great potential of oat protein-based inhibitors as a more natural source of antidiabetic compounds to delay α-amylase digestion of starch with the possibility of fewer side effects. However, further research is needed to address that aspect.

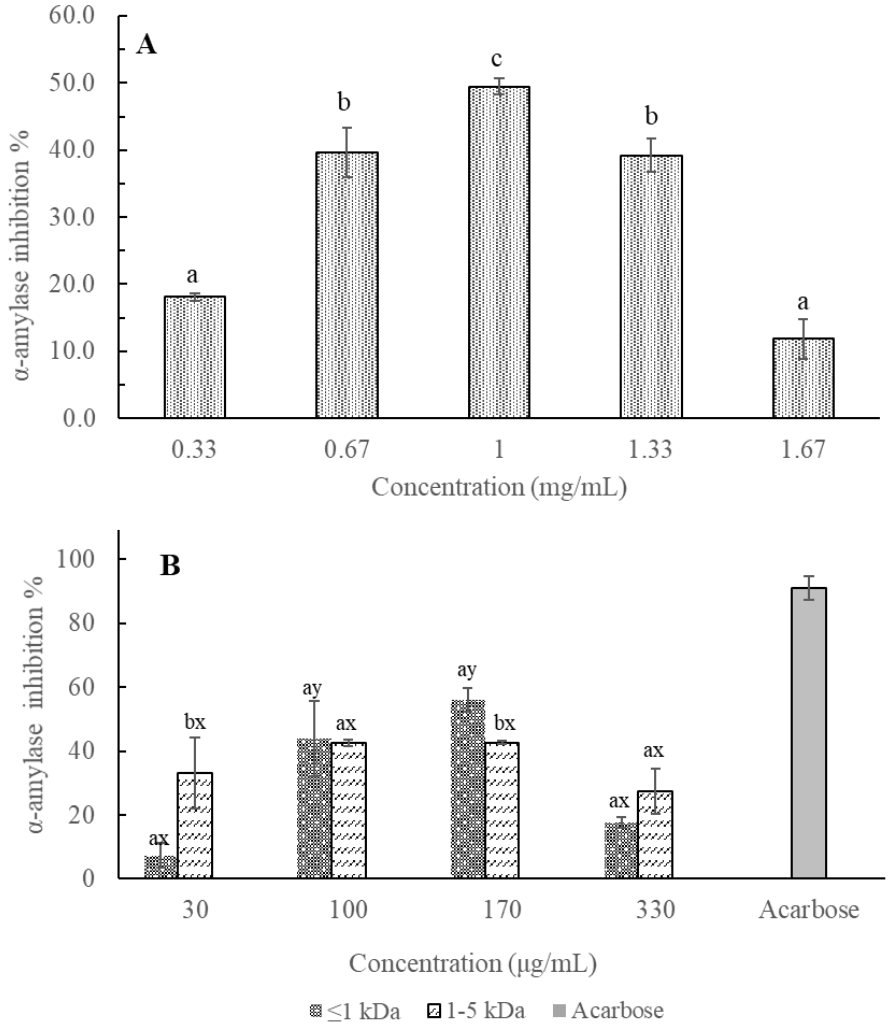


Figure 2.2 (A) Inhibitory effect of α-amylase by alcalase hydrolysate (AH) at different concentrations. Different letters on top of the bars represent significant difference between doses *($p < 0.05$)*. (B) Inhibitory effect of α-amylase by peptide

fractions from alcalase-flavourzyme hydrolysate (AFH) at different concentrations compared to the control, Acarbose at 30 μg/mL. Different letters (a-c) on top of the bars represent significant difference between groups at the same concentrations *(p <0.05)*. Different letters (x-z) on top of the bars represent significant difference between the same fractions at various concentrations *(p <0.05)*.

2.3.2.2 α-Glucosidase inhibitory assay

α-Glucosidase enzymes generate free glucose ready for absorption into circulation; therefore, its inhibition has been broadly studied to promote glucose regulation. Nowadays, some of the most popular α-glucosidase inhibitors are acarbose, voglibose, and miglitol (Lebovitz, 1997). In this study, the oat AH showed a significant difference in the inhibition rate over time (**Figure 2.3**). The enzyme kinetics in the first minute showed α-glucosidase inhibitory effects of up to 38%; however, the inhibitory effect significantly dropped by half after 5 minutes and then decreased to less than 10% after 10 minutes. Since α-glucosidase inhibitors are considered to be competitive, they bind to the same active site and interfere with the cleavage of oligosaccharides (Lebovitz, 1997). Hence, the results obtained in the enzyme kinetic assay suggests that the enzyme's affinity to its substrate increased with time, causing the peptide release from the active site and decreasing the inhibitory effect over time.

Inhibition of α-glucosidase has been observed for peptides from a greater variety of sources; for instance, inhibitory effect was obtained for chia seeds by alcalase-flavourzyme treatment, where ~40% inhibition was obtained for fractions >10 kDa at 0.68 mg/mL; the 1-3 kDa and <1 kDa fraction showed an inhibitory effect

of 18 and 8%, respectively. In contrast, chia pepsin-pancreatin hydrolysates showed no inhibitory effect for both 1-3 and <1 kDa M_W fractions, whereas 80% inhibitory effect was obtained by the 5-10 and >10 kDa fractions (Sosa Crespo, Laviada Molina, Chel-Guerrero, Ortiz-Andrade, & Betancur-Ancona, 2018). These results imply that the inhibition of α-glucosidase relies on the applied enzymes for the protein hydrolysis and peptide size to obtain potential sequences that can interact with α-glucosidase (Awosika & Aluko, 2019). α-Glucosidase inhibitory effect was also observed for alcalase hydrolysates from yellow field pea and walnut (~38%), but at higher concentrations of 20 mg/mL, and the inhibition increased in a dose-dependent mode (Awosika & Aluko, 2019; J. Wang et al., 2018). The sardine muscle alcalase hydrolysates showed IC_{50} of ~15 mg/mL for its most potent fractions; further purification generated two peptides with IC_{50} of 6.8 mg/mL and 2.0 mg/mL, respectively (Matsui et al., 1999; Matsui, Yoshimoto, Osajima, Oki, & Osajima, 1996). In this work, the obtained α-glucosidase inhibitory effect of 15 ± 3.1% could be observed for oat AH at a much lower concentration of 0.75 mg/mL, which is a positive finding to contribute to the antidiabetic activities of oat protein hydrolysates. One recent research also reported that an oat globulin derived peptide of 8 amino acids could inhibit α-glucosidase at the IC_{50} values of 78.58 μg/mL (F. Wang et al., 2018b).

These results suggest a potential for oat protein as a plant protein-based source to generate peptides as α-glucosidase inhibitors. It was also noticed that oat AFH fractions of ≤1 kDa and 1-5 kDa showed no obvious inhibitory effect (data not shown). These findings lead to the assumption that AFH in our research might be less

efficient to generate peptides with a strong α-glucosidase inhibition effect compared to trypsin hydrolysates.

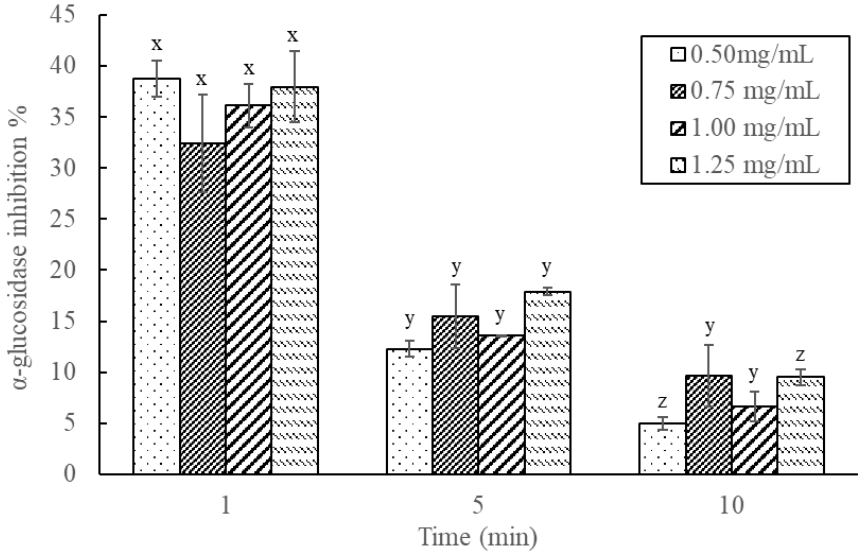


Figure 2.3 α-Glucosidase inhibitory effect of oat Alcalase hydrolysate (AH) using multiple concentrations at 1-, 5-, and 10-min incubation. Different letters (x-z) on top of the bars represent significant difference between times at the same concentrations *(p <0.05)*.

2.3.2.3 DPP-IV inhibitory assay

The main action of DPP-IV inhibitors is preventing the fast degradation of incretins like GLP-1 to produce a lasting effect on insulin stimulation. Oat AH tended to a dose-dependent DPP-IV inhibitory effect (**Figure 2.4A**) starting at 4.9 ± 1.5% and increasing to 48.7 ± 13% when increasing concentration from 100 to 500 µg/mL. However, the high inter-sample variability prevented from reaching statistical differences. Nevertheless, a dose-dependent inhibition was observed for the 1-5 kDa AFH fraction (**Figure 2.4B**), which showed an inhibition rate of 56.2 ± 4.3% when

the peptide concentration increased 500 μg/mL *($p < 0.05$)*. It has been reported studies on casein, soy and common bean proteins that peptides of low M_W had higher DPP-IV inhibitory effect, some of them with M_W of 10 kDa or 1 kDa (González-Montoya et al., 2018; Nongonierma & Fitzgerald, 2013; Oseguera Toledo et al., 2016). In line with the previous research, ultrafiltration of protein hydrolysates provided an effective approach for concentrating peptides with DPP-IV inhibition effect.

In previous work by Wang et al. (2015), hydrolysates of oat globulin showed good DPP-IV inhibition (IC_{50} 2.04 mg/mL) effect after 14 h of trypsin treatment. In our study, the continuous hydrolysis by alcalase and flavourzyme was more efficient to release bioactive peptides with strong DPP-IV inhibition capacity (IC_{50} of 0.413 ± 15 mg/mL) in only 6 h. It is likely related to the facts that alcalase belongs to the endoprotease classification, and flavourzyme exhibits both endo and exoprotease activity with non-specific cleavage; thus, such combination allowed extensive hydrolysis of oat protein for more rapid generation of DPP-IV inhibitory peptides. Porcine skin gelatin hydrolysates by alcalase and flavourzyme treatment demonstrated a DPP-IV inhibitory effect of approximately 60% at a concentration of 5 mg/mL (Huang et al., 2014). In comparison, oat protein hydrolysates presented a similar activity at a lower concentration of 500 μg/mL. Moreover, AFH fraction from oat protein was comparable to hydrolysates from lactoferrin and bovine serum albumin that displayed IC_{50} values of 0.379 and 0.513 mg/mL, respectively. DPP-IV positive control inhibitor, sitagliptin, had the highest inhibitory effect (86.8 % at a much smaller concentration of 50 μg/mL) as expected; however, findings in this study

confirm the greater potential to generate DPP-IV inhibitory peptides from oat protein compared to other plant protein sources.

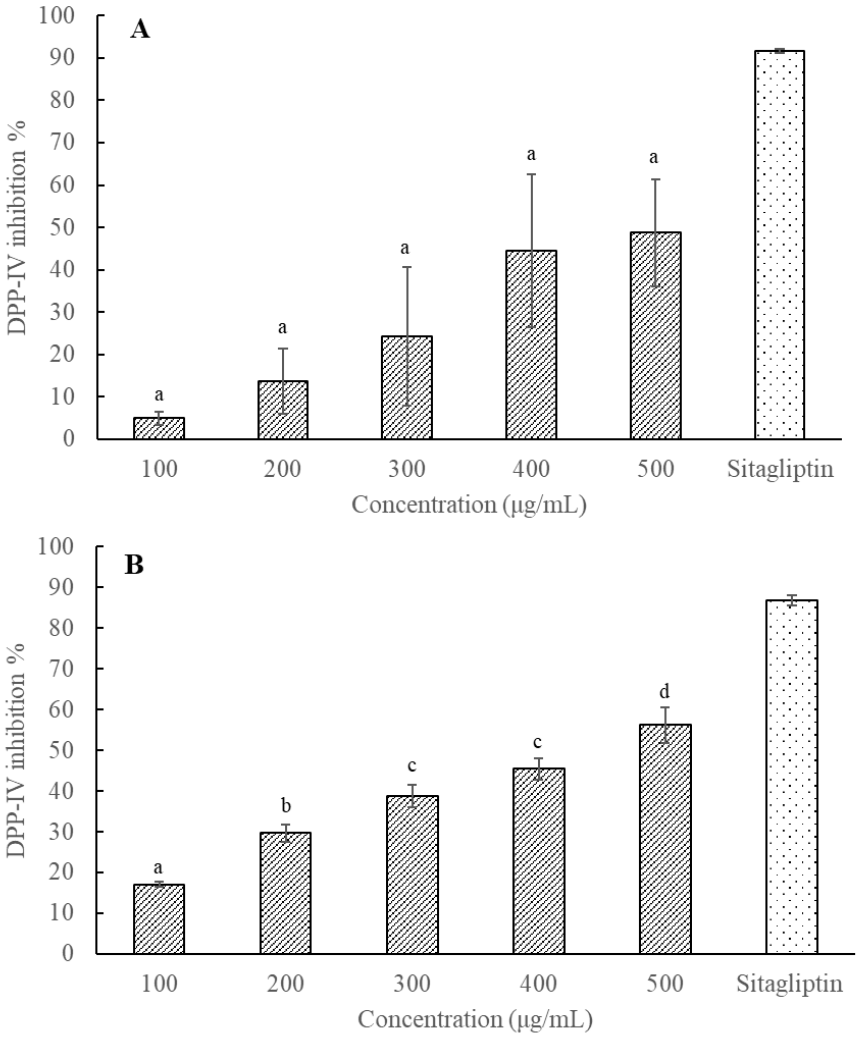


Figure 2.4 Oat alcalase hydrolysate (AH) (A) and alcalase-flavourzyme hydrolysate (AFH) 1-5 kDa fraction (B) concentration-dependent inhibitory effect of DPP-IV. Different letters on top of the bars represent significant difference between concentrations *($p < 0.05$)*.

2.3.3 RP-HPLC peptide fractionation

Since AFH fraction of 1-5 kDa showed overall positive inhibition results, it was chosen for further fractionation using RP-HPLC based on their different hydrophobicity. In this study, oat AFH was separated into four fractions (F1, F2, F3, and F4), as shown in **Figure 2.5**, with F1 being the most hydrophilic and F4 the most hydrophobic fraction. All four fractions were then collected and tested for their capacity to inhibit α-amylase, α-glucosidase, and DPP-IV.

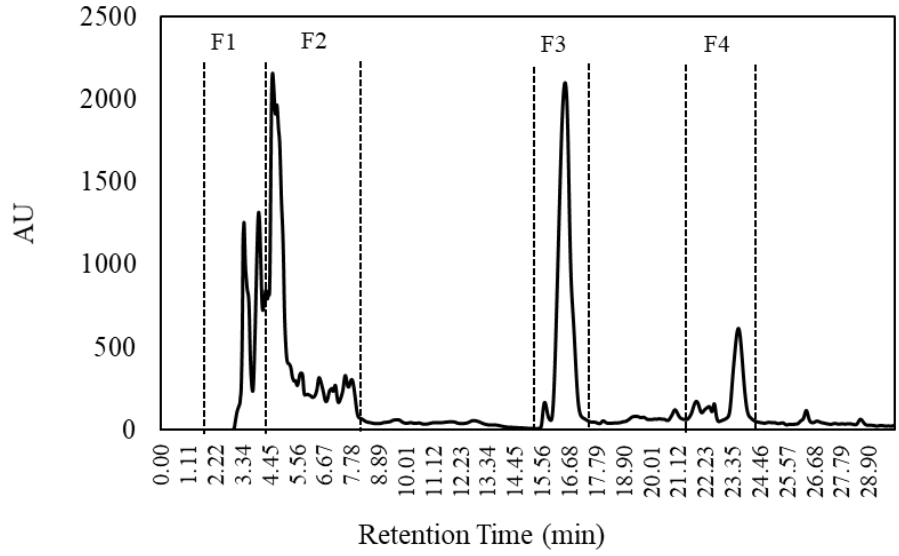


Figure 2.5 Reverse-phase chromatogram of four fractions obtained from AFH sample of 1-5 kDa.

Certain fractions exhibited better inhibitory effects. For instance, a higher α-amylase inhibition effect was observed for F2 and F3, as shown in **Figure 2.6A**. Specifically, the α-amylase inhibitory effect was 53.8 ± 5.6% for F2 even at a low concentration of 30 μg/mL compared to 33% for 1-5 kDa fraction at the same concentration. It was noticed that the inhibitory effect decreased with increasing concentration of the F2 to

170 μg/mL. For F3, the α-amylase inhibitory effect was 46.6 ± 6.3% at a concentration of 30 μg/mL. The highest inhibitory effect (57.3 ± 9.5%) was observed for F3 at the concentration of 100 μg/mL. The α-amylase inhibitory effect observed for F2 and F3 (F1 and F4 effect not shown) suggests that both hydrophobic and hydrophilic amino acids are necessary for interaction with the active site of α-amylase (González-Montoya et al., 2018). Studies where RP-HPLC is used to fractionate peptides with α-amylase inhibitory activity are lacking. However, limited works that analyzed the amino acid sequences of peptides have shown that both polar and non-polar amino acids are required in the peptide sequences to bind the active site of α-amylase for its inhibition. For example, α-amylase Tyr151, Lys200, Ala198, Leu162, Glu233, and Asp300 residues have been demonstrated to interact with acarbose and other non-proteinaceous molecules like Curcumin and Berberine (González-Montoya et al., 2018; Jhong, Riyaphan, Lin, Chia, & Weng, 2015; Z. Yu et al., 2012).

For the α-glucosidase assay, the inhibition values at 10 min were used to compare the effect of four fractions, as 10 min was the longest time that oat peptides generated an inhibitory activity. The results in **Figure 2.6B** showed the α-glucosidase inhibitory effect for F1 and F2 since F3, and F4 show little or no inhibitory effect. The inhibitory effect of F1 at a low concentration of 25 μg/mL was 19.7 %, which decreased with increasing concentration to 75 and 125 μg/mL. The inhibitory effect of F2 was 33.4 ± 1.8% at the highest concentration of 125 μg/mL, suggesting that the oat peptide fractions with more hydrophilic amino acids might better inhibit α-glucosidase. It has been reported that the α-glucosidase inhibitory effect ranged from 27 to 33% at 1000 μg/mL for peptide fractions generated from germinated soy protein

hydrolysate (González-Montoya et al., 2018). An inhibitory effect of 25% was observed for alcalase treated hemp seed protein hydrolysates at a concentration of 100 µg/mL (Ren et al., 2016). As a positive control, acarbose showed an α-glucosidase inhibitory effect of 37.1 % at a concentration of 125 µg/mL, which is in accordance with findings reported in the literature (Lordan, Smyth, Soler-Vila, Stanton, & Paul Ross, 2013; Moon et al., 2011). In our study, oat peptide fractions showed a comparable or higher α-glucosidase inhibitory effect when compared to peptides from other sources and were also comparable to the positive control acarbose. Previous studies report that the hydrophobicity of peptide and α-glucosidase inhibitory effect were poorly correlated (Ibrahim et al., 2018b). Therefore, the study of the peptide sequences in these fractions may allow a deeper understanding of the factors that contribute to better α-glucosidase inhibition effects.

F1, F2, F3, and F4 all exhibited DPP-IV inhibitory effects. Especially, F3 showed an inhibitory effect of 43.3 % at a relatively low concentration of 100 ug/mL **Figure 2.6C**. Further increasing concentration to 500 µg/mL led to an increased inhibitory effect of 78.0 %. The high inhibitory effect observed for F3 and F4 suggests that hydrophobic amino acids strongly contribute to peptides' DPP-IV inhibition. The existence of some hydrophilic amino acids might further improve the peptide bioactivity as F3 shows the highest inhibitory effect. Nongonierma & FitzGerald (2019) also reported that the hydrophobic or aromatic amino acids in peptide sequences contributed to DPP-IV inhibition. Moreover, structural analysis of known DPP-IV inhibitors suggests their capacity to bind to the active site of the enzyme by hydrogen bonding and hydrophobic interactions (Chakraborty et al., 2014). Thus, it

is believed that the exposure of hydrophobic and aromatic amino acids, obtained by the hydrolysate of low M_W (1-5 kDa), contributed to the strong capacity of oat peptides to inhibit DPP-IV and the fractionation by RP-HPLC to concentrate the peptides with DPP-IV inhibitory effects further.

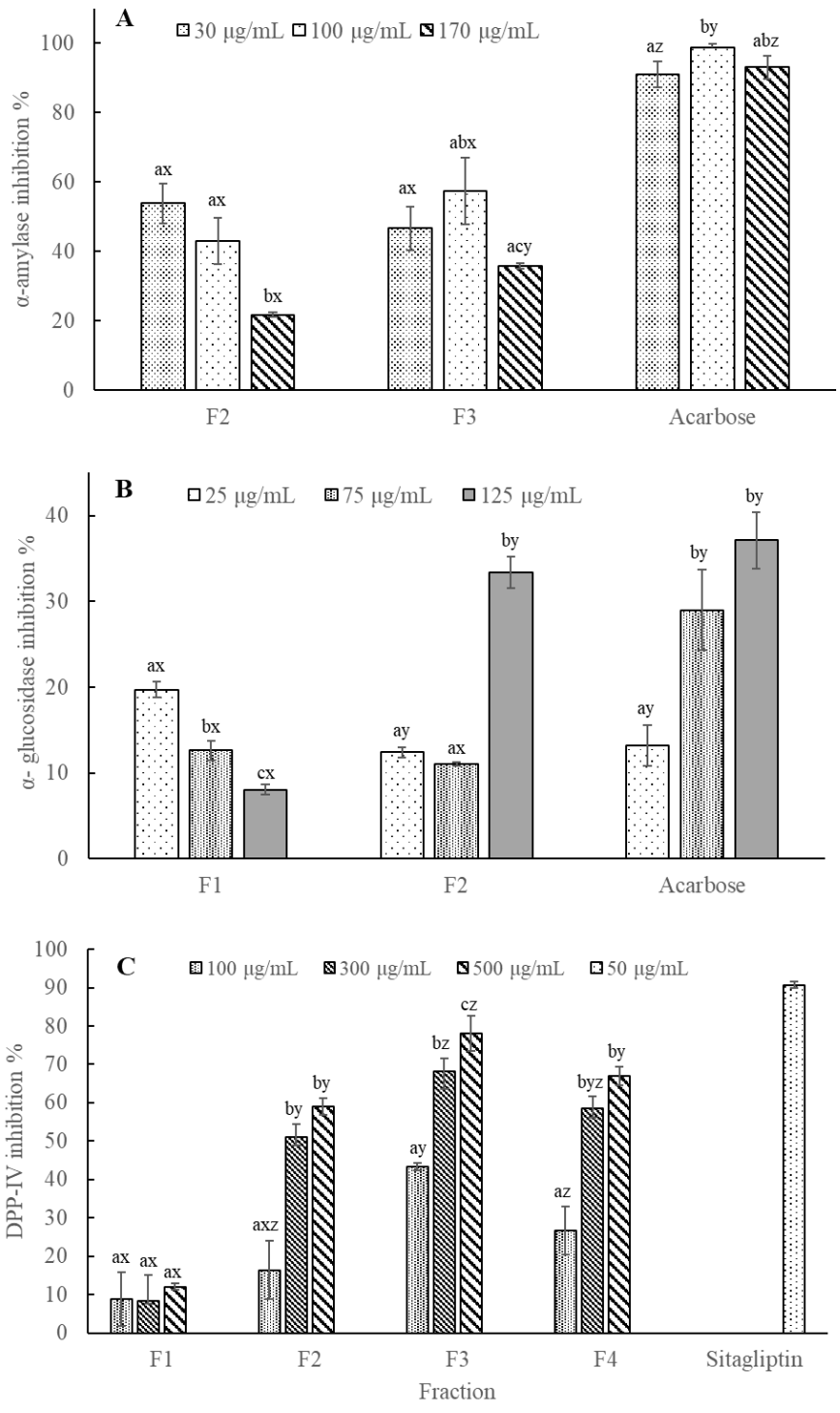


Figure 2.6 α-Amylase inhibition by fraction 2 (F2), fraction 3 (F3), and the control, Acarbose (A); α-glucosidase inhibition by fraction 1 (F1), fraction 2 (F2), and Acarbose after 10 min incubation (B); DPP-IV inhibitory effect by fraction 1 (F1),

fraction 2 (F2), fraction 3 (F3), fraction 4 (F4), and the control, sitagliptin. Different letters (a-c) on top of the bars represent significant difference between concentrations of the same fraction *(p <0.05)*. Different letters (x-z) on top of the bars represent significant difference between fractions at the same concentrations *(p <0.05)*

2.3.4 Amino acid content and peptide sequencing of the effective fractions

The amino acid compositions of oat protein and the fractions from its AHF hydrolysates are shown in **Table 2.1**. Glx (Glu + Gln) and Asx (Asp + Asn) decreased significantly after hydrolysis; this is consistent with the fact that both acidic and basic subunits of oat globulin are susceptible to endo and exopeptidases, as these oat polypeptides are rich in Glu and Asp, respectively (Burgess, Shewry, Matlashewski, Altosaar, & Miflin, 1983b; Liu et al., 2009b; Nieto-Nieto et al., 2014). Another study demonstrated that flavourzyme caused an increased exposure of aromatic amino acids, which is in line with the observed increment in Phe and Tyr in the AFH samples in our study (Walters et al., 2020). Alcalase, on the other hand, has preferential cleavage before and after hydrophobic amino acids (Esfandi, Willmore, & Tsopmo, 2018), which explains the significant increase in hydrophobic amino acids (e.g. Val, Ile, Leu, Phe) in the AFH fractions (53.9% and 47.5%) compared to the original oat protein (41.5%) *(p <0.05)*. The increased aromatic and hydrophobic amino acids provide a possible explanation of the improved inhibitory effects after enzymatic hydrolysis, together with reduced M_W to expose active groups.

For obtention of α-amylase inhibitory effect, it is considered important for the peptide chain to contain a Pro residue at the N- or C-terminus or both, as well as Gly or Phe at the N-terminus and Phe or Leu at the C-terminus (Ngoh & Gan, 2016). De

novo sequencing of our sample also identified new sequences (**Table 2.2**) varying from 4 to 7 amino acid peptides with similar characteristics in amino acid content and position. For example, Phe-Pro-Leu-Leu-Gln (FPLLQ), Phe-Pro-Leu-Leu-Phe (FPLLF), and Phe-Pro-Leu-Leu-Leu (FPLLL) all have Phe at the N-terminus and the presence of Leu and Phe at the C-terminus. Moreover, two peptide sequences of 8 amino acids each were identified from F3: Gly-Asp-Val-Val-Ala-Leu-Pro-Ala (GDVVALPA) and Asp-Val-Val-Ala-Leu-Pro-Ala-Gly (DVVALPAG). Both peptides are constituted by hydrophobic amino acids and one negatively charged amino acid (Asp). The high amount of Val, Ala, and Leu observed in the amino acid composition of samples (**Table 2.1**) matches their appearing frequency in the identified sequences. Reported sequences from seaweed Gly-Gly-Ser-Lys (GGSK) and Glu-Leu-Ser (ELS) exhibited a non-competitive type of inhibition when binding to the allosteric site of α-amylase, thus, creating conformational changes in the enzyme's structure and modifying the affinity to its substrate (Arise, 2016; Powers & Whitaker, 1978). These findings suggest that Glu_{pyro}-Val-Phe-Gly-Lys (E_{pyro}VFGK) peptide from F3 possess the ability to inhibit α-amylase due to the existence of similar amino acids in the sequences that have shown to bind the enzyme, although the exact mechanisms of action are yet to be investigated.

Table 2.1 Amino acid composition of oat protein and the fractions from its AHF hydrolysates

Residue	Oat protein	<1kDa Fraction	1-5 kDa Fraction
Asx*	8.47 ± 0.09 $_a$	5.86 ± 0.04$_b$	6.21 ± 0.01$_c$
Ser	5.86 ± 0.04$_{ab}$	5.68 ± 0.08$_a$	5.93 ± 0.01$_b$
Glx*	21.97 ± 0.09$_a$	13.48 ± 0.11$_b$	15.40 ± 0.09$_c$
Gly	7.52 ± 0.08$_a$	7.46 ± 0.09$_a$	7.40 ± 0.05$_a$
His	2.19 ± 0.03$_a$	3.03 ± 0.04$_b$	2.81 ± 0.02$_c$
Arg	6.39 ± 0.16$_a$	3.24 ± 0.07$_b$	4.73 ± 0.02$_c$
Thr	4.01 ± 0.01$_a$	4.38 ± 0.06$_b$	4.25 ± 0.03$_b$
Ala	6.90 ± 0.01$_a$	8.20 ± 0.10$_b$	7.01 ± 0.13$_a$
Pro	6.59 ± 0.14$_a$	3.72 ± 0.09$_b$	4.86 ± 0.03$_c$
Cys	2.61 ± 0.07$_a$	n.d.	1.30 ± 0.05$_c$
Tyr	3.45 ± 0.08$_a$	4.51 ± 0.01$_b$	3.75 ± 0.08$_c$
Val	6.92 ± 0.03$_a$	10.18 ± 0.15$_b$	8.62 ± 0.16$_c$
Met	1.94 ± 0.06$_a$	2.75 ± 0.01$_b$	2.13 ± 0.01$_c$
Lys	3.53 ± 0.02$_a$	2.20 ± 0.09$_b$	3.19 ± 0.07$_c$
Ile	4.54 ± 0.06$_a$	6.76 ± 0.12$_b$	6.08 ± 0.16$_c$
Leu	8.39 ± 0.00$_a$	11.77 ± 0.05$_b$	10.13 ± 0.07$_c$
Phe	5.31 ± 0.10$_a$	6.80 ± 0.35$_{ab}$	6.20 ± 0.12$_b$

*Asx includes aspartate + asparagine and Glx includes glutamine + glutamate. Different letters represent significant difference *($p < 0.05$)*.

For the α-glucosidase assay, molecular docking studies suggest that an albumin peptide Lys-Leu-Pro-Gly-Phe (KLPGF) is comparable to acarbose as a positive control for α-glucosidase inhibition with similar IC_{50} values of 33 and 39 mg/mL, respectively (Z. Yu et al., 2012). A similar pattern was observed in the effective sequences identified in this study. For example, Leu-Pro-Pro-Gln-Leu (LPPQL), Phe-Pro-Leu-Leu-Gln (FPLLQ), and Leu-Pro-Glu-Leu-Gln (LPELQ) (**Table 2**) both contain Leu-Pro or Pro-Leu as found in other peptides registered as α-glucosidase inhibitors (Minkiewic, Ivaniak, & Darewicz, 2019)

It was noticed that F2 from oat protein exhibited a fair degree of inhibition compared to that of acarbose (IC_{50} 137.7±23.2 μg/mL). According to an analysis of structural properties, α-glucosidase inhibition seems to rely more on the presence of hydrogen bonding. Thus, amino acids such as Ser, Thr, Tyr, Lys, or Arg at the N-terminus and/or a Pro at the penultimate position of the C-terminus and/or Met or Ala at the ultimate chain's position would contribute to the α-glucosidase inhibitory effect (Ibrahim et al., 2018b). This may explain some of the sequences identified from the oat protein fractions with high α-glucosidase inhibition effects, such as Tyr-Pro-Thr-Asn-Thr-Tyr (YPTNTY) and Gly-Asp-Val-Val-Ala-Leu-Pro-Ala (GDVVALPA). These sequences contained Tyr in both sides of the chain, and Thr, Ala, Pro in the suggested position and near the middle of the structure as pointed by Nishioka, Watanabe, Kawabata, & Ryoya (1997).

Diprotin A and B are strong DPP-IV inhibitors with IC_{50} values of 1.1 and 5.5 μg/mL with amino acid sequences: Ile-Pro-Ile and Val-Pro-Leu, respectively, as reported by Umezawa et al. (1984). Interestingly, de novo peptide sequencing of F3 disclosed the presence of sequences such as Leu-Pro-Val-Asp-Val (LPVDV), Leu-Pro-Leu-Pro-Gln (LPLPQ), and Tyr-Pro-Thr-Asn-Thr-Tyr (YPTNTY) (**Table 2**), which resemble the known inhibitors in that they both contain Val, Pro, and Leu in their N-terminal structures, and Pro in the chain's second position. In addition, dipeptides Leu-Pro and Ile-Pro have demonstrated to be some of the main DPP-IV inhibitors present in rice bran, and Tyr-Pro was found as an inhibitor in milk protein (Hatanaka et al., 2012; Nongonierma & Fitzgerald, 2014). Therefore, we can speculate that the presence of these dipeptides in oat peptide sequences such as Gly-

Asp-Val-Val-Ala-Leu-Pro-Ala (GDVVALPA) and Asp-Val-Val-Ala-Leu-Pro-Ala-Gly (DVVALPAG) might have greatly contributed to the AFH antidiabetic activity. Studies on binding modes of DPP-IV inhibitors to their active site showed that the presence of rings gave rigidity to gliptins structures; for instance, the cyclopropane component to the general cyanopyrrolidine structure allowed saxagliptin to hydrophobically interact at the S1 subsite of the enzyme (Nabeno et al., 2013). Therefore, it is believed that some of the peptide sequences structures obtained (**Figure 2.7**) may have contributed to the inhibitory activity due to the presence of rings and bulky groups that could provide rigidity to the peptide structures for better interaction with DPP-IV. Consequently, the cyclic structure in the pyro-glutamic acid found in the sequence Glu_{pyro}-Val-Phe-Gly-Lys (E_{pyro}VFGK) and other aromatic and cyclic amino acids like Tyr, Pro, and Phe, might have contributed to hydrophobic interactions and hydrogen bonding for DPP-IV inhibition. It is known that the ligand-Sitagliptin mechanism of action consists of hydrogen bonding and hydrophobic interactions at the active site of the enzyme, which gives it the characteristic of a competitive inhibitor (Chakraborty et al., 2014; Nongonierma & FitzGerald, 2019).

Due to its abundance in oat protein, Pro content is high in the identified sequences in our study, and it is possible that those peptides with antidiabetic effects could better resist gut enzymatic digestion; thus, maintaining their stability and bioavailability. Yet, the release of the smaller peptides from the identified sequences in this study after gut digestion needs to be investigated and their antidiabetic activities. Moreover, the peptides with antidiabetic activities identified have a relatively small M_W, with most of them having a 5 amino acid length. Thus the

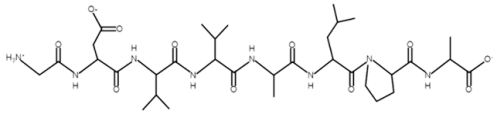
absorption of those peptides is possible through specific peptide transporters, paracellular transport, or transcytosis route (Oseguera-Toledo et al., 2014). In order to understand the underlying mechanisms of oat peptides digestion, absorption, and interactions with DPP-IV, studies using molecular docking as well as cell models and *in vivo* studies are required.

Table 2.2 Identification of amino acid sequences from the most potent oat peptide fractions by LC-MS/MS analysis with average local confidence > 90%.

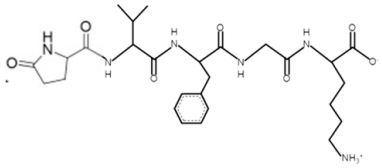
Fraction	Peptide	Tag Length	ALC (%)	m/z	RT	Mass
1 kDa	LPVDVL	6	97	655.44	18.71	654.40
	LPKYQ	5	96	648.37	13.5	647.36
	LPPQL	5	96	567.38	15.73	566.34
	E(-18.01)LFGK	5	95	575.32	22.42	574.31
	APGAGVY	7	95	634.35	13.21	633.31
	LPQYQ	5	95	648.37	13.58	647.33
	FPLLQ	5	94	617.39	18.14	616.36
	FPTLN	5	94	591.35	16.57	590.31
	FPLLF	5	94	636.41	22.44	635.37
	FPLLN	5	94	603.38	17.95	602.34
	LLVVLL	6	92	669.46	19.89	668.48
	FPLLL	5	92	602.43	21.69	601.38
	LPAL	4	91	413.30	16.27	412.27
	LPVL	4	90	441.34	17.41	440.30
	LSPLF	5	90	576.38	18.58	575.33
F2 (1-5 kDa)	LPPQL	5	93	567.41	14.70	566.34
	FPLLQ	5	92	617.44	17.73	616.36
	LPELQ	5	91	599.39	14.44	598.33
F3 (1-5 kDa)	GDVVALPA	8	*	741.48	16.12	740.41
	DVVALPAG	8	*	741.48	14.85	740.41
	YPTNTY	6	95	758.41	13.09	757.33
	DFPVY	5	94	640.35	18.42	639.29
	E(-18.01)VFGK	5	92	561.31	19.11	560.30
	LPVDV	5	91	542.33	15.24	541.31
	LPLPQ	5	90	567.37	18.05	566.34

Table 2.3 Concentration inducing 50% inhibition (IC50) of targeted enzymes.

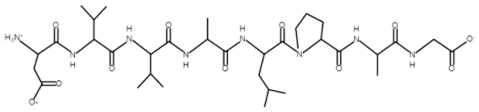
Enzyme	IC_{50} (µg/mL)	RP-HPLC Fraction
α-Amylase	101.0±51.7	F3
α-Glucosidase	274.9±44.8	F2
DPP-IV	178.7±19.9	F3



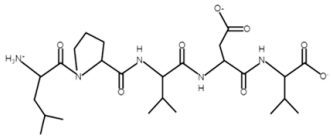
GDVVALPA



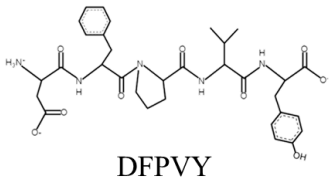
E_{pyro}VFGK



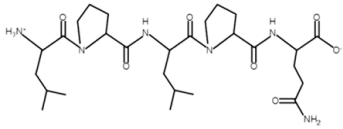
DVVALPAG



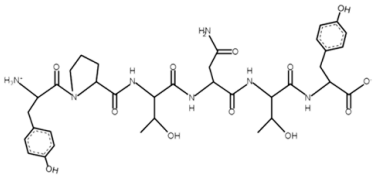
LPVDV



DFPVY



LPLPQ



YPTNTY

Figure 2.7 Peptide sequences primary structures found in F3 RP-HPLC oat sample using PepDraw tool at http://www.tulane.edu/~biochem/WW/PepDraw/.

2.4 Conclusion

This study found that peptides from oat protein, through continuous hydrolysis using alcalase and flavourzyme, successfully inhibited enzymes like DPP-IV, α-amylase, and α-glucosidase. Ultrafiltration and RP-HPLC fraction techniques were effective to concentrate DPP-IV and α-amylase inhibitory peptides from low M_W hydrolysates. LC-MS/MS analysis disclosed the presence of two 8 amino acid sequences from the most effective fractions, identified as GDVVALPA and DVVALPAG, as well as 25 new de novo sequences rich in hydrophobic and aromatic amino acids and proline. The results suggest that proline and hydrophobic amino acids play a crucial inhibitory role and may favor hydrophobic interactions and hydrogen bonding at the active site of these enzymes. Our data indicate that it is possible to generate antidiabetic peptides from oat protein which target three of the most studied enzymes for glucose regulation; a result that casts a new light for the study of oat peptides and their future in pharma- and nutraceutical applications for T2DM management. However, future research is needed to fully understand the interactions and mechanisms of action of the obtained peptide samples and potentially explore their *in vivo* effects. The presence of small amounts of other compounds like phytates and tannins in oat protein could impact the inhibitory effect, worthy of investigation in the future. Overall, oat is a good source of plant protein to generate peptides with antidiabetic activities. This knowledge may allow the industry to implement bioactive peptides in "diabetic-friendly" foods and general products in addition to oat β-glucans and phenolic compounds.

Chapter 3- Conclusion and recommendations

3.1 Summary and Conclusions

Type 2 diabetes is a worldwide issue that has represented a great challenge to overcome. Initiatives to reduce the burden of this condition have resulted in creating strategies to provide people with diabetes better life conditions. To provide an efficient treatment for diabetes, the generation of pharmacological agents to improve insulin secretion has shown great effectivity. However, the increased number of people living with diabetes is expected to increase in the future years. The prevalence of this condition in a 10-year time frame (2012-2022) for the Canadian population will represent a total health care cost of $15.36 billion, from which medications represent almost 17% of these costs (Bilandzic & Rosella, 2017). Moreover, adherence to treatments is influenced by many factors, including costs and the number of medications prescribed and their side effects. Diminishing risks of disease and complications is important on a population level, and most risk factors are related to lifestyle choices, so dietary modifications and exercise play a key role in preventing and treating type 2 diabetes. Besides, increasing physical activity and improving dietary habits are relatively low risk, low cost, and widely accessible actions toward diabetes management. Therefore, efforts in developing “diabetes-friendly” food products based on bioactive peptides from food protein have gained interest in regulating glucose metabolism in the last decade.

Although oat is considered to have health beneficial properties, most of its production is directed to animal feed applications, whereas human consumption of oats is still

low. Great part of the healthy benefits of oats is related to cholesterol and glucose regulating effects attributed to phenolic compounds and oat fiber components like β-glucans. Oat is also an important source of essential amino acids. It has been reported that oat protein can generate glucose regulating effects; however, research on this topic is still limited. Hence, the exploration of oat protein and its capacity to generate bioactive peptides with antidiabetic effects was investigated in this study.

Oat protein was first hydrolyzed using alcalase and assessed for its antidiabetic activity by inhibiting α-amylase, α-glucosidase, and DPP-IV enzymes. AH inhibitory effects showed a glimpse of the potential biological activity of oats hydrolysates; however, some of the concentrations assayed did not present a significant difference in their effect. Therefore, to generate better interactions with the enzymes with increased exposure to amino acid residues, continuous hydrolysis using alcalase and flavourzyme together with separation of fractions was done through ultrafiltration with M_W cut-off values of 5 and 1 kDa. The increased inhibitory effect was observed for α-amylase and DPP-IV, going from a 49.5% to 56% using the ≤1 kDa fraction at 100 μg/ml for α-amylase, and from 48.7% to 56.2% using the 1-5 kDa fraction at 500 μg/ml for DPP-IV. In contrast, α-glucosidase did not show an obvious inhibitory effect for AFH fractions. However, separation of the 1-5 kDa AFH into 4 fractions (F1, F2, F3, and F4) by RP-HPLC fractionation based on different hydrophobicity leads to an increased inhibitory effect, especially F3 for the inhibition of α-amylase and DPP-IV, and F2 for α-glucosidase. Positive controls like acarbose showed an inhibitory effect of 98.7% and 37.1% for α-amylase and α-glucosidase at 100 μg/mL and 125 μg/mL, respectively; likewise, sitagliptin showed 90.1% inhibitory effect for

the same for DPP-IV at 50 µg/mL. However, the highest inhibitory effects for α-amylase, α-glucosidase, and DPP-IV enzymes were: 57.3% at 100 µg/mL, 33.4% at 125 µg/mL, and 78.0% at 500 µg/mL, respectively. These results highlight the considerable effects that oat peptides can yield at relatively low concentrations compared to well-known enzyme inhibitors.

Peptide sequencing through LC-MS/MS analysis of the most active sequences allowed for a deeper understanding of the mechanisms of action of the obtained peptides and the role of certain amino acids situated in specific positions on the peptide chain. The obtained de novo sequences and the derived peptides from 12S oat globulin (Gly-Asp-Val-Val-Ala-Leu-Pro-Ala and Asp-Val-Val-Ala-Leu-Pro-Ala-Gly) showed that the presence of amino acids like Leu, Pro, Thr, Tyr, Lys, Gly, Phe, and Ala play an important role in the antidiabetic effect of oat peptides. Some of these residues may allow interactions with the target enzymes via hydrogen bonding and hydrophobic interactions at the active or allosteric sites of these enzymes. Our research show that oat peptides can inhibit three of the main targeted enzymes for glucose regulation in diabetes research.

3.2 Significance of research

This thesis is among the first research to prove that oat can be used as an ideal source of plant protein to generate peptides with antidiabetic activities with good potential for the management of type 2 diabetes. Hydrolyzing enzymes for the generation of effective peptides is an important method for the obtention of low molecular weight peptides and the exposure of active residues. This research demonstrated that oat protein hydrolysis using a combination of alcalase and flavourzyme and membrane

ultrafiltration enhances the enzymatic inhibitory effect compared to the effect of alcalase hydrolysate alone. This will allow future works to consider the use of endo and exopeptidases for an improved breakdown of oat protein into bioactive peptides. This is also the first research to systematically investigate the antidiabetic activities of oat protein hydrolysate fractions separated based on their different M_W and hydrophobicity through ultrafiltration and RP-HPLC. The antidiabetic effect was studied by inhibition of α-amylase, α-glucosidase, and DPP-IV, demonstrating that M_W difference and hydrophobicity of peptides impacted the inhibitory effect of the targeted enzymes with significant difference between some fractions and used concentrations. The achieved results may allow the industry to focus on the concentration of peptides with desirable structures to be used as antidiabetic ingredients in food applications. For instance, enzymes like DPP-IV and α-amylase showed better affinity to a more hydrophobic fraction such as F3; thus, the concentration of this particular fraction could provide the potential capacity to target two main mechanisms of action together, while these mechanisms are generally found in anti-diabetogenic drugs as two different classifications: DPP-IV inhibitors and α-amylase inhibitors.

LC-MS/MS analysis displayed in total 27 new *de novo* sequences from the most effective fractions. These findings suggest that peptides' mechanisms of action might rely on interactions with the active site or the targeted enzymes' allosteric sites. Disclosure of these sequences represents an opportunity to investigate their antidiabetic effect individually and fully understand the role of specific amino acids occupying certain positions within the peptide chain and the mechanisms involved in

their bioactivity. This research considers that fractionation and concentration of the most efficient sequence will promote an antidiabetic effect even if administered at a low dosage, being more efficient than the intake of its non fractionated protein version or oats per se. Some of the most effective sequences could favor the development of food products with low glycemic index characteristics since peptides' bioactivity could prevent starch breakdown into absorbable monosaccharides.

Overall, the present findings provide an insight into the creation of more natural antidiabetic agents derived from important plant protein sources like oats. Moreover, this research could lead to the use of peptides in a wider range of food product formulations destined for diabetes improvement and prevention. Likewise, this knowledge generates the opportunity to develop new value-added products to increase oat consumption as part of a healthy diet and promote additional revenue for the oat production and processing industry.

3.3 Recommendations

This research mainly focused on in vitro studies of oat peptides inhibitory effects through enzymatic assays; however, other in vitro studies in cellular and animal models could better demonstrate the role of bioactive peptides in more complex and physiological systems. It is also of great interest to better elucidate the inhibition mechanisms and interactions between the obtained peptides sequences and know more about their affinity to the enzyme's sites to classify their type of inhibition. Therefore, the recommendation for molecular docking *in silico* or kinetic assays could show how bioactive peptides bind to α-amylase, α-glucosidase, and DPP-IV for future work. Likewise, additional research is needed to understand how oat components like

polyphenols, fibers, and starch present in the oat protein extract could influence or interfere during the enzymatic inhibition process.

In addition, various sequences reported in this work might be resistant to gastrointestinal digestion due to its content in Pro content. In that regard, simulated gastrointestinal digestion of the obtained sequences can provide crucial information about the peptides' bioavailability and bioactivity. Ensuring that peptides with main activity at the intestinal brush border remain intact after digestion is essential to generate an antidiabetic effect. This provides a good starting point for developing delivery systems where bioactive peptides can be encapsulated to endure harsh gastrointestinal conditions. Future validation of the obtained antidiabetic effects in clinical trials will ensure their effectiveness and guarantee their use for its prospective food applications.

References

Abot, A., Cani, P. D., & Knauf, C. (2018, June 22). Impact of intestinal peptides on the enteric nervous system: Novel approaches to control glucose metabolism and food intake. *Frontiers in Endocrinology*, Vol. 9. https://doi.org/10.3389/fendo.2018.00328

Abot, A., Lucas, A., Bautzova, T., Bessac, A., Fournel, A., Le-Gonidec, S., … Knauf, C. (2018). Galanin enhances systemic glucose metabolism through enteric Nitric Oxide Synthase-expressed neurons. *Molecular Metabolism*, Vol. 10, pp. 100–108. https://doi.org/10.1016/j.molmet.2018.01.020

ADA. (2014). Diagnosis | ADA. Retrieved April 14, 2020, from American Diabetes Association website: https://www.diabetes.org/a1c/diagnosis

Admassu, H., Gasmalla, M. A. A., Yang, R., & Zhao, W. (2018). Identification of Bioactive Peptides with α-Amylase Inhibitory Potential from Enzymatic Protein Hydrolysates of Red Seaweed (Porphyra spp) [Research-article]. *Journal of Agricultural and Food Chemistry*, *66*(19), 4872–4882. https://doi.org/10.1021/acs.jafc.8b00960

Advanced Food Products - Oat Services Ltd. (2017). Retrieved November 20, 2020, from https://www.oat.co.uk/oat/food/

Aggarwal, A., & Drewnowski, A. (2019). Plant-and animal-protein diets in relation to sociodemographic drivers, quality, and cost: findings from the Seattle Obesity Study. *Am J Clin Nutr*, *110*, 451–460. https://doi.org/10.1093/ajcn/nqz064

Ahnen, R. T., Jonnalagadda, S. S., & Slavin, J. L. (2019). Role of plant protein in nutrition, wellness, and health. *Nutrition Reviews*, *77*(11), 735–747. https://doi.org/10.1093/nutrit/nuz028

Ahrén, B. (2007). DPP-4 inhibitors. *Best Practice and Research in Clinical Endocrinology and Metabolism*, *21*(4), 517–533. https://doi.org/10.1016/j.beem.2007.07.005

Alberti, K. G. M. M., Zimmet, P., & Shaw, J. (2006). Metabolic syndrome-a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabetic Medicine*, *23*(5), 469–480. https://doi.org/10.1111/j.1464-

5491.2006.01858.x

Alexander, C., Swanson, K. S., Fahey, G. C., & Garleb, K. A. (2019). Perspective: Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation. *Advances in Nutrition*, *10*(4), 576–589. https://doi.org/10.1093/advances/nmz004

Andriotis, V. M. E., Rejzek, M., Rugen, M. D., Svensson, B., Smith, A. M., & Field, R. A. (2016). Iminosugar inhibitors of carbohydrate-active enzymes that underpin cereal grain germination and endosperm metabolism. *Biochemical Society Transactions*, *44*, 159–165. https://doi.org/10.1042/BST20150222

Arendt, E. K., & Zannini, E. (2013). Cereal Grains for the Food and Beverage Industries. In *Cereal Grains for the Food and Beverage Industries*. https://doi.org/10.1533/9780857098924

Arise, R. O. (2016). In vitro antioxidant and α-amylase inhibitory properties of watermelon seed protein hydrolysates. *Environmental and Experimental Biology*, *14*(4), 163–172. https://doi.org/10.22364/eeb.14.23

Awosika, T. O., & Aluko, R. E. (2019). Inhibition of the in vitro activities of α-amylase, α-glucosidase and pancreatic lipase by yellow field pea (Pisum sativum L.) protein hydrolysates. *International Journal of Food Science and Technology*, *54*(6), 2021–2034. https://doi.org/10.1111/ijfs.14087

Bagchi, D., & Sreejayan, N. (2012). Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome. In *Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome*. https://doi.org/10.1016/C2010-0-66168-2

Bashary, R., Vyas, M., Nayak, S. K., Suttee, A., Verma, S., Narang, R., & Khatik, G. L. (2019). An Insight of Alpha-amylase Inhibitors as a Valuable Tool in the Management of Type 2 Diabetes Mellitus. *Current Diabetes Reviews*, *16*(2), 117–136. https://doi.org/10.2174/1573399815666190618093315

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., & Whelton, P. K. (2003). Dietary fiber intake and reduced risk of coronary heart disease in US men and women: The National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study. *Archives of Internal Medicine*, *163*(16), 1897–1904.

https://doi.org/10.1001/archinte.163.16.1897

Beck, E. J., Tapsell, L. C., Batterham, M. J., Tosh, S. M., & Huang, X. F. (2009). Increases in peptide Y-Y levels following oat beta-glucan ingestion are dose-dependent in overweight adults. *Nutrition Research (New York, N.Y.)*, *29*(10), 705–709. https://doi.org/10.1016/j.nutres.2009.09.012

Becker, L. C., Bergfeld, W. F., Belsito, D. V, Hill, R. A., Klaassen, C. D., Liebler, D. C., … Heldreth, B. (2019). Safety Assessment of Avena sativa (Oat)-Derived Ingredients As Used in Cosmetics. *International Journal of Toxicology*, *38*(3_suppl), 23S-47S. https://doi.org/10.1177/1091581819889904

Berdanier, C., Dwyer, J., & Heber, D. (2013). Handbook of Nutrition and Food, Third Edition. In *Handbook of Nutrition and Food, Third Edition*. https://doi.org/10.1201/b15294

Berger, J. P., SinhaRoy, R., Pocai, A., Kelly, T. M., Scapin, G., Gao, Y.-D., … Carr, R. D. (2018). A comparative study of the binding properties, dipeptidyl peptidase-4 (DPP-4) inhibitory activity and glucose-lowering efficacy of the DPP-4 inhibitors alogliptin, linagliptin, saxagliptin, sitagliptin and vildagliptin in mice. *Endocrinology, Diabetes & Metabolism*, *1*(1), e00002. https://doi.org/10.1002/edm2.2

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

Biörklund, M., van Rees, A., Mensink, R. P., & Önning, G. (2005). Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with β-glucans from oats or barley: A randomised dose-controlled trial. *European Journal of Clinical Nutrition*, *59*(11), 1272–1281. https://doi.org/10.1038/sj.ejcn.1602240

Bleakley, S., Hayes, M., O' Shea, N., Gallagher, E., & Lafarga, T. (2017). Predicted Release and Analysis of Novel ACE-I, Renin, and DPP-IV Inhibitory Peptides from Common Oat (Avena sativa) Protein Hydrolysates Using in Silico Analysis. *Foods*, *6*(12), 108. https://doi.org/10.3390/foods6120108

Blundell, J. E., & Bellisle, F. (2013). Satiation, satiety and the control of food intake. Theory and practice. In *Satiation, satiety and the control of food intake. Theory and practice*. Retrieved from https://eds-a-ebscohost-com.login.ezproxy.library.ualberta.ca/eds/ebookviewer/ebook/bmxlYmtfXzY3MTAxM19fQU41?sid=1c00fce4-ae99-4414-b04e-ac7e7c820ad6@pdc-v-sessmgr03&vid=4&format=EB&rid=1

Bozbulut, R., & Sanlier, N. (2019). Promising effects of β-glucans on glyceamic control in diabetes. *Trends in Food Science and Technology*, *83*(October 2018), 159–166. https://doi.org/10.1016/j.tifs.2018.11.018

Brayer, G. D., Luo, Y., & Withers, S. G. (1995). The structure of human pancreatic *α* -amylase at 1.8 Å resolution and comparisons with related enzymes. *Protein Science*, *4*(9), 1730–1742. https://doi.org/10.1002/pro.5560040908

Brückner-Gühmann, M., Banovic, M., & Drusch, S. (2019). Towards an increased plant protein intake: Rheological properties, sensory perception and consumer acceptability of lactic acid fermented, oat-based gels. *Food Hydrocolloids*, *96*(May), 201–208. https://doi.org/10.1016/j.foodhyd.2019.05.016

Brückner-Gühmann, M., Benthin, A., & Drusch, S. (2019). Enrichment of yoghurt with oat protein fractions: Structure formation, textural properties and sensory evaluation. *Food Hydrocolloids*, *86*, 146–153. https://doi.org/10.1016/j.foodhyd.2018.03.019

Burgess, S. R., Shewry, P. R., Matlashewski, G. J., Altosaar, I., & Miflin, B. J. (1983a). Characteristics of oat (Avena sativa L.) seed globulins. *Journal of Experimental Botany*, *34*(10), 1320–1332. https://doi.org/10.1093/jxb/34.10.1320

Burgess, S. R., Shewry, P. R., Matlashewski, G. J., Altosaar, I., & Miflin, B. J. (1983b). Characteristics Of Oat (Avena sativa L.) Seed Globulins. In *Journal of Experimental Botany* (Vol. 34). Retrieved from https://academic.oup.com/jxb/article/34/10/1320/497818

Butt, M. S., Tahir-Nadeem, M., Khan, M. K. I., Shabir, R., & Butt, M. S. (2008). Oat: Unique among the cereals. *European Journal of Nutrition*, *47*(2), 68–79. https://doi.org/10.1007/s00394-008-0698-7

Calbet, J. A. L., & Maclean, D. A. (2002). *Human Nutrition and Metabolism Plasma Glucagon and Insulin Responses Depend on the Rate of Appearance of Amino Acids after Ingestion of Different Protein Solutions in Humans 1,2*. Retrieved from https://academic.oup.com/jn/article/132/8/2174/4687709

Campbell, L. K., White, J. R., & Campbell, R. K. (1996). Acarbose: Its role in the treatment of diabetes mellitus. *Annals of Pharmacotherapy*, Vol. 30, pp. 1255–1262. https://doi.org/10.1177/106002809603001110

Casals-Casas, C., & Desvergne, B. (2011). Endocrine Disruptors: From Endocrine to Metabolic Disruption. *Annual Review of Physiology*, *73*(1), 135–162. https://doi.org/10.1146/annurev-physiol-012110-142200

Chakraborty, C., Hsu, M. J., & Agoramoorthy, G. (2014). Understanding the Molecular Dynamics of Type-2 Diabetes Drug Target DPP-4 and its Interaction with Sitagliptin and Inhibitor Diprotin-A. *Cell Biochemistry and Biophysics*, *70*(2), 907–922. https://doi.org/10.1007/s12013-014-9998-0

Cheung, I. W. Y., Nakayama, S., Hsu, M. N. K., Samaranayaka, A. G. P., & Li-Chan, E. C. Y. (2009). Angiotensin-I Converting Enzyme Inhibitory Activity of Hydrolysates from Oat (Avena sativa) Proteins by In Silico and In Vitro Analyses. *J. Agric. Food Chem*, *57*, 9234–9242. https://doi.org/10.1021/jf9018245

Choudhuri, S., & Chanderbhan, R. F. (2016). Models and Mechanisms in Evaluation of the Biology of Nutrients : Genetic and Molecular Principles. In *Nutraceuticals : efficacy, safety and toxicity* (pp. 209–225).

Cicero, A. F. G., Fogacci, F., & Colletti, A. (2017). Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review. *British Journal of Pharmacology*, *174*(11), 1378–1394. https://doi.org/10.1111/bph.13608

Comerford, K. B., & Pasin, G. (2016). Emerging evidence for the importance of dietary protein source on glucoregulatory markers and type 2 diabetes: Different effects of dairy, meat, fish, egg, and plant protein foods. *Nutrients*, *8*(8). https://doi.org/10.3390/nu8080446

de Carvalho, K. M. B., Pizato, N., Botelho, P. B., Dutra, E. S., & Gonçalves, V. S. S.

(2020, September 1). Dietary protein and appetite sensations in individuals with overweight and obesity: a systematic review. *European Journal of Nutrition*, Vol. 59, pp. 2317–2332. https://doi.org/10.1007/s00394-020-02321-1

de Sales, P. M., de Souza, P. M., Simeoni, L. A., Magalhães, P. de O., & Silveira, D. (2012, January 25). α-amylase inhibitors: A review of raw material and isolated compounds from plant source. *Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 15, pp. 141–183. https://doi.org/10.18433/j35s3k

de Souza Rocha, T., Hernandez, L. M. R., Mojica, L., Johnson, M. H., Chang, Y. K., & González de Mejía, E. (2015). Germination of Phaseolus vulgaris and alcalase hydrolysis of its proteins produced bioactive peptides capable of improving markers related to type-2 diabetes in vitro. *Food Research International*, *76*(P1), 150–159. https://doi.org/10.1016/j.foodres.2015.04.041

Dia, V. P., & Mejia, E. G. de. (2010). Lunasin promotes apoptosis in human colon cancer cells by mitochondrial pathway activation and induction of nuclear clusterin expression. *Cancer Letters*, *295*(1), 44–53. https://doi.org/10.1016/j.canlet.2010.02.010

Dickinson, E. (2003, January 1). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, Vol. 17, pp. 25–39. https://doi.org/10.1016/S0268-005X(01)00120-5

Drucker, D. J., & Nauck, M. A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *The Lancet*, *368*(9548), 1696–1705. https://doi.org/10.1016/S0140-6736(06)69705-5

Dun, X. P., Wang, J. H., Chen, L., Lu, J., Li, F. F., Zhao, Y. Y., … Bergman, T. (2007). Activity of the plant peptide aglycin in mammalian systems. *FEBS Journal*, *274*(3), 751–759. https://doi.org/10.1111/j.1742-4658.2006.05619.x

Environment and Climate Change Canada. (2019). Greenhouse Gas Sources and Sinks: Executive Summary 2019. Retrieved April 21, 2020, from aem website: https://www.canada.ca/en/environment-climate-change/services/climate-change/greenhouse-gas-emissions/sources-sinks-executive-summary-2019.html#toc3

Esfandi, R., Willmore, W. G., & Tsopmo, A. (2018). *Peptidomic analysis of hydrolyzed oat bran proteins, and their in vitro antioxidant and metal chelating properties*. https://doi.org/10.1016/j.foodchem.2018.11.110

Family, A., Ripsin, C. M., Kang, H., & Urban, R. J. (2009). *Management of Blood Glucose in Type 2 Diabetes Mellitus* (Vol. 79). Retrieved from www.aafp.org/afp

Fauser, F., & Jonikas, M. (2018). *Plant Chemical Genomics*. https://doi.org/10.1017/CBO9781139021500

Fowler, J., & Silverberg, N. (2008). Active Naturals Have a Key Role in Atopic Dermatitis. *Seminars in Cutaneous Medicine and Surgery*, *27*(3 SUPPL. 1), 8–10. https://doi.org/10.1016/S1085-5629(08)70005-1

Franco, O. L., Rigden, D. J., Melo, F. R., & Grossi-de-Sá, M. F. (2002). Plant α-amylase inhibitors and their interaction with insect α-amylases: Structure, function and potential for crop protection. *European Journal of Biochemistry*, *269*(2), 397–412. https://doi.org/10.1046/j.0014-2956.2001.02656.x

Futterer, T., Rush, A., Meyer, K., & Crider, C. C. (2019). Assessment of skin microbiome diversity and skin health in dry skin and dry, itchy skin: A bilateral, controlled clinical trial using oat-containing lotions and wash. *Journal of the American Academy of Dermatology*, *81*(4), AB23. https://doi.org/10.1016/j.jaad.2019.06.121

García-Pérez, L.-E., ´ Lvarez •, M. A., Dilla, T., Gil-Guillén, V., Orozco-Beltrán, D., García-Pérez, L.-E., … Dilla, Á. T. (2013). Adherence to Therapies in Patients with Type 2 Diabetes. *Diabetes Ther*, *4*, 175–194. https://doi.org/10.1007/s13300-013-0034-y

Ghani, U. (2020). Cyclitols and miscellaneous inhibitors. In *Alpha-Glucosidase Inhibitors* (pp. 137–161). https://doi.org/10.1016/b978-0-08-102779-0.00006-x

Goldbach, L., Vermeulen, B. J. A., Caner, S., Liu, M., Tysoe, C., Van Gijzel, L., … Jongkees, S. A. K. (2019). Folding Then Binding vs Folding through Binding in Macrocyclic Peptide Inhibitors of Human Pancreatic α-Amylase. *ACS Chemical Biology*, *14*(8), 1751–1759. https://doi.org/10.1021/acschembio.9b00290

Goldenberg, R. M., & Face, F. (n.d.). Management of Unmet Needs in Type 2 Diabetes Mellitus: The Role of Incretin Agents. In *Canadian Journal of*

Diabetes.

González-Montoya, M., Hernández-Ledesma, B., Mora-Escobedo, R., & Martínez-Villaluenga, C. (2018). Bioactive peptides from germinated soybean with anti-diabetic potential by inhibition of dipeptidyl peptidase-IV, α-amylase, and α-glucosidase enzymes. *International Journal of Molecular Sciences*, *19*(10). https://doi.org/10.3390/ijms19102883

Gorissen, S. H. M., Crombag, J. J. R., Senden, J. M. G., Waterval, W. A. H., Bierau, J., Verdijk, L. B., & van Loon, L. J. C. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids*, *50*(12), 1685–1695. https://doi.org/10.1007/s00726-018-2640-5

Gorrell, M. D. (2005). Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clinical Science*, *108*(4), 277–292. https://doi.org/10.1042/CS20040302

Goudarzi, M., & Madadlou, A. (2013). Influence of whey protein and its hydrolysate on prehypertension andpostprandial hyperglycaemia in adult men. *International Dairy Journal*, *33*(1), 62–66. https://doi.org/10.1016/j.idairyj.2013.06.006

Gough, S. (2016). Handbook of incretin-based therapies in type 2 diabetes. In *Handbook of Incretin-based Therapies in Type 2 Diabetes*. https://doi.org/10.1007/978-3-319-08982-9

Greenfield, J. R., Farooqi, I. S., Keogh, J. M., Henning, E., Habib, A. M., Blackwood, A., … Gribble, F. M. (2009). Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese, and type 2 diabetic subjects. *American Journal of Clinical Nutrition*, *89*(1), 106–113. https://doi.org/10.3945/ajcn.2008.26362

Habibi Zarabadi, M., Kadivar, M., & Keramat, J. (2018). Production and evaluation the properties of laminated oat protein film and electrospun nylon. *Journal of Food Processing and Preservation*, *42*(2). https://doi.org/10.1111/jfpp.13513

Hall, J. E. (2016a). Insulin, Glucagon, and Diabetes Mellitus- ClinicalKey. In *Guyton and Hall Textbook of Medical Physiology* (pp. 983–999). Retrieved from https://www-clinicalkey-com.login.ezproxy.library.ualberta.ca/#!/content/book/3-s2.0-

B9781455770052000792?indexOverride=GLOBAL

Hall, J. E. (2016b). Metabolism of Carbohydrates and Formation of Adenosine Triphosphate - ClinicalKey. In *Guyton and Hall Textbook of Medical Physiology* (p. 1 21). Retrieved from https://www-clinicalkey-com.login.ezproxy.library.ualberta.ca/#!/content/book/3-s2.0-B9781455770052000688?scrollTo=%23hl0000319

Han, R., Maycock, J., Murray, B. S., & Boesch, C. (2019). Identification of angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory peptides derived from oilseed proteins using two integrated bioinformatic approaches. *Food Research International*, *115*(May 2018), 283–291. https://doi.org/10.1016/j.foodres.2018.12.015

Hao, X., Li, J., Shi, Q., Zhang, J., He, X., & Ma, H. (2009). Characterization of a Novel Legumin α-Amylase Inhibitor from Chickpea (Cicer arietinum L.) Seeds. *Bioscience, Biotechnology, and Biochemistry*, *73*(5), 1200–1202. https://doi.org/10.1271/bbb.80776

Hatanaka, T., Inoue, Y., Arima, J., Kumagai, Y., Usuki, H., Kawakami, K., … Mukaihara, T. (2012). Production of dipeptidyl peptidase IV inhibitory peptides from defatted rice bran. *Food Chemistry*, *134*(2), 797–802. https://doi.org/10.1016/j.foodchem.2012.02.183

Health Canada. (2019). Canada's Dietary Guidelines for Health Professionals and Policy Makers. In *Health Canada*.

Hii, S. L., Tan, J. S., Ling, T. C., & Ariff, A. Bin. (2012). Pullulanase: Role in starch hydrolysis and potential industrial applications. *Enzyme Research*, Vol. 2012. https://doi.org/10.1155/2012/921362

Holst, J. J. (2003). Implementation of GLP-1 based therapy of type 2 diabetes mellitus using DPP-IV inhibitors. *Advances in Experimental Medicine and Biology*, *524*, 263–279. https://doi.org/10.1007/0-306-47920-6_33

Hopkins, M., Andrews, R., Salem, V., Taylor, R., Roux, C. W., Robertson, E., & Burns, E. (2020). Improving understanding of type 2 diabetes remission: research recommendations from Diabetes UK's 2019 remission workshop. *Diabetic Medicine*, *37*(11), 1944–1950. https://doi.org/10.1111/dme.14358

Hsieh, C. H., Wang, T. Y., Hung, C. C., Chen, M. C., & Hsu, K. C. (2015). Improvement of glycemic control in streptozotocin-induced diabetic rats by Atlantic salmon skin gelatin hydrolysate as the dipeptidyl-peptidase IV inhibitor. *Food and Function*, *6*(6), 1887–1892. https://doi.org/10.1039/c5fo00124b

Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G., & Willett, W. C. (2001). Diet, Lifestyle, and the Risk of Type 2 Diabetes Mellitus in Woman. In *The New England Journal of Medicine* (Vol. 345). Retrieved from www.nejm.org

Huang, S. L., Hung, C. C., Jao, C. L., Tung, Y. S., & Hsu, K. C. (2014). Porcine skin gelatin hydrolysate as a dipeptidyl peptidase IV inhibitor improves glycemic control in streptozotocin-induced diabetic rats. *Journal of Functional Foods*, *11*(C), 235–242. https://doi.org/10.1016/j.jff.2014.09.010

Ibrahim, M. A., Bester, M. J., Neitz, A. W. H., & Gaspar, A. R. M. (2018a). Structural properties of bioactive peptides with α-glucosidase inhibitory activity. *Chemical Biology and Drug Design*, *91*(2), 370–379. https://doi.org/10.1111/cbdd.13105

Ibrahim, M. A., Bester, M. J., Neitz, A. W. H., & Gaspar, A. R. M. (2018b). Structural properties of bioactive peptides with α-glucosidase inhibitory activity. *Chemical Biology and Drug Design*, *91*(2), 370–379. https://doi.org/10.1111/cbdd.13105

Idrees, S., Sarfraz, R. A., Riaz, M., Khera, R. A., Zia, A., Hanif, M. A., & Suleman, M. (2017). Evaluation of antidiabetic, antioxidant, antimicrobial and cytotoxicity properties of avena sativa roots extracts. *Oxidation Communications*, *40*(2), 613–623.

International Diabetes Federation. (2019). *IDF Diabetes Atlas*.

Jhong, C. H., Riyaphan, J., Lin, S. H., Chia, Y. C., & Weng, C. F. (2015). Screening alpha-glucosidase and alpha-amylase inhibitors from natural compounds by molecular docking in silico. *BioFactors*, *41*(4), 242–251. https://doi.org/10.1002/biof.1219

Ji, L. L., Lay, D., Chung, E., Fu, Y., & Peterson, D. M. (2003). Effects of avenanthramides on oxidant generation and antioxidant enzyme activity in exercised rats. *Nutrition Research*, *23*(11), 1579–1590. https://doi.org/10.1016/S0271-5317(03)00165-9

Jin, Y., Yan, J., Yu, Y., & Qi, Y. (2015). Screening and identification of DPP-IV inhibitory peptides from deer skin hydrolysates by an integrated approach of LC–MS/MS and in silico analysis. *Journal of Functional Foods*, *18*(1), 344–357. https://doi.org/10.1016/j.jff.2015.07.015

Jodayree, S., Smith, J. C., & Tsopmo, A. (2011). *Use of carbohydrase to enhance protein extraction efficiency and antioxidative properties of oat bran protein hydrolysates*. https://doi.org/10.1016/j.foodres.2011.12.004

Jönsso, B., Lund, M., & Barroso da Silva, F. L. (2007). 9.4.1 Interaction of Two Peptides - Knovel. In *Food Colloids- Self-Assembly and Material Science*. Retrieved from https://app-knovel-com.login.ezproxy.library.ualberta.ca/web/view/khtml/show.v/rcid:kpFCSAMS01/cid:kt00C6ZQE1/viewerType:khtml//root_slug:941-interaction-of-two-peptides/url_slug:interaction-two-peptides?kpromoter=federation&toc-within=peptides&b-toc-cid=

Jorge, S. S., Raúl, R. B., Isabel, G. L., Edith, P. A., Bernardo, E. B. H., César, A. P. J., … Rubén, R. R. (2015). Dipeptidyl peptidase iv inhibitory activity of protein hydrolyzates from Amaranthus hypochondriacus L. Grain and their influence on postprandial glycemia in streptozotocin-induced diabetic mice. *African Journal of Traditional, Complementary and Alternative Medicines*, *12*(1), 90–98. https://doi.org/10.4314/ajtcam.v12i1.13

Kalogeropoulou, D., LaFave, L., Schweim, K., Gannon, M. C., & Nuttall, F. Q. (2008). Leucine, when ingested with glucose, synergistically stimulates insulin secretion and lowers blood glucose. *Metabolism: Clinical and Experimental*, *57*(12), 1747–1752. https://doi.org/10.1016/j.metabol.2008.09.001

Kannan, A., Hettiarachchy, N. S., & Marshall, M. R. (2011). Food proteins and peptides as bioactive agents. *Bioactive Food Proteins and Peptides: Applications in Human Health*, 1–28. https://doi.org/10.1201/b11217-2

Khang, D. T., Vasiljevic, T., & Xuan, T. D. (2016). Bioactive compounds, antioxidant and enzyme activities in germination of oats (Avena sativa L.). *International Food Research Journal*, *23*(5), 1980–1987.

Kilara, A., & Panyam, D. (2003). Critical Reviews in Food Science and Nutrition

Peptides From Milk Proteins and Their Properties Peptides From Milk Proteins and Their Properties. *Critical Reviews in Food Science and Nutrition*, *43*(6), 607–633. https://doi.org/10.1080/10408690390251138

Kim, J. M., Noh, E. M., Kwon, K. B., Hwang, B. M., Hwang, J. K., You, Y. O., … Lee, Y. R. (2013). Dihydroavenanthramide D prevents UV-irradiated generation of reactive oxygen species and expression of matrix metalloproteinase-1 and -3 in human dermal fibroblasts. *Experimental Dermatology*, *22*(11), 759–761. https://doi.org/10.1111/exd.12243

Kumari, A. (2018). Chapter 1 – Glycolysis. *Sweet Biochemistry*, 1–5. https://doi.org/10.1016/B978-0-12-814453-4.00001-7

Kwon, Y. I. I., Vattem, D. A., & Shetty, K. (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition*, *15*(1), 107–118.

Lacroix, I. M. E., & Li-Chan, E. C. Y. (2013). *Inhibition of Dipeptidyl Peptidase (DPP)-IV and α-Glucosidase Activities by Pepsin-Treated Whey Proteins*. https://doi.org/10.1021/jf401000s

Lafarga, T., O'connor, P., & Hayes, M. (2014). Identification of novel dipeptidyl peptidase-IV and angiotensin-I-converting enzyme inhibitory peptides from meat proteins using in silico analysis. *Peptides*, *59*, 53–62. https://doi.org/10.1016/j.peptides.2014.07.005

Lammi, C., Zanoni, C., & Arnoldi, A. (2015). Three peptides from soy glycinin modulate glucose metabolism in human hepatic HepG2 cells. *International Journal of Molecular Sciences*, *16*(11), 27362–27370. https://doi.org/10.3390/ijms161126029

Lazaridou, A., & Biliaderis, C. G. (2007). Molecular aspects of cereal β-glucan functionality: Physical properties, technological applications and physiological effects. *Journal of Cereal Science*, *46*(2), 101–118. https://doi.org/10.1016/j.jcs.2007.05.003

Lebovitz, H. E. (1997). Alpha-Glucosidase Inhibitors. *Endocrinology and Metabolism Clinics of North America*, *26*(3), 539–551. https://doi.org/10.1016/S0889-8529(05)70266-8

Lebovitz, H. E., Irl Hirsch, M. R., & Vassello, J. (1997). *Current therapies for diabetes*.

Ley, S. H., Hamdy, O., Mohan, V., & Hu, F. B. (2014). Prevention and management of type 2 diabetes: Dietary components and nutritional strategies. *The Lancet*, Vol. 383, pp. 1999–2007. https://doi.org/10.1016/S0140-6736(14)60613-9

Li-Chan, E. C. Y., Hunag, S. L., Jao, C. L., Ho, K. P., & Hsu, K. C. (2012). Peptides derived from Atlantic salmon skin gelatin as dipeptidyl-peptidase IV inhibitors. *Journal of Agricultural and Food Chemistry*, *60*(4), 973–978. https://doi.org/10.1021/jf204720q

Li, X., Cai, X., Ma, X., Jing, L., Gu, J., Bao, L., … Li, Y. (2016). Short-and Long-Term Effects of Wholegrain Oat Intake on Weight Management and Glucolipid Metabolism in Overweight Type-2 Diabetics: A Randomized Control Trial. *Nutrients*, *8*, 549. https://doi.org/10.3390/nu8090549

Liu, G., Li, J. I., Shi, K. E., Wang, S. U., Chen, J., Liu, Y., & Huang, Q. (2009a). Composition, Secondary Structure, and Self-Assembly of Oat Protein Isolate. *J. Agric. Food Chem*, *57*, 4552–4558. https://doi.org/10.1021/jf900135e

Liu, G., Li, J. I., Shi, K. E., Wang, S. U., Chen, J., Liu, Y., & Huang, Q. (2009b). Composition, Secondary Structure, and Self-Assembly of Oat Protein Isolate. *J. Agric. Food Chem*, *57*, 4552–4558. https://doi.org/10.1021/jf900135e

Lordan, S., Smyth, T. J., Soler-Vila, A., Stanton, C., & Paul Ross, R. (2013). The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food Chemistry*, *141*(3), 2170–2176. https://doi.org/10.1016/j.foodchem.2013.04.123

Lu, J., Zeng, Y., Hou, W., Zhang, S., Li, L., Luo, X., … Xiang, M. (2012). The soybean peptide aglycin regulates glucose homeostasis in type 2 diabetic mice via IR/IRS1 pathway. *Journal of Nutritional Biochemistry*, *23*(11), 1449–1457. https://doi.org/10.1016/j.jnutbio.2011.09.007

Lu, Y., Lu, P., Wang, Y., Fang, X., Wu, J., & Wang, X. (2019). A novel dipeptidyl peptidase IV inhibitory tea peptide improves pancreatic β-cell function and reduces α-cell proliferation in streptozotocin-induced diabetic mice. *International Journal of Molecular Sciences*, *20*(2). https://doi.org/10.3390/ijms20020322

Ma, C. Y., & Harwalkar, V. R. (1984). Chemical Characterization and Functionality Assessment of Oat Protein Fractions. In *Tischer, W.; Strotmann, H. Biochim. Biophys. Acta* (Vol. 32). Retrieved from UTC website: https://pubs.acs.org/sharingguidelines

Ma, S., Zhang, M., Bao, X., & Fu, Y. (2020). Preparation of antioxidant peptides from oat globulin. *CYTA - Journal of Food*, *18*(1), 108–115. https://doi.org/10.1080/19476337.2020.1716076

Ma, X., Gu, J., Zhang, Z., Jing, L., Li, Y., Xu, M., … Li, Y. (2013). Effects of Avena nuda L. on metabolic control and cardiovascular disease risk among Chinese patients with diabetes and meeting metabolic syndrome criteria: Secondary analysis of a randomized clinical trial. *European Journal of Clinical Nutrition*, *67*(12), 1291–1297. https://doi.org/10.1038/ejcn.2013.201

Mäkinen, O. E., Sozer, N., Ercili-Cura, D., & Poutanen, K. (2016). Protein From Oat: Structure, Processes, Functionality, and Nutrition. *Sustainable Protein Sources*, 105–119. https://doi.org/10.1016/B978-0-12-802778-3.00006-8

Manders, R. J. F., Praet, S. F. E., Meex, R. C. R., Koopman, R., De Roos, A. L., Wagenmakers, A. J. M., … Van Loon, L. J. C. (2006). *Protein Hydrolysate/Leucine Co-Ingestion Reduces the Prevalence of Hyperglycemia in Type 2 Diabetic Patients*. https://doi.org/10.2337/dc06-1424

Marks, V. (2005). Glucose | Metabolism and maintenance of blood glucose level. In *Encyclopedia of Human Nutrition* (pp. 398–404). https://doi.org/10.1016/B0-12-226694-3/00153-8

Matsui, T., Oki, T., & Osajima, Y. (1999). Isolation and identification of peptidic α-glucosidase inhibitors derived from sardine muscle hydrolyzate. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *54*(3–4), 259–263. https://doi.org/10.1515/znc-1999-3-417

Matsui, T., Yoshimoto, C., Osajima, K., Oki, T., & Osajima, Y. (1996). In vitro survey of α–glucosidase inhibitory food components. *Bioscience, Biotechnology and Biochemistry*, *60*(12), 2019–2022. https://doi.org/10.1271/bbb.60.2019

Mendes, O., Koetzner, L., & Chen, J. (2018). Nutraceutical Impact on the Pathophysiology of Diabetes Mellitus. In *Nutritional and Therapeutic*

Interventions for Diabetes and Metabolic Syndrome (pp. 329–341). https://doi.org/10.1016/b978-0-12-812019-4.00026-x

Menon, R., Gonzalez, T., Ferruzzi, M., Jackson, E., Winderl, D., & Watson, J. (2016). *Oats — From Farm to Fork*. *77*.

Mentlein, R. (1999). Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regulatory Peptides*, *85*(1), 9–24. https://doi.org/10.1016/S0167-0115(99)00089-0

Minkiewic, P., Ivaniak, A., & Darewicz, M. (2019). Database of Bioactive Peptides: Current Opportunities. *International Journal of Molecular Sciences*, *20*. Retrieved from http://www.uwm.edu.pl/biochemia/index.php/pl/biopep

Mohamed, A., Biresaw, G., Xu, J., Hojilla-Evangelista, M. P., & Rayas-Duarte, P. (2009). Oats protein isolate: Thermal, rheological, surface and functional properties. *Food Research International*, *42*(1), 107–114. https://doi.org/10.1016/j.foodres.2008.10.011

Mojica, L., Luna-Vital, D. A., & González de Mejía, E. (2017). Characterization of peptides from common bean protein isolates and their potential to inhibit markers of type-2 diabetes, hypertension and oxidative stress. *Journal of the Science of Food and Agriculture*. https://doi.org/10.1002/jsfa.8053

Möller, N. P., Scholz-Ahrens, K. E., Roos, N., & Schrezenmeir, J. (2008). Bioactive peptides and proteins from foods: Indication for health effects. *European Journal of Nutrition*. https://doi.org/10.1007/s00394-008-0710-2

Moon, H. E., Islam, N., Ahn, B. R., Chowdhury, S. S., Sohn, H. S., Jung, H. A., & Choi, J. S. (2011). Protein Tyrosine Phosphatase 1B and α-Glucosidase Inhibitory Phlorotannins from Edible Brown Algae, Ecklonia stolonifera and Eiseniaï¿¿bicyclis. *Biotechnology, and Biochemistry*, *75*(8), 1472–1480. https://doi.org/10.1271/bbb.110137

Moran, T. H., & Dailey, M. J. (2011). *Intestinal feedback signaling and satiety*. https://doi.org/10.1016/j.physbeh.2011.02.005

Morato, P. N., Lollo, P. C. B., Moura, C. S., Batista, T. M., Camargo, R. L., Carneiro, E. M., & Amaya-Farfan, J. (2013). Whey Protein Hydrolysate Increases Translocation of GLUT-4 to the Plasma Membrane Independent of Insulin in

Wistar Rats. *PLoS ONE*, *8*(8), 853–859. https://doi.org/10.1371/journal.pone.0071134

Murphy, J. P., & Hoffman, L. A. (1992). The Origin, History, and Production of Oat. *H. Marshall, M. Sorrells (Eds.), Oat Science and Technology, American Society of Agronomy and Crop Science Society of America*, 1–28. https://doi.org/10.2134/agronmonogr33.c1

Nabeno, M., Akahoshi, F., Kishida, H., Miyaguchi, I., Tanaka, Y., Ishii, S., & Kadowaki, T. (2013). *A comparative study of the binding modes of recently launched dipeptidyl peptidase IV inhibitors in the active site*. https://doi.org/10.1016/j.bbrc.2013.03.010

Nahoum, V., Ve Roux, G. ', Ronique Anton, V. !, Rouge ! ‡, P., Puigserver, A., Bischoff, H., … Payan, F. (2000). Crystal structures of human pancreatic α-amylase in complex with carbohydrate and proteinaceous inhibitors. In *Biochem. J* (Vol. 346).

Naicker, K., Manuel, D., Øverland, S., Skogen, J. C., Johnson, J. A., Sivertsen, B., & Colman, I. (2018). Population attributable fractions for Type 2 diabetes: an examination of multiple risk factors including symptoms of depression and anxiety. *Diabetol Metab Syndr*, *10*, 84. https://doi.org/10.1186/s13098-018-0387-5

Nakajima, Y., Ito, K., & Yoshimoto, T. (2008). *Dipeptidyl-peptidase IV*. (2006), 3374–3379.

Nauck, M. A. (2009). Unraveling the Science of Incretin Biology. *AJM*, *122*(S3), S3–S10. https://doi.org/10.1016/j.amjmed.2009.03.012

Nelson, D., & Cox, M. (2005). *Lehninger Principles of Biochemistry* (4th editio). New York: W.H. Freeman.

Newsholme, P., Brennan, L., & Bender, K. (2006). *Amino Acid Metabolism,-Cell Function, and Diabetes*. https://doi.org/10.2337/db06-S006

Ngoh, Y.-Y., & Gan, C.-Y. (2016). Enzyme-assisted extraction and identification of antioxidative and a-amylase inhibitory peptides from Pinto beans (Phaseolus vulgaris cv. Pinto). *Food Chemistry*, (190), 331–337. https://doi.org/10.1016/j.foodchem.2015.05.120

Nieto-Nieto, T. V., Wang, Y. X., Ozimek, L., & Chen, L. (2014). Effects of partial hydrolysis on structure and gelling properties of oat globular proteins. *Food Research International*, *55*, 418–425. https://doi.org/10.1016/j.foodres.2013.11.038

Nishioka, T., Watanabe, J., Kawabata, J., & Ryoya, N. (1997). Isolation and Activity of N-p-Coumaroyltyramine, an α-Glucosidase Inhibitor in Welsh Onion (Allium fistulosum). *Bioscience, Biotechnology, and Biochemistry*, *61*(7), 1138–1141.

Nongonierma, A. B., & Fitzgerald, R. J. (2013). *Inhibition of dipeptidyl peptidase IV (DPP-IV) by proline containing casein-derived peptides*. https://doi.org/10.1016/j.jff.2013.09.012

Nongonierma, A. B., & Fitzgerald, R. J. (2014). *Susceptibility of milk protein-derived peptides to dipeptidyl peptidase IV (DPP-IV) hydrolysis*. https://doi.org/10.1016/j.foodchem.2013.08.097

Nongonierma, A. B., & FitzGerald, R. J. (2019). Features of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides from dietary proteins. *Journal of Food Biochemistry*, *43*(1), 1–12. https://doi.org/10.1111/jfbc.12451

Nongonierma, A. B., Paolella, S., Mudgil, P., Maqsood, S., & Fitzgerald, R. J. (2017). *Identification of novel dipeptidyl peptidase IV (DPP-IV) inhibitory peptides in camel milk protein hydrolysates*. https://doi.org/10.1016/j.foodchem.2017.10.033

Olokoba, A. B., Obateru, O. A., & Olokoba, L. B. (2012). Type 2 diabetes mellitus: A review of current trends. *Oman Medical Journal*, Vol. 27, pp. 269–273. https://doi.org/10.5001/omj.2012.68

Orsini Delgado, M. C., Tironi, V. A., & Añón, M. C. (2011). Antioxidant activity of amaranth protein or their hydrolysates under simulated gastrointestinal digestion. *LWT - Food Science and Technology*, *44*(8), 1752–1760. https://doi.org/10.1016/j.lwt.2011.04.002

Osain, M. (2018). Gastrointestinal physiology: Development, principles and mechanisms of regulation. In *Gastrointestinal Physiology: Development, Principles and Mechanisms of Regulation*. https://doi.org/10.1007/978-3-319-91056-7

Oseguera-Toledo, M. E., Gonzalez de Mejia, E., & Amaya-Llano, S. L. (2015). Hard-to-cook bean (Phaseolus vulgaris L.) proteins hydrolyzed by alcalase and bromelain produced bioactive peptide fractions that inhibit targets of type-2 diabetes and oxidative stress. *Food Research International*, *76*, 839–851. https://doi.org/10.1016/j.foodres.2015.07.046

Oseguera-Toledo, M. E., González de Mejía, E., Reynoso-Camacho, R., Cardador-Martínez, A., & Amaya-Llano, S. L. (2014). Proteins and bioactive peptides. *Nutrafoods*, *13*(4), 147–157. https://doi.org/10.1007/s13749-014-0052-z

Oseguera Toledo, M. E., Gonzalez de Mejia, E., Sivaguru, M., & Amaya-Llano, S. L. (2016). Common bean (Phaseolus vulgaris L.) protein-derived peptides increased insulin secretion, inhibited lipid accumulation, increased glucose uptake and reduced the phosphatase and tensin homologue activation in vitro. *Journal of Functional Foods*, *27*, 160–177. https://doi.org/10.1016/j.jff.2016.09.001

Owusu-Apenten, R. (2010). Bioactive peptides: Applications for improving nutrition and health. In *Bioactive Peptides: Applications for Improving Nutrition and Health*. https://doi.org/10.1201/EBK1439813621

Patil, P., Mandal, S., Tomar, S. K., & Anand, S. (2015). Food protein-derived bioactive peptides in management of type 2 diabetes. *European Journal of Nutrition*, Vol. 54, pp. 863–880. https://doi.org/10.1007/s00394-015-0974-2

Penders, J., Thijs, C., Vink, C., Stelma, F. F., Snijders, B., Kummeling, I., … Stobberingh, E. E. (2006). Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics*, *118*(2), 511–521. https://doi.org/10.1542/peds.2005-2824

Perez-Puyana, V., Felix, M., Romero, A., & Guerrero, A. (2016). *Short Communication Development of pea protein-based bioplastics with antimicrobial properties*. https://doi.org/10.1002/jsfa.8051

Peterson, D. M. (2011). Storage Proteins. In *Oats: Chemistry and Technology: Second Edition* (pp. 123–142). https://doi.org/10.1016/B978-1-891127-64-9.50013-0

Pick, M. E., Hawrysh, Z. J., Gee, M. I., Toth, E., Garg, M. L., & Hardin, R. T. (1996). Oat bran concentrate bread products improve long-term control of diabetes.pdf.

Journal of the American Dietetic Association, *96*(12), 1254–1261.

Poretsky, L. (2010). Principles of diabetes mellitus. In *Principles of Diabetes Mellitus*. https://doi.org/10.1007/978-0-387-09841-8

Powers, J. R., & Whitaker, J. R. (1978). Effect of Several Experimental Parameters on Combination of Red Kidney Bean (Phaseolus Vulgaris) α -amylase inhibitor with porcine pancreatic α -amylase. *Journal of Food Biochemistry*, *1*(3), 239–260. https://doi.org/10.1111/j.1745-4514.1978.tb00184.x

Prairie Oat Growers Association. (2021). Oat Grower Manual. Retrieved February 10, 2021, from https://poga.ca/about-oats

PrOatein oat protein | Lantmännen Oats. (2020). Retrieved November 21, 2020, from https://www.lantmannenoats.com/proatein/

Rains, T. M., & Maki, K. C. (2013). The Effects of Oats and β-Glucan on Blood Pressure and Hypertension. In *Oats Nutrition and Technology* (pp. 239–254). https://doi.org/10.1002/9781118354100.ch10

Raj, P., Ames, N., Joseph Thandapilly, S., Yu, L., & Netticadan, T. (2020). The effects of oat ingredients on blood pressure in spontaneously hypertensive rats. *Journal of Food Biochemistry*, *44*(10). https://doi.org/10.1111/jfbc.13402

Ren, Y., Liang, K., Jin, Y., Zhang, M., Chen, Y., Wu, H., & Lai, F. (2016). Identification and characterization of two novel α-glucosidase inhibitory oligopeptides from hemp (Cannabis sativa L.) seed protein. *Journal of Functional Foods*, Vol. 26, pp. 439–450. https://doi.org/10.1016/j.jff.2016.07.024

Roig-Zamboni, V., Cobucci-Ponzano, B., Iacono, R., Ferrara, M. C., Germany, S., Bourne, Y., … Sulzenbacher, G. (2017). Structure of human lysosomal acid α-glucosidase-A guide for the treatment of Pompe disease. *Nature Communications*, *8*(1). https://doi.org/10.1038/s41467-017-01263-3

Sabato, S. F., Ouattara, B., Yu, H., D'aprano, G., Le Tien, C., Mateescu, M. A., & Lacroix, M. (2001). *Mechanical and Barrier Properties of Cross-Linked Soy and Whey Protein Based Films*. https://doi.org/10.1021/jf0005925

Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S., & Mauri, A. N. (2010). Biodegradable sunflower protein films naturally activated with antioxidant

compounds. *Food Hydrocolloids*, *24*(5), 525–533. https://doi.org/10.1016/j.foodhyd.2009.12.002

Samocha-Bonet, D., Wong, O., Synnott, E.-L., Piyaratna, N., Douglas, A., Gribble, F. M., … Greenfield, J. R. (2011). Glutamine Reduces Postprandial Glycemia and Augments the Glucagon-Like Peptide-1 Response in Type 2 Diabetes Patients 1-3. *The Journal of Nutrition Biochemical, Molecular, and Genetic Mechanisms J. Nutr*, *141*, 1233–1238. https://doi.org/10.3945/jn.111.139824

Savoie, L., Agudelo, R. A., Gauthier, S. F., Marin, J., & Pouliot, Y. (2005). In vitro determination of the release kinetics of peptides and free amino acids during the digestion of food proteins. *Journal of AOAC International*, *88*(3), 935–948. https://doi.org/10.1093/jaoac/88.3.935

Seifert, R. (2019). Drugs for Treatment of Diabetes Mellitus. In *Basic Knowledge of Pharmacology* (pp. 241–251). https://doi.org/10.1007/978-3-030-18899-3_19

Shao, Y., & Tang, C. H. (2016). Gel-like pea protein Pickering emulsions at pH3.0 as a potential intestine-targeted and sustained-release delivery system for β-carotene. *Food Research International*. https://doi.org/10.1016/j.foodres.2015.11.025

Singh, B., & Kaur, A. (2016). Antidiabetic potential of a peptide isolated from an endophytic *Aspergillus awamori*. *Journal of Applied Microbiology*, *120*(2), 301–311. https://doi.org/10.1111/jam.12998

Smeets, A. J., Soenen, S., Luscombe-Marsh, N. D., Ueland, Ø., & Westerterp-Plantenga, M. S. (2008). Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *Journal of Nutrition*, *138*(4), 698–702. https://doi.org/10.1093/jn/138.4.698

Sosa Crespo, I., Laviada Molina, H., Chel-Guerrero, L., Ortiz-Andrade, R., & Betancur-Ancona, D. (2018). Inhibitory effect of peptide fractions derivatives from chia (Salvia hispanica) hydrolysis against α-amylase and α-glucosidase enzymes. *Nutr Hosp*, *35*(4), 928–935.

Strychar, R. (2011). World Oat Production, Trade, and Usage. In *Oats: Chemistry and Technology*. Retrieved from

https://books.google.ca/books?hl=en&lr=&id=HhIvDAAAQBAJ&oi=fnd&pg=PA1&dq=Canada+oat+production&ots=B5Tao6SBCu&sig=7ZUJG3Bu-n0JNiKogfd-sXJIYQY&redir_esc=y#v=onepage&q=Canada oat production&f=false

Tiefenbacher, K. F. (2017). Technology of Main Ingredients - Water and Flours -. In *Technology of Wafers and Waffles I*. Retrieved from https://app-knovel-com.login.ezproxy.library.ualberta.ca/web/view/khtml/show.v/rcid:kpTWWIOA01/cid:kt012EAGB4/viewerType:khtml//root_slug:2-technology-of-main-ingredients---water-and-flours/url_slug:technology-main-ingredients?kpromoter=federation&b-toc-c

Tiwari, U., & Cummins, E. (2011). Meta-analysis of the effect of β-glucan intake on blood cholesterol and glucose levels. *Nutrition*, *27*(10), 1008–1016. https://doi.org/10.1016/j.nut.2010.11.006

Tosh, S. M., & Miller, S. S. (2015). Oats. In *Encyclopedia of Food and Health* (pp. 119–125). https://doi.org/10.1016/B978-0-12-384947-2.00497-9

U.S. Department of Agriculture (USDA). (2019). FoodData Central. *FoodData Central*, p. fdc.nal.usda.gov. Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/1101825/nutrients

Umezawa, H., Aoyagi, T., Ogawa, K., Naganawa, H., Hamada, M., & Takeuchi, T. (1984). Diprotins A and B, inhibitors of dipeptidyl aminopeptidase IV, produced by bacteria. *The Journal of Antibiotics*, *37*, 422–425.

USDA, H. (2015). *2015 Dietary Guidelines Advisory Committee Report*.

Van Smoorenburg, A. N., Hertroijs, D. F. L., Dekkers, T., Elissen, A. M. J., & Melles, M. (2019). Patients' perspective on self-management: Type 2 diabetes in daily life. *BMC Health Services Research*, *19*(1). https://doi.org/10.1186/s12913-019-4384-7

Van Vliet, T., & Walstra, P. (2017). Dispersed systems basic considerations. In *Fennema's Food Chemistry* (pp. 467–539). https://doi.org/10.1201/9781315372914

Vanvi, A., & Tsopmo, A. (2016). Pepsin digested oat bran proteins: Separation, antioxidant activity, and identification of new peptides. *Journal of Chemistry*,

2016. https://doi.org/10.1155/2016/8216378

Velarde-Salcedo, A. J., Barrera-Pacheco, A., Lara-González, S., Montero-Morán, G. M., Díaz-Gois, A., González De Mejia, E., & Barba De La Rosa, A. P. (2013). In vitro inhibition of dipeptidyl peptidase IV by peptides derived from the hydrolysis of amaranth (Amaranthus hypochondriacus L.) proteins. *Food Chemistry*, *136*(2), 758–764. https://doi.org/10.1016/j.foodchem.2012.08.032

Walters, M. E., Willmore, W. G., & Tsopmo, A. (2020). Antioxidant, physicochemical, and cellular secretion of glucagon-like peptide-1 properties of oat bran protein hydrolysates. *Antioxidants*, *9*(6), 1–15. https://doi.org/10.3390/ANTIOX9060557

Wang, Bei, Atungulu, G. G., Khir, R., Geng, J., Ma, H., Li, Y., & Wu, B. (2015). Ultrasonic Treatment Effect on Enzymolysis Kinetics and Activities of ACE-Inhibitory Peptides from Oat-Isolated Protein. *Food Biophysics*, *10*(3), 244–252. https://doi.org/10.1007/s11483-014-9375-y

Wang, Bo, Xie, N., & Li, B. (2019). Influence of peptide characteristics on their stability, intestinal transport, and in vitro bioavailability: A review. *Journal of Food Biochemistry*, *43*(1), 1–6. https://doi.org/10.1111/jfbc.12571

Wang, F., Yu, G., Zhang, Y., Zhang, B., & Fan, J. (2015a). *Dipeptidyl Peptidase IV Inhibitory Peptides Derived from Oat (Avena sativa L.), Buckwheat (Fagopyrum esculentum), and Highland Barley (Hordeum vulgare trif urcatum (L.) Trofim) Proteins*. https://doi.org/10.1021/acs.jafc.5b04016

Wang, F., Yu, G., Zhang, Y., Zhang, B., & Fan, J. (2015b). Dipeptidyl Peptidase IV Inhibitory Peptides Derived from Oat (Avena sativa L.), Buckwheat (Fagopyrum esculentum), and Highland Barley (Hordeum vulgare trifurcatum (L.) Trofim) Proteins. *Journal of Agricultural and Food Chemistry*, *63*(43), 9543–9549. https://doi.org/10.1021/acs.jafc.5b04016

Wang, F., Zhang, Y., Yu, T., He, J., Cui, J., Wang, J., … Fan, J. (2018a). Oat globulin peptides regulate antidiabetic drug targets and glucose transporters in Caco-2 cells. *Journal of Functional Foods*, *42*(September 2017), 12–20. https://doi.org/10.1016/j.jff.2017.12.061

Wang, F., Zhang, Y., Yu, T., He, J., Cui, J., Wang, J., … Fan, J. (2018b). Oat globulin

peptides regulate antidiabetic drug targets and glucose transporters in Caco-2 cells. *Journal of Functional Foods*, *42*(November 2017), 12–20. https://doi.org/10.1016/j.jff.2017.12.061

Wang, J., Du, K., Fang, L., Liu, C., Min, W., & Liu, J. (2018). Evaluation of the antidiabetic activity of hydrolyzed peptides derived from Juglans mandshurica Maxim. fruits in insulin-resistant HepG2 cells and type 2 diabetic mice. *Journal of Food Biochemistry*, *42*(3), 1–10. https://doi.org/10.1111/jfbc.12518

Wang, P., Chen, H., Zhu, Y., McBride, J., Fu, J., & Sang, S. (2015). Oat Avenanthramide-C (2c) Is Biotransformed by Mice and the Human Microbiota into Bioactive Metabolites. *The Journal of Nutrition*, *145*(2), 239–245. https://doi.org/10.3945/jn.114.206508

Ward, O. P. (2019). Proteases. *Comprehensive Biotechnology*, *3*, 604–615. https://doi.org/10.1016/B978-0-444-64046-8.00187-7

Whitney, E. N., & Rolfes, S. R. (2013). *Understanding nutrition*. Wadsworth, Cengage Learning.

World Health Organization. (2007). Protein and Amino Acid Requirements in Human Nutrition Report of a joint FAO/WHO/UNU expert consultation (WHO Technical Report Series 935). In *World Health Organization Food and Agriculture Organization of the United Nations United Nations University*. Retrieved from www.who.int/bookorders

Wu, W., He, W., Tan, Y., Tian, Z., Chen, L., Hu, F.-Q., & Wu, W. (2011). Food protein-stabilized nanoemulsions as potential delivery systems for poorly water-soluble drugs: preparation, in vitro characterization, and pharmacokinetics in rats. *International Journal of Nanomedicine*, 521. https://doi.org/10.2147/ijn.s17282

Xia, Y., Wang, Y., & Chen, L. (2011). Molecular structure, physicochemical characterization, and in vitro degradation of barley protein films. *Journal of Agricultural and Food Chemistry*, *59*(24), 13221–13229. https://doi.org/10.1021/jf203157a

Xu, Y., Galanopoulos, M., Sismour, E., Ren, S., Mersha, Z., Lynch, P., & Almutaimi, A. (2020). Effect of enzymatic hydrolysis using endo- and exo-proteases on

secondary structure, functional, and antioxidant properties of chickpea protein hydrolysates. *Journal of Food Measurement and Characterization*, *14*(1), 343–352. https://doi.org/10.1007/s11694-019-00296-0

Yaghoubi, M., Mansell, K., Vatanparast, H., Steeves, M., Zeng, W., & Farag, M. (2019). Prevalence of Type 1 and Type 2 Diabetes-Related Complications and Their Association With Determinants Identified in Canada's Survey on Living With Chronic Diseases—Diabetes Component. *Canadian Journal of Diabetes*, *44*. https://doi.org/10.1016/j.jcjd.2019.09.001

Yan, W., Fetch, J. M., Frégeau-Reid, J., Rossnagel, B., & Ames, N. (2011). Genotype × location interaction patterns and testing strategies for oat in the Canadian prairies. *Crop Science*, *51*(5), 1903–1914. https://doi.org/10.2135/cropsci2010.09.0548

Yang, C., Wang, Y., & Chen, L. (2017). Fabrication, characterization and controlled release properties of oat protein gels with percolating structure induced by cold gelation. *Food Hydrocolloids*, *62*, 21–34. https://doi.org/10.1016/j.foodhyd.2016.07.023

Yang, C., Wang, Y., Lu, L., Unsworth, L., Guan, L. L., & Chen, L. (2018). Oat protein-shellac beads: Superior protection and delivery carriers for sensitive bioactive compounds. *Food Hydrocolloids*, *77*, 754–763. https://doi.org/10.1016/j.foodhyd.2017.11.017

Yibchok-Anun, S., Adisakwattana, S., Yao, C. Y., Sangvanich, P., Roengsumran, S., & Hsu, W. H. (2006). Slow acting protein extract from fruit pulp of Momordica charantia with insulin secretagogue and insulinomimetic activities. *Biological and Pharmaceutical Bulletin*. https://doi.org/10.1248/bpb.29.1126

Yu, G., Wang, F., Zhang, B., & Fan, J. (2016). In vitro inhibition of platelet aggregation by peptides derived from oat (Avena sativa L.), highland barley (Hordeum vulgare Linn. var. nudum Hook. f.), and buckwheat (Fagopyrum esculentum Moench) proteins. *Food Chemistry*, *194*, 577–586. https://doi.org/10.1016/j.foodchem.2015.08.058

Yu, Z., Yin, Y., Zhao, W., Liu, J., & Chen, F. (2012). Anti-diabetic activity peptides from albumin against α-glucosidase and α-amylase. *Food Chemistry*, *135*(3),

2078–2085. https://doi.org/10.1016/j.foodchem.2012.06.088

Yu, Z., Yin, Y., Zhao, W., Yu, Y., Liu, B., Liu, J., & Chen, F. (2011). Novel peptides derived from egg white protein inhibiting alpha-glucosidase. *Food Chemistry*, *129*(4), 1376–1382. https://doi.org/10.1016/j.foodchem.2011.05.067

Yue, J., Gu, Z., Zhu, Z., Yi, J., Ohm, J. B., Chen, B., & Rao, J. (2021). Impact of defatting treatment and oat varieties on structural, functional properties, and aromatic profile of oat protein. *Food Hydrocolloids*, *112*(June 2020). https://doi.org/10.1016/j.foodhyd.2020.106368

Zarkadas, C. G., Yu, Z., & Burrows, V. D. (1995). Protein Quality of Three New Canadian-Developed Naked Oat Cultivars Using Amino Acid Compositional Data. In *Food Chem* (Vol. 43). Retrieved from https://pubs.acs.org/sharingguidelines

Zhang, H., & Wang, J. (2015). Peptides Derived from Oats Improve Insulin Sensitivity and Lower Blood Glucose in Streptozotocin-Induced Diabetic Mice. *Journal of Biomedical Sciencies*, *04*(01), 1–7. https://doi.org/10.4172/2254-609x.100007

Zheng, Y., Wang, X., Zhuang, Y., Li, Y., Shi, P., Tian, H., … Chen, X. (2020). Isolation of novel ACE-inhibitory peptide from naked oat globulin hydrolysates *in silico* approach: Molecular docking, *in vivo* antihypertension and effects on renin and intracellular endothelin-1. *Journal of Food Science*, *85*(4), 1328–1337. https://doi.org/10.1111/1750-3841.15115

Zhong, L., Ma, N., Wu, Y., Zhao, L., Ma, G., Pei, F., & Hu, Q. (2019). Gastrointestinal fate and antioxidation of β-carotene emulsion prepared by oat protein isolate-Pleurotus ostreatus β-glucan conjugate. *Carbohydrate Polymers*, *221*(April), 10–20. https://doi.org/10.1016/j.carbpol.2019.05.085

Zwer, P. K. (2015). Oats: Overview. In *Encyclopedia of Food Grains: Second Edition* (Vol. 1–4, pp. 173–183). https://doi.org/10.1016/B978-0-12-394437-5.00013-9