STRUCTURE-FUNCTION PROPERTIES OF PEA PROTEIN-DERIVED PEPTIDES WITH INHIBITORY ACTIVITIES AGAINST GASTROINTESTINAL ENZYMES

By

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A Thesis

Submitted to the Faculty of Graduate Studies

of the University of Manitoba

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

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ABSTRACT

Obesity and Type 2 diabetes mellitus (T2DM) are chronic disease conditions of public health significance. An approach to combating these conditions is the inhibition of digestive enzymes. Synthetic compounds, such as Acarbose and Orlistat, have been developed for enzyme inhibition; however, they tend to possess negative side effects ranging from diarrhea to hepatotoxicity. With the global prevalence of both conditions, identification of alternative enzyme inhibitors becomes imperative. Compounds of natural sources (such as dietary components) are more desirable as they are thought to possess a lower risk of negative side effects when compared to the synthetic inhibitors. Therefore, the overall aim of this study was to produce yellow field pea protein-derived peptides that can inhibit activities of α-amylase, α-glucosidase, pancreatic lipase, trypsin and chymotrypsin, the main enzymes responsible for carbohydrate, protein and lipid digestion within the human gastrointestinal tract. Protein hydrolysates were produced via enzymatic hydrolysis of pea protein isolate using four different enzymes: alcalase, pepsin, trypsin, and chymotrypsin. Each hydrolysate was then fractionated into different peptide sizes (<1, 1-3, 3-5 and 5-10 kDa) by membrane ultrafiltration. The unfractionated hydrolysates and peptide fractions were analyzed for their ability to inhibit *in vitro* activities of the gastrointestinal enzymes. The mode and kinetics of enzyme inhibition were then determined using the most active peptide fractions. Results indicate that the fractionated peptides were better inhibitors of the digestive enzymes (except for pancreatic lipase) compared to the unfractionated hydrolysates. The mode of inhibition was identified to be non-competitive for α-glucosidase and competitive for α-amylase, trypsin, and chymotrypsin. Findings from this study suggest that pea-protein derived peptides have the potential to be developed into functional foods and/or nutraceuticals for management of caloric intake with respect to obesity and T2DM.

ACKNOWLEDGMENTS

First and foremost, I would like to thank the Almighty God for seeing me through the period of this program. I thank God for endowing me with strength, wisdom, knowledge, and understanding to complete this program.

My deepest appreciation goes to my supervisor, Dr. Rotimi Aluko, for taking a chance on me and giving me the opportunity to carry out my graduate program. I thank him for his guidance, unwavering support, and patience throughout the program. I will also like to acknowledge the contributions of my thesis committee members, Dr. Sijo Joseph and Dr. Athar Ata, for taking the time to read and evaluate this thesis.

I am thankful for the financial support of the sponsoring organizations: University of Manitoba and Natural Sciences and Engineering Research Council (NSERC).

Special thanks to my parents who have been pillars of strength. Thank you for your prayers and words of encouragement. I appreciate you both for always standing by me and making the process easy. God bless you.

My gratitude goes to Dr. Adeola Alashi for her guidance and to my lab colleagues for their support.

To my husband, Dr. Peter Awosika, and son, Joshua Awosika, thank you for the constant support and sacrifices you both had to make to enable me to complete my graduate program. Thank you for always being there for me and making everything worthwhile. I will always love you both forever and a day more.

DEDICATION

I dedicate this thesis to God Almighty who has enabled and seen me through this phase of my life.

I also dedicate this thesis to my son, Joshua, who was born in the course of my program. Thank you for always giving me a reason to laugh and filling my heart with joy.

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LIST OF ABBREVIATIONS

AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride
Asp	Aspartic acid
BMI	Body mass index
BSA	Bovine serum albumin
C4O6HKNa	Sodium potassium tartrate
CuSO4.5H20	Copper sulfate
Cacl2	Calcium chloride
DMSO	Dimethyl sulfoxide
EC	Enzyme Commission number
F	fraction
FPLC	Fast Protein Liquid Chromatography
GI	Glycemic index
GIT	Gastrointestinal tract
Gly	Glycine
HCl	Hydrochloric acid
HDL	High-density lipoprotein
LC-MS/MS	Liquid chromatography-tandem mass spectrometry

LDL	Low-density lipoprotein
Legumin MS	Legumin Minor small
LMW	Low Molecular Weight
Met	Methionine
MS	Mass spectrometry
MW	Molecular weight
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
RP-HPLC	Reverse-Phase High-Performance Liquid Chromatography
SDS	Sodium dodecyl sulfate
Ser	Serine
T2DM	Type 2 diabetes mellitus
Trp	Tryptophan
UV	Ultraviolet
VLDL	Very low-density lipoprotein
YFP	Yellow Field Pea
YFPP	Yellow Field Pea Protein

YPAH	Yellow Pea Alcalase Hydrolysate
YPCH	Yellow Pea Chymotrypsin Hydrolysate
YPP	Yellow Pea Protein
YPPI	Yellow Field Pea Protein isolate
YPPH	Yellow Pea Pepsin Hydrolysate
YPTH	Yellow Pea Trypsin Hydrolysate

1. INTRODUCTION

Obesity is a "condition characterized by the accumulation of fat that presents a risk to health" (WHO, 2017b). It is likely to be a result of the complex interaction (which is poorly understood) between genetics, dietary intake, physical activity, lifestyle and environmental factors, which results in long-term positive energy balance and ultimately in the increase of body fat mass; therefore obesity can be said to be a multi-factorial condition (Butland et al., 2007; Lau et al., 2007). However, from a biological point of view, obesity can be preventable as it is due to an imbalance between energy input and output (increase in intake of energy-dense foods & increase in physical inactivity) (WHO, 2017b). Obesity has been established as a risk factor for several chronic diseases such as type 2 diabetes mellitus, musculoskeletal disorders (osteoarthritis), hypertension; stroke, cardiovascular diseases, and certain types of cancers (endometrial, breast, ovarian, liver, gallbladder, colon, kidney and prostate), which are the leading causes of death worldwide (Kumanyika et al., 2002; Biro and Wien, 2010). Of importance is the association between obesity and type 2 diabetes mellitus (T2DM), "a condition characterized by increased concentrations of glucose in the blood" and has been identified as a global public health concern (WHO, 2017a). Both obesity and T2DM have been identified as public health concerns and termed global epidemics, in the sense that, the prevalence (number of individuals with these conditions) is still rapidly increasing despite evidence showing their association with adverse health effects. According to the World Health Organization, statistics reveal that as of 2014, 13% of the world's adult population were obese (with 11% being male and 15% being female) and that the global prevalence of diabetes in adults aged 18 years and over is 8.5%; in addition, 1.6 million deaths were

directly caused by diabetes mellitus (WHO, 2016; WHO, 2017a). In Canada, 20.2% of Canadians adults (18+ yrs), that is approximately 5.3 million adults were reported to be obese as of 2014 (Statistics Canada, 2015) and as of 2015, 2.1 million Canadians (12+ yrs) were reported to have diabetes (Statistics Canada, 2017). Both conditions were once considered to be a problem only in western countries; however, it is now seen to be rising in both low and middle-income countries (Caballero, 2007; WHO, 2016; WHO, 2017a). Despite current strategies to combat these conditions, they are still seen to be on the rise, therefore, there is a need to search for other approaches in their prevention and/or management, which would be beneficial and effective.

Obesity accounts for 80-85% of the risk of developing type 2 diabetes mellitus, the association between obesity and diabetes is strong such that a term called "diabesity" has been coined (Golay and Ybarra, 2005). Type 2 diabetes mellitus occurs as a result of a defect in insulin secretion together with a progressive rise in insulin resistance and is largely as a result of excess body weight and physical inactivity (Golay and Ybarra, 2005; WHO, 2017a). Obesity (increase in overall fatness, especially abdominal fat) is said to contribute to the development of diabetes mellitus via the release of pro-inflammatory chemicals by the fat cells, which in turn makes the body become less sensitive to insulin action. The insulin-sensitive cells and tissues become less responsive, thereby increasing insulin resistance, which is a hallmark of type 2 diabetes mellitus (Dandona et al., 2004; Golay and Ybarra, 2005). Body Mass Index (BMI) is the commonly used measure of body weight and method of detecting obesity; it is calculated by dividing a person's weight in kilograms by the square of their height in meters. Other measures include waist circumference, waist-hip ratio, and skinfold thickness. A person is said to be overweight when they have a BMI equal to or

greater than 25; obese if the BMI is equal or greater than 30 (Lunagariya et al., 2014; WHO, 2016). Obesity is further classified into Class 1 (BMI = 30-34.9); Class 2 (BMI = 35-39.9) and Class 3 (BMI ≥ 40) (Lunagariya et al., 2014). On the other hand, type 2 diabetes mellitus is diagnosed by random blood glucose concentration >11.1 mmol/L or fasting blood glucose >7.0 mmol/L (fasting = no caloric intake for at least 8 hours) or 2 hr blood glucose >11.1 mmol/L with oral glucose tolerance test or glycated hemoglobin (HbA1C) > 6.5% (Inzucchi, 2012).

Both conditions have similar prevention and management strategies, which includes eating a healthy diet, maintaining normal body weight and regular physical activities (WHO, 2016; WHO, 2017a). It has been established that a reduction in weight by 5-10% is beneficial to health and also with regards to type 2 diabetes mellitus; it has a significant impact in improving insulin sensitivity and thus may contribute in its management (Wing et al., 2011). The management of obesity includes lifestyle modifications (diet and physical activity); pharmacological therapy (e.g. orlistat, lorcaserin, sibutramine) and surgery (bariatric surgery, which is recommended for BMI equal to or greater than 40) (Lau et al., 2007; Seyedan et al., 2015). The management of type 2 diabetes mellitus includes lifestyle modifications and pharmacotherapy (e.g. acarbose, metformin) (Patil et al., 2015). Lifestyle modification is the first option for obesity and type 2 diabetes mellitus management, followed by pharmacotherapy (Lau et al., 2007). Pharmacotherapy produces modest weight loss; however, it comes with the issue of costs, compliance and side effects ranging from diarrhea to hepatotoxicity (Lunagariya et al., 2014; Seyedan et al., 2015; Tysoe et al., 2016). The use of bariatric surgery for the management of obesity is said to be the most effective, but comes with the issues of costs, being invasive and complications associated with surgeries (Lau et

al., 2007). However, from these current strategies, lifestyle modification remains the key element in the management of obesity as well as in type 2 diabetes mellitus because it aims at reducing energy intake and increasing energy expenditure (Lau et al., 2007).

It has been established that the use of dietary modification or nutritional intervention in the prevention and/or management of obesity and type 2 diabetes mellitus, in order to promote weight loss (which helps in reducing calorie intake and maintaining energy balance as well as maintaining a normal body weight) is an important element (Patil et al., 2015). An approach to the reduction of energy/calorie intake is the inhibition of digestive enzymes. Among existing treatments, the development of nutrient digestion and absorption inhibitors is considered an important strategy in the effort to decrease energy intake via gastrointestinal mechanisms (Kim et al., 2012; Patil et al., 2015). This strategy in managing diabetes mellitus includes suppressing the digestion of carbohydrates in the gut by the inhibition of carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase, in turn reducing the amount of glucose absorbed into the body (Lacroix and Li-Chan, 2013). This is because these enzymes including α-amylases (salivary amylase and pancreatic amylase) and α-glucosidases are involved in the metabolism of carbohydrates. α-amylases are responsible for the breakdown of complex dietary carbohydrates into oligosaccharides and disaccharides as they cleave α-1,4 glycosidic bonds. The resultant products are further degraded by α-glucosidase into absorbable monosaccharides such as glucose and fructose (Shinde et al., 2008; Tysoe et al., 2016). It has been argued that the inhibition of α-glucosidase is more important than that of α-amylase in the management of type 2 diabetes mellitus (Uraipong and Zhao, 2016b). This is because α-glucosidase is the key enzyme responsible for the production of glucose, which is the end product of carbohydrate digestion (Uraipong and

Zhao, 2016b; Ercan and El, 2016). Therefore, inhibition of α-glucosidase activity is considered to be an effective strategy for the control of diabetes by diminishing the amount of glucose available for absorption (Yu et al., 2011). The key enzyme responsible for the gastrointestinal digestion of dietary lipids is pancreatic lipase, which breaks down triacylglycerides into the absorbable products: 2-monoglyceride and free fatty acids (Kim et al., 2012; Seyedan et al., 2015). Inhibition of pancreatic lipase reduces the digestion of triacylglycerides and in turn the efficiency of fat absorption in the small intestine, and thereby initiates modest long-term reductions in body weight. Therefore, suppression or delay of dietary lipid digestion and absorption through inhibition of pancreatic lipase activity has been targeted for the development of anti-obesity agents because the enzyme is responsible for hydrolysis of 50–70% of total dietary fats in the human digestive system (Siow et al., 2016). The metabolism of dietary proteins involves a number of enzymes: pepsin, which is responsible for the partial hydrolysis of dietary proteins into a mixture of polypeptides, small oligopeptides and free amino acids in the stomach. Trypsin, chymotrypsin and carboxypeptidase function in the duodenum and further hydrolyze the pepsin products and dietary proteins into a mixture of smaller oligopeptides and free amino acids, which are then absorbed by the body (Erickson and Kim, 1990). Figure 1 illustrates the enzymes involved in nutrient digestion.

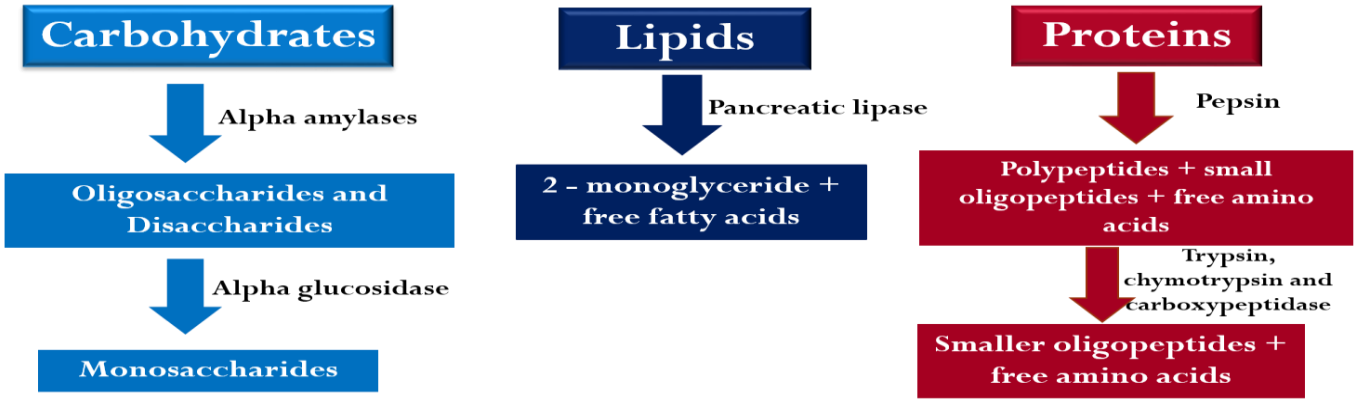

Figure 1. Key enzymes involved in nutrient digestion.

Although this research's main focus is on the inhibition of enzymes involved in the digestion of carbohydrates and lipids, the study is also interested in studying the inhibition of the enzymes involved in protein digestion as it is known that excessive protein intake can cause weight gain (Bray et al., 2012). This is because excess dietary amino acids are used to synthesize acetyl- CoA which is a precursor for the synthesis of triglycerides, which in turn is stored as fat in the body; on the other hand, the excess amino acids can be used as substrates for the production of glucose (gluconeogenesis) (Wu, 2013). In addition, high protein diet can stress or overwork the kidney as an increased breakdown of proteins produce high levels of ammonia or urea which are waste products the kidney has to remove from the body to prevent toxicity (Wu, 2009). Furthermore, for bioactive peptides to exert their effects, there is a need to preserve their bioactivity during the digestive process, as structural degradation by proteases during passage through the gastrointestinal tract can render them less active or even inactive (Segura-Campos et al., 2011; Aluko, 2012). Therefore, the inhibition of proteases such as trypsin and chymotrypsin serve as a strategy to reduce calorie intake as well as to preserve the structure and bioactivity of the bioactive peptides during passage through the gastrointestinal tract. To prevent this structural degradation, trypsin and

chymotrypsin inhibiting peptides could be added as adjuvants during oral feeding of the bioactive peptides.

Gastrointestinal enzyme inhibitors act via two mechanisms: (a) blocking the active site of the digestive enzyme so it cannot take up and bind to the substrate or (b) binding to the enzyme's non-active site causing a distortion in the shape of the enzyme, so that the substrate is unable to fit into the active site, in turn leading to reduced nutrient digestibility (Abd El-latif, 2015). The inhibition of a digestive enzyme catalysis leads to a reduced rate of nutrient digestion coupled with delayed nutrient digestion and absorption (increased overall digestion time). Since the dietary carbohydrates, proteins, and lipids are not rapidly broken down during enzyme inhibition, the food will have less caloric value than when enzyme inhibitors are absent (Patil et al., 2015). Therefore, inhibition of these enzymes constitutes a major approach to the management of obesity as well as type 2 diabetes mellitus.

With the continual increase in the global prevalence of obesity and type 2 diabetes mellitus, it is paramount to explore the development and production of anti-obesity and anti-diabetic agents from natural sources as an alternative strategy which would be effective, safe and have minimal side effects (Patil et al., 2015).

2. LITERATURE REVIEW

2.1 Bioactive Peptides

Introduction: Proteins are a source of energy and amino acids, which are essential for growth and maintenance; they are also known to carry a wide range of nutritional, functional and biological properties (Korhonen and Pihlanto, 2003). Proteins exert their physiological actions either directly or upon digestion *in vivo* or *in vitro*. Dietary proteins are a rich source of biologically active peptides and they possess specific biological properties which make them potential ingredients of functional or health-promoting foods (Korhonen and Pihlanto, 2006). Many of these properties are attributed to bioactive peptides encrypted within the sequence of the parent protein (Udenigwe and Aluko, 2012). Bioactive peptides can be defined as "specific protein fragments that have a positive impact on body functions or conditions and may ultimately influence health" (Kitts and Weiler, 2003). These peptides influence health in a number of ways and are known to have multifunctional properties (Meisel and FitzGerald, 2003). The bioactivity of a peptide is dependent primarily on its structural properties (e.g. chain length) and physicochemical characteristics of the amino acid residues (e.g., hydrophobicity, molecular charge) (Udenigwe and Aluko, 2012). Depending on the amino acid sequence and size, these peptides exert a number of different activities *in-vivo*, affecting the systems in the body e.g. cardiovascular, endocrine, immune and nervous systems in addition to nutrient utilization (Korhonen and Pihlanto, 2006).

Bioactive peptides can be used in the formulation of therapeutic products and have been identified as health-promoting agents against various human health and disease conditions (Aluko, 2008; Udenigwe and Aluko, 2012). Research shows that application of bioactive

peptides are preferred as therapeutic ingredients or health-promoting agents because they do not have the toxic or severe negative side effects that are associated with therapeutic drugs, even though they may have less physiological effectiveness (Aluko, 2008). The use of bioactive peptides in the formulation of diets or therapeutic products have advantages such as their low cost, safety and the additional nutritional benefits they provide (Sarmadi and Ismail, 2010). However, the function of these bioactive peptides is dependent on their structure as well as their absorption and bioavailability in the target tissues (Udenigwe and Aluko, 2012). Peptides are beneficial because they can cross the digestive epithelial barriers and travel to the site at which they have an impact (Li et al., 2006). Protein absorption in the form of short chain peptides is considered a more efficient method of amino acid absorption compared with an equivalent amount of free amino acids. This is due to the availability of peptide-specific transport systems and the fact that peptides are less hypertonic than free amino acids mixtures, enabling good absorption of other dietary components and eliminating osmotic problems (Clemente, 2000; Peñas et al., 2006).

There exists a wide range of food sources from which bioactive peptides have been identified. Examples include milk, eggs, gelatin, fish muscle, corn, wheat, rice, soy, meat, legumes, cereals, vegetables, marine products, seaweed, yeast and fungi (Korhonen and Pihlanto, 2006; Moughan et al., 2014). There are different types of bioactive peptides produced and this is as a result of the kind of food protein used to generate them; some food proteins may be rich sources of these peptides, while some are poor sources (Korhonen and Pihlanto, 2003). However, the most studied protein source shown to produce bioactive peptides is milk and the two most studied group of peptides identified from milk are the ACE-inhibitory peptides and the family of opioid-like peptides (Moughan et al., 2014). For example, the best known ACE-inhibitory peptides Val-

Pro-Pro (VPP) and Ile-Pro-Pro (IPP) have been identified in milk (Sipola et al., 2002). Similarly, a research study showed that 33 mg of β-casomorphin–7 (an opioid peptide) can be derived from the digestion of 1 g of β-casein in milk; the amount of peptide produced was stated to be potentially significant physiologically (Meisel and Fitzgerald, 2000).

Production of bioactive peptides: Bioactive peptides are encrypted within the primary structure of proteins and thus have to be released for them to become active, as evidence suggests that protein hydrolysates and peptides have a higher bioactivity in comparison to their parent protein (Aluko, 2008; Udenigwe and Aluko, 2012). Bioactive peptides remain inactive as long as they remain within the primary structure of their parent protein; they only become active when liberated. Bioactive peptides are produced or released from their parent protein via various methods such as microbial fermentation, food processing and enzyme-catalyzed proteolysis *in-vitro* or within the gastrointestinal tract after human consumption (Aluko, 2008; Udenigwe and Aluko, 2012). Most bioactive peptides are produced via hydrolysis (by enzyme, alkali or acid treatment) or fermentation (Sarmadi and Ismail, 2010; Singh et al., 2014). Studies that investigate health effects of bioactive peptides apply them in 2 different forms either as hydrolysates of precursor proteins or as bioactive peptides (Sarmadi and Ismail, 2010). Hydrolysate is a mixture that is mainly composed of peptides and amino acids which are produced via protein hydrolysis or fermentation; on the other hand, bioactive peptides are several linked amino acids purified from hydrolysates (Sarmadi and Ismail, 2010). In cases where protein hydrolysis is induced by endogenous proteases, the term “autolysate” is usually used rather than hydrolysate (Sarmadi and Ismail, 2010). Enzymatic protein hydrolysates contain short-chain peptides with characteristic amino acid composition and defined molecular size. They are composed of free amino acids, short peptides (di and tripeptides) and longer peptides

that normally contain between 2-20 amino acid residues (molecular mass ≈ 1500 – 6000 Da) (Singh et al., 2014; Capriotti et al., 2015). Protein hydrolysates show technological advantages such as improved solubility, heat stability and relatively high resistance to precipitation by many agents such as pH or metal ions (Alashi et al., 2013; Tavano, 2013).

Protein digestion to produce hydrolysates or bioactive peptides can be carried out via enzymes, acid or alkali. Acid and alkali hydrolysis (both termed as chemical hydrolysis) tend to be difficult processes to control and yield products with reduced nutritional qualities. For example, acidic treatment destroys tryptophan, glutamine, and asparagine, while alkali treatment destroys cysteine, serine, threonine and produce toxic by-products (e.g. lysine-alanine and D-amino acids) (Clemente, 2000). On the other hand, enzymatic hydrolysis enables a more efficient tailoring of peptide products without the formation of toxic products or destruction of amino acids. It is also the most common and preferred method because of its moderate cost, high quality of the end products and also due to the fact that the overall amino acid composition of enzymatic protein hydrolysates is similar to that of the starting material (Aluko, 2008; Udenigwe and Aluko, 2012). Enzymatic hydrolysis of the parent protein is performed using either single or multiple proteases to release peptides of interest (Udenigwe and Aluko, 2012). Proteolytic enzymes hydrolyze the peptide linkage between amino acids to yield a mixture of peptides with different molecular sizes (Li-Chan, 2015). Proteolytic enzymes are classified into 2 groups according to their mechanism of hydrolysis: exoproteases and endoproteases. Endoproteases hydrolyze the peptide bonds within protein molecules to produce relatively large peptides, while exoproteases systematically remove amino acids from either the N-terminus or the C-terminus by hydrolyzing the terminal peptide bonds (Gofferjé et al., 2015). Other digestive enzymes and different enzyme combinations of proteinases such as alcalase, chymotrypsin, pancreatin, thermolysin as well as

enzymes from bacterial and fungal sources have also been used for the generation of these bioactive peptides (Korhonen and Pihlanto, 2006).

Certain factors are put into consideration when producing bioactive peptides and they include hydrolysis time, the degree of hydrolysis of the proteins; enzyme-substrate ratio and pre-treatment of the protein prior to hydrolysis (Udenigwe and Aluko, 2012). It is important to note that enzymatic hydrolysis cannot produce a suitable product per se without the need for post-hydrolysis treatments. Therefore after hydrolysis, the resulting peptide product is further processed based on physiochemical and structural properties of the constituent peptides in a bid to enhance bioactivity (Udenigwe and Aluko, 2012). Examples of post-hydrolysis treatment that are commonly used are listed in Table 1. These post-hydrolysis processes often result in appreciable peptide yield depending on the prevalence of the amino acid residues or peptides of interest within the hydrolysate. It has been documented that bioactive peptides are generally safe because they are products of hydrolysis of natural proteins. For instance, the breakdown of proteins to low molecular weight peptides can reduce the allergenic properties during membrane ultrafiltration of hydrolysates; however, it is of utmost importance that care should be taken to avoid processing techniques that would negatively affect peptide quality and safety (Moure et al., 2005; Udenigwe and Aluko, 2012).

Table 1. Common post-hydrolysis processes

Post-hydrolysis modification process	Description	Reference
Ultrafiltration	Most efficient post-hydrolysis procedure used to remove residual high-molecular weight peptides and proteins.	(Aoife et al., 2013)
Membrane ultrafiltration and size- exclusion chromatography	Used to concentrate peptides of defined molecular weight ranges. To obtain fractions containing low molecular weight peptides that can resist further *in-vivo* proteolytic digestion. The use of an ultrafiltration membrane with a specific molecular weight cut-off value is to reduce the antigen content of hypoallergenic formulas.	(Udenigwe and Aluko, 2012) (Lacroix and Li-Chan, 2013)
Reverse-phase High-Performance Liquid Chromatography (RP-HPLC)	To fractionate peptides based on their hydrophobic properties (usually on a hydrophobic column matrix). Useful for studying the structure-function properties of peptides	(Girgih et al., 2013) (Alu et al., 2017)
Chromatography using selective ion-exchange columns	To obtain peptide fractions of particular net charges. This process is useful when molecular disease targets are inactivated by molecules with strong net positive or negative charges.	(Pownall et al., 2011) (Lafarga and Hayes, 2014)
Activated carbon column	Involves passing of hydrolysates through a column packed with activated carbon or in situ mixing with activated carbon. Useful in obtaining peptide fractions rich in branched-chained amino acids and low in aromatic amino acids. Can also be used to reduce the bitterness of protein hydrolysates.	(Udenigwe and Aluko, 2010) (Aoife et al., 2013)
Electrodialysis-Ultrafiltration (EDUF)	Useful for separating cationic, anionic and neutral peptides of defined molecular sizes. Can also be used to concentrate low molecular weight bioactive peptides with net charges.	(Doyen et al., 2014) (He et al., 2016)

2.2 Functions of Bioactive Peptides

The interest of bioactive peptides in foods lies in their potential pharmaceutical and/or nutraceutical benefits (Maestri et al., 2016). Bioactive peptides have been documented to possess activities and/or functions that are beneficial to human health (Lafarga and Hayes, 2014); thus, they have attracted substantial interest from researchers. The health benefits attributed to bioactive peptides are based on their inherent amino acid composition and sequence as well as the size of active sequences, which vary from 2–20 amino acid residues (Korhonen and Pihlanto, 2006). They have been shown to affect body systems by possessing potent biological activities such as antihypertensive, antioxidant, antithrombotic, immunomodulatory, anticancer, antimicrobial, mineral binding, opiate-like and lipid-lowering activities (Aoife et al., 2013; Moughan et al., 2014; Lafarga and Hayes, 2014).

Hypocholesterolemic and Hypolipidemic Peptides: Bioactive peptides derived from foods such as soybean, milk, fish, buckwheat, and egg white have been shown to possess hypocholesterolemic and hypolipidemic activities (Udenigwe and Aluko, 2012). However, most of the peptides in the literature with these bioactivities are derived from soybean protein. These peptides have the ability to reduce cholesterol and lipid levels via various mechanisms including stimulating the secretion of bile acids; modification of lipid metabolism in the liver; reducing production and release of triglycerides (Ruiz Ruiz et al., 2014). Other mechanisms reported include the enhancement of HDLs, reduction of LDLs and VLDLs, interaction with hormones and cholesterol receptors as well as the induction of expression of genes encoding LDL receptors in the liver cells (Lovati et al., 2000; Yamauchi et al., 2003; Lin et al., 2011; Liu et al., 2012; Jensen-Urstad and Semenkovich, 2012; Ben Khaled et al., 2012; Howard and Udenigwe, 2012;

Udenigwe and Aluko, 2012). It has been reported that the most functional amino acid residues in these peptides are those with hydrophobic groups (leucine, tryptophan, and tyrosine). This is because they are able to establish interactions with lipids and the hydrophobic components of bile compounds; on the other hand, the hydrophilic amino acid residues are able to inhibit the function of various biosynthetic enzymes (Maestri et al., 2016). For example, a group of researchers fed 40 Sprague-Dawley rats a diet containing 16% hydrolysate obtained via protamex hydrolysis of freshwater clam over a period of 4 weeks (Lin et al., 2011). The results showed 65.8% and 26.1% reductions in plasma triglyceride and cholesterol respectively, in addition to 66% increase in HDL. There was also a corresponding 60% decrease in LDL and VLDL, a reduction of 27.6% and 50.5% in hepatic triglycerides and total cholesterol, respectively with increased fecal excretion of lipids and bile acids. Similarly, a recent study (Soares et al., 2015) showed that the hydrolysis of amaranth (*Amaranth cruentus*) proteins using pepsin and trypsin generated 3 peptides GGV, IVG and VGVL. These peptides showed strong inhibitory activities (40-45%) against 3-hydroxyl-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), a key enzyme in the cholesterol biosynthesis pathway, indicating a potential hypocholesterolemic effect.

Opioid-like Peptides: Peptides showing opioid-like activity have been termed “exorphins” as they have structural similarity to endorphins and enkephalins (Maestri et al., 2016). These peptides have been identified in soybean and wheat proteins. They possess a binding activity similar to that of endogenous ligands and have a direct influence over gut function via interaction with smooth muscles and increasing electrolyte absorption (Hayes et al., 2007; Garcia et al., 2013). For instance, a 10 mg/kg dose of Soymorphin-5, a μ-opioid pentapeptide with amino acid sequence YPFVV derived from soy-β-conglycinin β-subunit of major soy protein (7S fraction)

via enzymatic hydrolysis with pancreatic elastase and leucine aminopeptidase has been documented to suppress intestinal contractions and possess anxiolytic activity in mice (Maestri et al., 2016; Ohinata et al., 2007). YPFVV has also been shown in mouse to exhibit antidiabetic effects by altering glucose and lipid metabolism (Yamada et al., 2012).

Antihypertensive Peptides: Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase that converts the decapeptide angiotensin I to the octapeptide angiotensin II (Maestri et al., 2016). Regulation of the renin-angiotensin pathway via the inhibition of ACE activity has antihypertensive effects. It has been noted that peptide-induced inhibition of ACE activity involves both competitive and non-competitive modes of action (Duan et al., 2014). ACE inhibitory peptides are noted to be short in chain length and carry hydrophobic residues (including proline) along with some positively charged residues (Udenigwe and Aluko, 2012). Also, the presence of aromatic amino acid residues (phenylalanine, tyrosine, and tryptophan) at the C-terminus of peptides promotes ACE inhibition (Saleh et al., 2014). Some peptides obtained from hydrolysis of food proteins such as flaxseed, pea, and hemp are seen to directly inhibit renin activity, an enzyme secreted by the kidneys that converts angiotensinogen to angiotensin I (Udenigwe and Aluko, 2012). Other peptides have vasoprotective effects, mainly by inducing a reduction in the intracellular concentration of calcium in the smooth muscle cells of blood vessels; these set of peptides are seen to be prominent in pulse protein hydrolysates (Kumrungsee et al., 2014). Evidence reveals that a 5 mg/kg dose of two tripeptides VNP and VWP purified from rice protein hydrolysate (derived with alcalase-trypsin combination) had antihypertensive effects when administered orally to spontaneously hypertensive rats. The tripeptides were shown to reduce systolic blood pressure by 29 and 38 mmHg, respectively (190 mmHg to 161 and 152 mmHg) and the antihypertensive effect was potent and long-lasting with a minimum duration of

8 hours (Chen et al., 2013). Antihypertensive effects of peptides have also been documented in humans (Turpeinen et al., 2013; Fekete et al., 2015). For instance, two lactotripeptides (IPP and VPP) derived from casein hydrolysate were added to fruit juice and administered to 164 participants in a randomized double-blind clinical trial at a dose of 3 mg (1 mg of IPP and 2 mg of VPP) for 4 weeks (Cicero et al., 2012). The results showed significant reductions ($p < 0.001$) only in the lactotripeptide-treated patients in both systolic blood pressure (-3.42 mm Hg) and diastolic blood pressure (-2.35 mm Hg) when compared with the baseline values.

Anti-oxidative Peptides: Peptides with anti-oxidative activity can be found in wheat germ as well as in a number of other food proteins (Sarmadi and Ismail, 2010). Antioxidant peptides suppress the damage caused by ROS (reactive oxygen species), in turn restricting the peroxidation of essential fatty acids (Maestri et al., 2016). Cysteine, lysine, histidine, methionine, and tryptophan are all effective as radical scavengers (Udenigwe and Aluko, 2012). Antioxidant peptides have been shown to confer protection against Alzheimer's disease in animal models (Martorell et al., 2013). Metal chelation is an important component of the inhibition of free radical formation and some peptides, especially those containing cysteine, histidine, aspartic acid and glutamic acid are effective as chelators (Guo et al., 2014). These anti-oxidative peptides have been identified in chickpea, hemp, sesame, sunflower, and soybean protein hydrolysates (Guo et al., 2014). For instance, Girgih et al. (2014) identified from the pepsin-pancreatin digest of hemp seed proteins, two peptides WVYY and PSLPA with 67% and 58% DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and metal chelation activities of 94% and 96%, respectively.

Immunomodulatory Peptides: These peptides contribute to the host's defence response but they do not interact directly with the pathogens. They are thought to strengthen the host immune system by enhancing the proliferation and maturation of immune cells, especially through

stimulation of NK cells (natural killer) and macrophage phagocytosis. They can also up-regulate the production of antibodies, chemokines, and cytokines as well as inactivate inflammatory compounds (Maestri et al., 2016). Conversely, peptides that exhibit an inhibitory effect on lymphocyte proliferation are also considered to be immunomodulatory, because they are able to reduce hypersensitivity and allergic reactions (Baldi et al., 2005). Peptides that possess antimicrobial activity have been suggested as potential alternatives to conventional antibiotics for the treatment of bacterial infections (Maestri et al., 2016). Peptides that stimulate the proliferation of human lymphocytes in peripheral blood contain glycine along with other residues (Maestri et al., 2016). For example, Lunasin (a 43 residue peptide derived from soybean) has been documented to have several immunomodulatory effects such as inhibition of histone acetylation, enhanced activation of NK cells via regulation of gene expression, prevention of cell transformation induced by carcinogens or viral oncogenes in addition to inhibition of inflammation via the suppression of NF-κB pathway (Hernández-Ledesma et al., 2011; Hsieh et al., 2011; Singh et al., 2014).

Anticancer / Antiproliferative Peptides: In cancer therapy research, natural biomaterials such as protein hydrolysates might exhibit a pivotal biological role against cancer cells by targeting them and preventing their proliferation (Liu et al., 2016). The identification of bioactive peptides with anticancer activity could be a new strategy to develop novel anticancer therapeutic agents with low toxicity. Through modulation of apoptosis and the cell cycle, anticancer bioactive peptides suppress human gastric cancer growth (Su et al., 2010). Peptides with anticancer activity have been isolated from enzymatic hydrolysates obtained from vegetable, rice, soy and marine algae proteins (Rizzello et al., 2016). This is exemplified by a pentapeptide EQRPR isolated from rice bran, which exhibited antiproliferative activities on different cancer cell lines at 600–700 μg/mL

dose. The peptide had 84% inhibition of colon cancer cell multiplication (Caco-2, HCT-116), 80% against breast cancer cells (MCF-7, MDA-MB-231) and 84% against liver (HepG-2) cancer cells (Kannan et al., 2010).

Multifunctional Peptides: Some food protein hydrolysates have been discovered to contain multifunctional peptides that exhibit more than one physiologically important bioactive property (Udenigwe and Aluko, 2012). For example, peptides derived from chymotrypsin digestion of bovine milk α-casein exhibited several *in vitro* bioactivities such as ACE and propyl endopeptidase inhibition in addition to antioxidant (DPPH), zinc-binding and antibacterial activities (Srinivas and Prakash, 2010). Similarly, peptides obtained from quinoa/pea proteins have also exhibited multifunctionalities such as ACE-inhibition and antioxidant activity (Aluko and Monu, 2003; Humiski and Aluko, 2007). In addition, alcalase-derived peptides from hen egg white lysozyme also had antioxidant and calmodulin-dependent phosphodiesterase inhibitory activities (You et al., 2010). These multifunctional peptides provide an alternative strategy in the amelioration of more than one disease target or multiple symptoms of a disease, as many human diseases are interrelated in terms of etiology and progression (Udenigwe and Aluko, 2012).

Foods represent not just a source of calories, energy and the building blocks for cellular processes, but also a source of compounds important for promoting health and sustaining a range of physiological processes. Studies on food-derived peptides with various physiological and biological activities have redefined the nutritional value of food proteins. Food protein bioactive peptides possess remarkable multifunctional activities relevant to the sustenance of human health. Delivering bioactive peptides through food is an attractive alternative to administering synthetic drugs; in some cases, the peptide binds highly selectively to its target and generally the risk of toxic metabolites is low. However, compounds/tools, such as nanocarriers and

microencapsulation that can effectively prevent degradation of bioactive peptides as well as the safety of the peptide-based products must be determined to improve bioavailability and maintain peptide potency (Udenigwe and Aluko, 2012; Maestri et al., 2016; Liu et al., 2016).

2.3 Structure and mode of action of gastrointestinal enzymes

Enzymes are "biological molecules made up of proteins or polypeptides that catalyze or accelerate chemical, biological, and metabolic reactions that occur within the human body" (Ho and Gibaldi, 2013). They function under two main conditions: having a specific and suitable substrate to which the enzyme can attach itself to form an enzyme-substrate complex and ultimately a product, and having a proper environmental condition to catalyze the reaction in terms of acidity or alkalinity (DeLuca and Lyndal York, 2013). The active site of an enzyme (sometimes referred to as the catalytic center) is that portion of the enzyme that interacts with the substrate and converts it into a product. Two distinct models of how an enzyme binds its substrate have been proposed: the lock-and-key (complementary) model of Fischer and the induced fit (conformational change) model of Koshland (DeLuca and Lyndal York, 2013). Enzymes are classified into three main groups: Metabolic enzymes, Digestive enzymes and Food enzymes (Kaur and Sekhon, 2012). Metabolic enzymes are those enzymes that catalyze various chemical reactions within the cells such as detoxification and energy production. Food enzymes are naturally present in raw foods and act as an exogenous source of digestive enzymes. In contrast, digestive enzymes, also known as gastrointestinal enzymes are secreted along the gastrointestinal tract to break down food into nutrients that can be easily absorbed into the bloodstream (Kaur and Sekhon, 2012). The main digestive enzymes that the body uses include protease, amylase, sucrase, lipase, lactase, and maltase (Ho and Gibaldi, 2013).

This section will focus on the catalytic activities of digestive enzymes, specifically α-amylase, α-glucosidase, chymotrypsin, pancreatic lipase and trypsin as these are the enzymes this research study aims to inhibit. α-amylase (α-1,4-ᴅ-glucan-4-glucanohydrolase, EC 3.2.1.1) is a member of

glycoside hydrolase family 13 (GH13) of the sequence-based classification of glycoside hydrolases (Janeček et al., 2014). It catalyzes the hydrolysis of the internal α-1,4-glycosidic linkages in starch, glycogen and various malto-oligosaccharides into low molecular weight products such as glucose, maltose, and maltotriose units by adopting a double displacement mechanism with retention of anomeric configuration (Kim et al., 2014). α-amylase is subdivided into two forms: salivary amylase (ptyalin) found in the saliva, breaks starch into maltose and dextrin while pancreatic α-amylase, produced by the pancreas completes the hydrolysis of starch, producing glucose, fructose, and oligosaccharides of varying lengths (Lordan et al., 2013). Structurally, amylases are noted to have the $(\beta/\alpha)_8$ barrel-fold structure with active sites that contain a trio of acidic groups, specifically two aspartic residues and one glutamic acid residue that are presumed to be the catalytic residues, which work together to cleave the bonds between sugar units (Kagawa et al., 2003). α-amylase is known as a calcium metalloenzyme because it is completely unable to function in the absence of calcium and its active site contains calcium ion which stabilizes the structure of the enzyme, it also contains chloride ion bound underneath the active site that assists in the activity and stability of the enzyme (Tiwari et al., 2015).

On the other hand, α-glucosidase (EC 3.2.1.20), a member of glycoside hydrolase family GH31 is located in the brush-border surface membrane of intestinal cells and activates the final step of the carbohydrate digestive process (Kim et al., 2014). It is a carbohydrate-hydrolase that releases α-glucose from the non-reducing end by hydrolyzing both α-1,4- and α-1,6-glucosidic linkages (Ho and Gibaldi, 2013), in other words, it catalyzes the hydrolysis of complex carbohydrates and disaccharides to absorbable monosaccharides such as glucose. Structurally, it is a monomeric glycoprotein and also noted to have the $(\beta/\alpha)_8$ barrel-fold structure as α-amylase, the Trp-516 and Asp-518 residues have been deemed critical for the enzyme's catalytic functionality (Roig-

Zamboni et al., 2017). Like α-amylase, it is a calcium-containing enzyme and it performs its catalysis via a double displacement reaction mechanism with retention of the anomeric carbon configuration in the product (Azam et al., 2012).

Trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1), also known as serine proteases are enzymes that cleave peptide bonds in proteins, in which serine serves as the nucleophilic amino acid at the enzyme's active site (Naffin-Olivos et al., 2017). Both enzymes are classified as endopeptidases because they cleave peptide bonds within the polypeptide chain rather than at the terminal amino acids located at the ends of polypeptides. Both enzymes are known to function in the duodenum and break down proteins into smaller peptides. Chymotrypsin is composed of 241 amino acid residues with 3 peptide chains (A chain: 13 residues, B chain: 131 residues and C chain: 97 residues) while trypsin is a single chain polypeptide of 223 amino acid residues held together by 2 disulfide bridges formed by 8 homologous cysteine residues in each molecule (Desnuelle et al., 2014). Trypsin is produced in the small intestine when its proenzyme form, trypsinogen produced by the pancreas is activated by the enzyme enteropeptidase (Weiss, 2014). On the other hand, chymotrypsin is synthesized in the pancreas as a precursor called chymotrypsinogen that is activated by trypsin to produce chymotrypsin (Hedstrom, 2002). Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, while chymotrypsin cleaves peptide amide bonds where the side-chain of the amino acid is a large hydrophobic amino acid (tyrosine, tryptophan, and phenylalanine) (Horn et al., 2014). These amino acids contain an aromatic ring in their sidechain that fits into a 'hydrophobic pocket' (Ser-198, Gly-216, and Gly-226) of the chymotrypsin enzyme (Chow, 2014). Both enzymes contain a catalytic triad consisting of histidine-57, aspartate-102, and serine-195 and contain an "oxyanion hole" formed by Gly-193 and Ser-195 that serves to stabilize the enzymes (Hedstrom,

2002). In addition, trypsin contains an aspartate residue (Asp 189) located in its catalytic pocket, which is responsible for the specificity of the enzyme as it attracts and stabilizes positively charged lysine and/or arginine. Furthermore, evidence shows that Ser-21 and Met-192 are responsible for substrate binding on chymotrypsin (Desnuelle et al., 2014)

Pancreatic lipase (EC 3.1.1.3) belongs to the family of lipolytic enzymes that hydrolyze ester linkages of triglycerides. Pancreatic lipase is secreted from the pancreas (pancreatic acinar cells) and is the primary lipase enzyme that breaks down dietary fat (triglycerides) in the human digestive system to monoglycerides and free fatty acids, which are then absorbed by the body (Lunagariya et al., 2014). It is secreted in its final form, however, it becomes effective only in the presence of colipase in the duodenum (Mukherjee, 2003). Structurally, pancreatic lipase is noted to have a tertiary structure suggested by its conserved disulfide bonds and its active site is centered on a serine residue which has been shown to participate with an histidine and an aspartic acid residue, in a charge relay system (Birari and Bhutani, 2007).

All the above enzymes described in this section are vital for nutrient digestion and absorption in the human body. However, increase in the catalytic activity of these enzymes may result in the inception of certain symptoms of disease conditions such as T2DM and obesity. Thus, the inhibition of these gastrointestinal enzymes is an important therapeutic strategy in a bid to ameliorate the disease symptoms.

2.4 Bioactive Peptides with inhibitory activities against gastrointestinal enzymes

A range of various natural compounds and food sources have been investigated and shown to possess inhibitory activities against gastrointestinal enzymes. Examples include finger millet, milk, kidney bean, corn, rosemary leaves, amaranth seeds, alginates and pulses (lentils, chickpeas, cowpea and pinto bean) (Niwa et al., 2003; Strugala et al., 2005; Apostolidis et al., 2007; Shobana et al., 2009; Campos-Vega et al., 2010; Roy et al., 2010; Ali et al., 2014; Chater et al., 2015). Several studies have investigated the use of various bioactive compounds (e.g polysaccharides, polyphenols, saponins, tannins, flavonoids, methanolic extracts from plants) to influence the digestive system as an approach in the management of obesity and diabetes mellitus (He et al., 2007; Seyedan et al., 2015; Ercan and El, 2016). This can be achieved by inhibiting the key enzymes involved in food digestion (Lacroix and Li-Chan, 2013). For example, a chickpea extract was investigated as an inhibitory agent against the digestive enzymes involved in carbohydrate and lipid metabolism (α-amylase, α-glucosidase, and lipase) (Ercan and El, 2016). The results showed that the chickpea extract inhibited α-amylase (72.6% ± 0.84), lipase (85.4% ± 0.73) and α-glucosidase (10.9% ± 1.32); the inhibitory activity was attributed to the saponin content.

However, this section will be focused on bioactive peptides that can inhibit digestive enzymes. The body of literature is quite scarce on the inhibition of certain enzymes (such as chymotrypsin, trypsin, and pepsin) by bioactive peptides. Current literature information indicates much focus is on α-amylase, α-glucosidase and pancreatic lipase inhibitions, which is quite understandable, given the concern with obesity and diabetes mellitus. A current research study showed that peptides derived from pinto bean were able to inhibit α-amylase (Ngoh and Gan, 2016). The

hydrolysates produced by Protamex were subjected to ultrafiltration to separate peptides based on molecular weight. Six fractions were produced and then tested for the inhibitory activity of α-amylase. Results showed that fractions with molecular weight (MW) less than 3 kDa had the highest inhibitory activity against α-amylase (62.10% ± 3.49). The < 3 kDa fraction was then subjected to mass spectrometry, which identified 7 peptides (PPHMLP, PPMHLP, PLPWGAGF, GDAACCGLPLLP, PPHMGGP, PLPPHDLL, and FNPFPSPHTP). Similarly, peptides present in rice bran albumin hydrolysates inhibited *in vitro* activity of α-glucosidase (Uraipong and Zhao, 2016a). Four proteases (Alcalase, Protamax, Neutrase, and Flavourzyme) were used for the rice albumin hydrolysis, with alcalase hydrolysates having the highest α-glucosidase inhibitory activity. Alcalase hydrolysates were then subjected to ultrafiltration to separate peptides based on molecular weight. Results showed that the <3 kDa fractions had the highest inhibitory activities against α-glucosidase (47.9% ± 2.6). The <3 kDa fraction was then further separated by ion exchange chromatography to yield 2 peaks, with AAIE1 having the highest α-glucosidase inhibitory activity. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) of AAIE1 identified 40 peptides and the mode of inhibition of AAIE1 was determined to be of the non-competitive mixed type. In addition, another study (Yu et al., 2011) revealed seven novel peptides derived from the hydrolysis of egg white via alcalase, however, only 2 of these peptides, RVPSLM and TPSPR with IC_{50} values of 23.1 and 40.0 μM, respectively were noted to possess α-glucosidase inhibitory activity.

Bioactive peptides from marine products have also been documented to inhibit digestive enzymes. For example, Nazumazoles D-F (1, 2, 3) (which are cyclic pentapeptides in structure) isolated from marine sponge (*Theonella swinhoei*) inhibited chymotrypsin activity with IC_{50} values of 2, 3, 10 μM respectively (Fukuhara et al., 2016). In another study (Siow et al., 2016),

three novel peptides (CSPs) obtained via protamex hydrolysis of cumin seed and labeled as CSP1 (FFRSKLLSDGAAAAKGALLPQYW), CSP2 (RCMAFLLSDGAAAAQQLLPQYW) and CSP3 (DPAQPNYPWTAVLVFRH) were identified and tested for inhibition of pancreatic lipase (PL) activity. All three peptides at 20 mg/ml concentration had inhibitory activity against PL with CSP1 and CSP2 having > 50% inhibition (54.6% and 50.1%) and CSP3 with 22.6% inhibition.

Furthermore, 2 peptides with 50% trypsin inhibitory activity at a concentration of 3.75 µg/ml were identified from *Opuntia joconostle* Weber (xoconostle, an acid cactus pear) (Aguirrezabala-Cámpano et al., 2013). The two identified peptides OjTI 1 and OjTI 2 had low molecular weights of 4.26 and 4.17 kDa as determined by mass spectrometry. For OjTI1, the sequence obtained was RGQSCNPYEGIECCGDILCIQPRIWPPVPGRCA; while OjTI2 gave several peptides with the following sequences: GDILCIQPR, GQSCNPYEG, PYEGIECCGDILCIQPR, PPVGPR, and ATVSLPR.

Evidence from scientific literature has shown that bioactive peptides possess significant inhibitory activities against gastrointestinal enzymes. When the amount of bioactive peptides that can be safely consumed is taken into consideration, these peptides have the potential to be developed into non-drug supplements for the management of obesity and type 2 diabetes mellitus (Uraipong and Zhao, 2016b).

2.5 Yellow field Pea

Yellow field pea seeds (*Pisum sativum L.*), contain proteins and have been identified as one of the most valued pulse crops produced by Canada, which is the largest producer and exporter worldwide (Agboola et al., 2010; Pulse Canada, 2017). Other top pea producing and exporter countries include Russian Federation, China, Australia, USA and India (Agboola et al., 2010; Dahl et al., 2012). Yellow field pea seeds have been identified to be a rich source of protein, containing 18-30% on a dry basis with a variety of amino acid sequences (Aluko, 2008; Adebiyi and Aluko, 2011), as well as carbohydrate (in form of starch-amylose), resistant starch, fibre, vitamins and minerals such as iron, zinc, selenium, folate and other B vitamins (Dahl et al., 2012). It has a low glycemic index (GI = 32) and is low in fat and energy; it has a high content lysine, an essential amino acid which is low in cereal-based diets (Dahl et al., 2012; Adolphe et al., 2015). In addition, yellow pea seed starch contains amylose, which is a resistant type of starch as it is digested slowly and thus has impact on post-prandial glucose response and insulin resistance, suggesting potential use in the management of type 2 diabetes mellitus (Dahl et al., 2012). Fibre from the seed coat and cell walls of yellow pea contribute to gastrointestinal function and health as they have a beneficial effect on intestinal motility (Dahl et al., 2003; Agboola et al., 2010). The seed vitamin and mineral contents play important roles in the prevention of deficiency-related diseases, specifically those that involve selenium and folate deficiencies (Dahl et al., 2012). This makes pea seed a valuable and a good source of food ingredients to be used in the production of healthy foods as it is capable of meeting dietary needs and has an advantage in terms of economic costs because it is cheap, widely distributed and readily available (Agboola et al., 2010; Dahl et al., 2012). In addition, yellow field pea protein seems to be more suitable than soy or whey protein (which contain gluten and gliadin) because it

is hypoallergenic and has a mild pleasantly sweet taste (Ndiaye et al., 2012). Therefore, there is a need for food manufacturers to be able to incorporate the yellow field pea into acceptable food products; however, knowledge of the functional properties is essential for successful application (Agboola et al., 2010).

Bioactive peptides derived from pulses are generating interest, as they are more cost-effective than those derived from animal sources (Pownall et al., 2010). Moreover, no toxicity of plant protein peptides has been reported even at high doses (Pownall et al., 2010; Li et al., 2011); this gives it an advantage over drugs that have toxic/side effects. Existing literature shows that yellow field pea protein and its hydrolytic products such as hydrolysates and peptides can positively influence human health via various important nutritional and functional effects. These studies showed that pea protein hydrolysates have anti-inflammatory, anti-oxidant, anti-bacterial, angiotensin-1-converting enzyme inhibitory and immuno-modulatory activities (Barbana and Boye, 2010; Niehues et al., 2010; Pownall et al., 2010; Pownall et al., 2011; Ndiaye et al., 2012). Evidence suggests that they have the ability to improve cardiovascular diseases by lowering blood pressure and improving serum lipid levels (Li et al., 2011; Aluko et al., 2015). Pea protein hydrolysates can also promote weight loss by increasing the duration of satiety (Lunde et al., 2011), delay or prevent the risk of kidney disease (Dahl et al., 2012), reduce insulin resistance (Marinangeli and Jones, 2011) and influence gastrointestinal function through increased bowel movement (Dahl et al., 2003).

For example, a research conducted on activated macrophages showed that the hydrolysates from yellow field pea protein have anti-oxidant and anti-inflammatory activities. This is because the hydrolysates inhibited activation of pro-inflammatory substances (such as nitric oxide, interleukin-6, cytokines and tumor necrosis factor alpha) in addition to immunomodulating

activities when tested in a murine model (Ndiaye et al., 2012). A single-blinded crossover clinical trial with 22 participants (Marinangeli et al., 2009), compared the effects of food products made with either 100% whole yellow pea flour or 100 % whole wheat flour on glycemic response. The results showed that foods made with whole yellow pea flour reduced postprandial glucose significantly when compared to that of whole wheat flour. Similarly, another study (Marinangeli and Jones, 2011) revealed that muffins made with 26.4g of whole yellow pea flour and 6g of fractionated yellow pea flour reduced insulin resistance by 25% and fasting insulin by 13.5 % and 9.8%, respectively, in comparison to the control group who received muffins made with white wheat flour. Thus, the authors concluded that given the results, yellow field pea might play a role in the management of type 2 diabetes mellitus. Moreover, in a recent randomized crossover trial (Mollard et al., 2014), 15 participants were subjected to a diet of various pea seed product treatments, which included (i) pea hull fibre (7 g), (ii) pea protein (10 g), (iii) pea hull fibre (7 g) plus pea protein (10g), and (iv) whole yellow peas (406 g). Results revealed that at 30, 45 and 75 min post-consumption, protein plus fibre and whole peas led to lower blood glucose concentrations and at 155 min, the whole peas led to lower blood glucose concentrations. This study provides evidence and supports the use of pea components as value-added ingredients in foods designed to improve glycemic control. In addition, the effects of yellow pea protein on short-term food intake as well as subjective appetite, and glycemic response were investigated (Smith et al., 2012). Results showed that although pea protein diets did not affect subjective appetite, they led to the suppression of food intake and reduced glycemic response. This study provides an evidence for the usefulness of yellow pea proteins as a value-added ingredient aimed at suppressing food intake and controlling blood glucose.

Yellow pea components are of interest because they are the least expensive and most abundant pulse, but their consumption is noted to be extremely low, especially in countries where the rates of diabetes and obesity are high such as Europe and North America (Mudryj et al., 2012; Smith et al., 2012; Mollard et al., 2014). Despite its under-consumption, it is an important food product, as yellow pea protein and its hydrolytic products possess high nutritional and functional values as well as potential disease-preventing properties (Li et al., 2011). Thus, yellow field pea products have the potential to be developed into a valuable ingredient for functional foods and nutraceuticals.

However, to the best of our knowledge, information is scarce on the inhibitory properties of yellow field pea protein-derived peptides against gastrointestinal enzymes. Against this backdrop, this research work was carried out to add useful information to scientific literature. Specifically, the work provides new data on the potential application of bioactive peptides derived from yellow field pea protein as potential agents against obesity and diabetes. The pea protein hydrolysate may be an ingredient to formulate functional foods and nutraceuticals for the prevention or treatment of obesity and diabetes-related disease symptoms.

3. STUDY RATIONALE, HYPOTHESES, AIM, AND OBJECTIVES

3.1 STUDY RATIONALE

Currently, known medications such as Acarbose and Orlistat are used in the treatment of type 2 diabetes mellitus (T2DM) and obesity, respectively. The mechanism of action of these drugs is via the inhibition of digestive enzymes. For example, acarbose is an α-glucosidase inhibitor, while orlistat is a pancreatic lipase inhibitor. However, these drugs come with the issues of cost, compliance and negative side effects ranging from diarrhea to hepatotoxicity. Therefore, it was proposed to investigate if a natural product, specifically, bioactive peptides from a food source (yellow field pea) can have the same effect i.e. inhibit digestive enzymes. This is because food-derived peptides could be cheaper, safer and have minimal side effects in comparison to their synthetic counterparts. Yellow peas were chosen because they are also safe, inexpensive and its food-grade protein fractions are commercially available, of high quality and approved for human consumption.

3.2 HYPOTHESES

a) Peptides with inhibitory activities against digestive enzymes (α-amylase, α-glucosidase, pancreatic lipase, trypsin and chymotrypsin) can be isolated after hydrolysis of pea proteins with alcalase, pepsin, trypsin, and chymotrypsin.

b) Activity of enzymatic protein hydrolysates will be inversely related to peptide size.

c) Peptide inhibition of gastrointestinal tract (GIT) enzymes will involve both competitive and non-competitive modes.

3.3 AIM AND OBJECTIVES

The overall aim of this research was to produce peptides derived from yellow field pea protein that can negatively influence *in vitro* activities of GIT enzymes responsible for nutrient digestion.

Therefore, the specific objectives of the study were to:

a) Optimize enzymatic production of yellow field pea protein-derived peptides with *in vitro* inhibitory properties against α-amylase, α-glucosidase, trypsin, chymotrypsin and pancreatic lipase.
b) Determine the effect of peptide size on the GIT enzyme inhibitory activities of the enzymatic pea protein hydrolysates.
c) Determine the mode and kinetics of enzyme inhibition by the most active peptide fraction(s).
d) Determine the amino acid sequence of peptides present in the enzymatic hydrolysates using mass spectrometry.

4. MATERIALS AND METHODS

4.1 Materials

Yellow field pea (YFP) protein isolate was purchased from Nutri-Pea Limited (Portage La Prairie, Manitoba, Canada). Enzymes and other chemical reagents were purchased from Sigma-Aldrich (St Louis, Missouri, USA) and Fisher Scientific Company (Ottawa, Ontario, Canada), respectively. All chemicals were of analytical grade and were used without further purification. Glass (double)-distilled water was used for the preparation of the reagents.

4.2 Methods

4.2.1 Protein Content Determination

The protein content of yellow field pea protein isolate (YPPI) was determined using the modified Lowry method with bovine serum albumin (BSA) as the standard (Markwell et al., 1978). Briefly, 100 parts of Reagent A (which consisted of 2% Na_2CO_3, 0.4% NaOH, 0.16% C_4O_6HKNa and 1% SDS) was mixed homogeneously with 1 part of Reagent B (4% $CuSO_4.5H_20$) to form Reagent C. Reagent D was a mixture of 1 part of Folin-Ciocalteu phenol reagent and 1 part of double distilled water. A stock solution of BSA (in distilled water) and protein sample (dissolved in 0.1 M NaOH) at 10 mg/ml was used to prepare serial dilutions of 10-100 µg/ml. A 3 ml aliquot of reagent C was then added to each tube, mixed and incubated at room temperature for 1 hr. After incubation, 0.3 ml of reagent D was added to each tube, vortexed thoroughly and left to stand in the dark for 45 min. Finally, absorbance was read at 660 nm using UV-visible spectrophotometer (Biochrom Ultrospec 4300 Pro, Hollister, MA, USA). The analysis was done in triplicates and protein content calculated using the following equation:

$(\text{Absorbance}_{\text{sample}}) / (\text{Absorbance}_{\text{standard}}) \times \text{Concentration}_{\text{standard}} = \text{Corresponding sample concentration}$

$\text{Concentration}_{\text{sample}} / \text{Concentration}_{\text{standard}} \times 100 = \text{Protein content (\%)}$

4.2.2 Enzymatic Hydrolysis

Enzymatic hydrolysis of the pea protein isolate was carried out using four different proteases (Alcalase, Pepsin, Trypsin, and Chymotrypsin). These enzymes were chosen because they have food grade status and are compliant with regulatory laws. Moreover, the enzymes have different proteolysis mode of action and would produce peptides that differ in chain length and amino acid composition, which enhances the diversity of potentially active peptides. These four enzymes are endoproteases (i.e. their cleavage action occurs within the polypeptide chain). Alcalase is an endoprotease with broad and random enzymatic specificity, which liberates small peptides and thus increases the numbers of terminal amino group residues in the hydrolysates (Xia et al., 2012). Trypsin cleaves peptide bonds on the carboxyl side of basic amino acids, specifically arginine and lysine (Yin et al., 2008). Pepsin and chymotrypsin are endopeptidases that mainly cleave peptide bonds involving hydrophobic and aromatic amino acids (such as phenylalanine, tyrosine, tryptophan), thus they generate peptides having hydrophobic and/or aromatic amino acid as terminal groups (Boschin et al., 2014).

Briefly, in a 1 L beaker, the pea protein isolate was suspended in double distilled water to obtain 5% (w/v) mixture. Under constant stirring, the beaker containing the protein mixture was placed on a magnetic stirring hot plate equipped with an external temperature probe for accurate temperature control. The mixture was heated to the optimal temperature and adjusted to the optimal pH of each protease using either 1 M NaOH or 1 M HCl. The optimal temperature and pH of each protease are shown in Table 2. Hydrolysis was initiated by the addition of each protease at a ratio of 4% (on basis of protein content w/w). Once an enzyme has been added, the temperature and pH of the reaction was monitored and maintained constant for 4 hrs, after which hydrolysis was terminated by adjusting to pH 5.0 with either 2 M NaOH or 2 M HCl followed by

heating at 95°C for 15 min to ensure complete denaturation of the enzymes. The reaction mixture was then cooled to room temperature and centrifuged at 10,000g for 15 min at 4°C using an Allegra^{TM} 6R centrifuge (Beckman Coulter, Mississauga, ON) to separate the soluble hydrolyzed materials (peptides) from the unhydrolyzed residue (mainly undigested proteins). The clear supernatant was collected as the hydrolysate and a portion freeze-dried and stored at -20°C until further analysis, while the remainder was fractionated using membrane ultrafiltration. Hydrolysis was carried out in duplicates.

Table 2. Optimal temperature and pH of proteases used for enzymatic hydrolysis

Enzyme	Temperature (^{o}C)	pH
Alcalase	55	8
Trypsin	37	8
Chymotrypsin	37	8
Pepsin	37	2

4.2.3 Ultrafiltration

The collected supernatant (hydrolysate) was then passed through an Amicon stirred ultrafiltration cell using 1 kDa, 3 kDa, 5 kDa and 10 kDa molecular weight (MW) cut-off membranes respectively, to produce peptides of different sizes.

Briefly, the supernatant was first passed through the ultrafiltration cell using 1 kDa membrane, the permeate (<1 kDa) was collected and freeze-dried, while the retentate was kept in the fridge. Distilled water was then added to the retentate and passed through a 3 kDa membrane; the permeate (1-3 kDa) was collected and freeze-dried, while the retentate was kept in the fridge. This process was repeated by passing the 3 kDa retentate through a 5 kDa membrane to collect a permeate (3-5 kDa). The 5 kDa retentate was then passed through a 10 kDa membrane and the permeate (5-10 kDa) collected. The resulting permeates and 10 kDa retentate were lyophilized and stored at -20°C for further analysis.

The freeze-dried hydrolysates, membrane fractions, and retentates were weighed and their protein content was determined using the modified Lowry method as described above. The yield of the hydrolysates and peptide fractions were calculated by dividing the final weight of the protein hydrolysates and peptide fractions after ultrafiltration by the initial weight of the protein used for enzymatic hydrolysis. The protein yield (%) was derived using the following formula:

Protein yield (%) = [(Protein content / 100) **x** Yield] x 100.

4.2.4 *In vitro* Enzyme Inhibition Assays

The hydrolysates and membrane fractions were tested for *in vitro* inhibition of the following digestive enzymes: α-amylase, α-glucosidase, trypsin, chymotrypsin and pancreatic lipase. The appropriate assay methods to determine the activity of each enzyme was obtained from the literature and adapted for the samples. All assays were conducted in triplicates.

4.2.4.1 α-Amylase Inhibition Assay

α-amylase inhibitory activity was assayed following the method described by Siow et al. (2017) with slight modifications. Briefly, lyophilized protein hydrolysates and membrane fractions were re-dissolved in 1 ml of 0.02 M sodium phosphate containing 0.006 M NaCl, pH 6.9. A 100 µl aliquot of each sample (final peptide concentration = 50-225 µg/ml) and 100 µl of α-amylase solution (final concentration = 28.57 µg/ml) were added to test tubes and allowed to incubate for 10 min at 25^{o}C. After incubation, 100 µl of 1% starch solution (dissolved in the above buffer) was added and incubated at 25^{o}C for 10 min. The reaction was terminated by adding 200 µl of dinitrosalicylic acid (DNSA) colour reagent (96 mM DNSA, 2 M sodium potassium tartrate tetrahydrate and 2 M NaOH) followed by incubation in a boiling water bath at 100^{o}C for 5 min. The reaction mixture was allowed to cool to room temperature, after which 3 ml of double distilled water was added. A 200 µl aliquot of the reaction mixture was then transferred to a 96-well microplate and the absorbance read at 540 nm using a Synergy™ H4 Hybrid microplate reader (Biotek™, Vermont, USA) set at 25^{o}C. A blank reading (addition of buffer to replace enzyme) was subtracted from each well. The enzyme activity was quantified by measuring the maltose equivalents released from starch at 540 nm. The pharmacological α-amylase inhibitor

acarbose was assayed the same way and used as a positive control. The α-amylase inhibitory activity (%) was calculated using the equation:

Inhibition (%) = [((Ac − (As − Asb)) / Ac] * 100

Ac = Absorbance of the control

As = Absorbance of the sample

Asb = Absorbance of the sample blank

4.2.4.2 α-Glucosidase Inhibition Assay

α-glucosidase inhibitory activity was assayed according to previously described methods (Shobana et al., 2009; Ranilla et al., 2010; Uraipong and Zhao, 2016b) with slight modifications. Briefly, 300 mg of rat intestinal acetone powder was homogenized in 9 ml of 0.9% NaCl solution and centrifuged at 12,000 x g for 30 min; the clear supernatant was used as a source of α-glucosidase enzyme. Lyophilized protein hydrolysates and membrane fractions were redissolved in 0.1 M sodium phosphate buffer, pH 6.9. Fifty µl of samples (final peptide concentration = 5-20 mg/ml) were pre-mixed with 50 µl of α-glucosidase enzyme (final concentration = 8.33 mg/ml) in a 96-well microplate and incubated at 37^{o}C for 10 min. Following incubation, 100 µl of 5 mM 4-nitrophenyl α-D-glucopyranoside (PNP-glycoside) solution (in 0.1 M sodium phosphate buffer, pH 6.9) was added to each well and absorbance read continuously at 405 nm for 30 min (at every 30 seconds interval) using a microplate reader set at 37^{o}C. A blank reading (no enzyme added) was subtracted from each well. α-glucosidase activity was quantified by measuring the p-nitrophenol released from the PNP-glycoside at 405nm The pharmacological α-

glucosidase inhibitor acarbose was assayed using the same protocol and served as a positive control.

The α-glucosidase inhibitory activity (%) was calculated using the equation:

Inhibition (%) = [((Ac -Acb) − (As -Asb)) / [Ac − Acb] *100

Ac = Absorbance of the control

Acb = Absorbance of the control blank

As = Absorbance of the sample

Asb = Absorbance of the sample blank

4.2.4.3 Trypsin Inhibition Assay

Trypsin inhibitory activity was determined following the method described by Souza et al. (2016). Briefly, 200 µl of trypsin (dissolved in 20 mM Tris-HCl buffer, pH = 7.5; final concentration = 60 µg/ml) was premixed with 200 µl of samples (re-dissolved in the above buffer; final peptide concentration = 1-10mg/ml) and incubated for 5 min at 37°C. The reaction was started by the addition of 500 µl of 1 mM BApNA (N-Benzoyl-D-L arginine paranitroanilide) prepared in Tris-HCl buffer (pH 7.5) that contained 1% (v/v) dimethyl sulfoxide. Following incubation for 10 min at 37°C, the reaction was terminated by the addition of 100 µl of 30 % (v/v) acetic acid. A 200 µl aliquot of the reaction mixture was transferred to a 96-well microplate and the absorbance measured using a microplate reader at 410 nm. Trypsin inhibitory activity was determined by measuring the release of p-nitroaniline from the substrate

BApNA. 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) was used as a positive control. The 50% inhibition concentration (IC_{50}) values of the protein hydrolysates and membrane fractions were obtained by non-linear regression analysis of a plot of trypsin percentage inhibition versus the sample concentrations using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

The trypsin inhibitory activity (%) was calculated using the equation:

Inhibition (%) = [Ac − As] /Ac] *100

Ac = Absorbance of the control

As = Absorbance of the sample

4.2.4.4 Chymotrypsin Inhibition Assay

Chymotrypsin inhibitory activity was determined following the method described by Abd El-latif (2015). Briefly, 400 µl of chymotrypsin (dissolved in 0.01 M Tris-HCl buffer containing 0.02 M $CaCl_2$, pH=8; final concentration = 20 µg/ml) and 400 µl of samples (re-dissolved in the same buffer; final peptide concentration = 2-6 mg/ml) were pre-mixed and incubated for 15 min at 37ºC. The reaction was started by the addition of 1 ml of 1 mM BTpNA (N-Benzoyl-L-tyrosine p-nitroanilide) prepared in 0.01 M Tris-HCl buffer containing 0.02 M $CaCl_2$ and 40% (v/v) ethanol, pH 8.0. Following incubation for 15 min at 37ºC, the reaction was terminated by the addition of 200 µl of 30 % (v/v) acetic acid. A 200 µl aliquot of the reaction mixture was transferred to a 96-well microplate and the absorbance measured using a microplate reader at 410 nm. Chymotrypsin inhibitory activity was determined by measuring the release of p-nitroaniline

from the substrate, BTpNA. 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) was used as a positive control. The chymotrypsin inhibitory activity (%) was calculated using the equation:

Inhibition (%) = [Ac − As] /Ac] x 100

Ac = Absorbance of the control

As = Absorbance of the sample

4.2.4.5 Pancreatic Lipase Inhibition Assay

Pancreatic lipase inhibitory activity was assayed following the method described by Tang et al. (2016) with slight modifications. Pancreatic lipase inhibitory activity was determined by measuring the release of 4-methylumbelliferone (4-MU) from the substrate 4-methylumbelliferyl oleate (4-MU oleate). A 25 µl aliquot of samples (final peptide concentration = 5-15 mg/ml) dissolved in Tris buffer (13 mM Tris-HCl, 150 mM NaCl and 1.3 mM $CaCl_2$, pH = 8) and 225 µl of a 0.5 mM 4-MU oleate solution were mixed in a 96-well microplate and incubated for 15 min at 37°C. After incubation, 25 µl of pancreatic lipase solution (final concentration = 3.125 U/ml) was added to start the enzyme reaction and then incubated at 37ºC for 1 hr. After incubation, the amount of 4-methylumbelliferone released by the lipase was measured with a microplate reader at a wavelength of 400 nm. The pharmacological pancreatic lipase inhibitor (orlistat) was used as a positive control. The 50% inhibition concentration (IC_{50}) values of the protein hydrolysates and membrane fractions were obtained by non-linear regression analysis of a plot of pancreatic lipase

percentage inhibition versus the sample concentrations using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

The pancreatic lipase inhibitory activity (%) was calculated using the equation:

Inhibition (%) = [Ac − As] /Ac] * 100

Ac = Absorbance of the control

As = Absorbance of the sample

4.2.5 Kinetics of Enzyme Inhibition

The mode of inhibition of the enzymes α-amylase, α-glucosidase, trypsin, and chymotrypsin by protein hydrolysates and membrane fractions were determined by studying the kinetics of enzyme reaction measured at various substrate and peptide concentrations using the previously described methods. The inhibition pattern was then determined from the double reciprocal Lineweaver-Burk plots, in which the inverse of the initial rate was plotted against the inverse of the substrate concentration in the presence or absence of the peptides. PNP-glycoside in the concentration range 0.2-3 mM; soluble starch in the range 0.04-0.5%; BApNa in the range of 0.5-2.5 mM and BTpNa in the range of 0.05-0.3 mM were used as substrates for α-glucosidase, α-amylase, trypsin, and chymotrypsin, respectively. The range of concentrations of peptides used for the inhibitory kinetics were 50-225 µg/ml (α-amylase), 5-20 mg/ml (α-glucosidase), 0.25-2.5 mg/ml (trypsin) and 2-8 mg/ml (chymotrypsin). The Lineweaver-Burk plots and kinetic parameters were obtained using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

4.2.6 Size Exclusion Fast Protein Liquid Chromatography (FPLC) Analysis

Determination of the molecular weight distribution of yellow pea protein isolate and its four enzymatic hydrolysates was carried out using previously described methods (Alashi et al., 2014; He et al., 2014). In summary, the isolate (20 mg/ml) and hydrolysates (10 mg/ml; except for pepsin hydrolysate at 15 mg/ml) were dissolved in 0.05 M sodium phosphate buffer containing 0.15 M NaCl (pH 7.2) and then filtered through a 0.2 μm syringe filter. The filtered samples were then injected onto a Superdex Peptide 10/300 (10 x 300 mm) GL column attached to an FPLC system (AKTA purifier), equipped with a UV detector. The injection volume was 1 ml and the mobile phase was 0.05 M sodium phosphate buffer containing 0.15 M NaCl (pH 7.2) that was run isocratically at 0.5 ml/min flow rate. Elution was monitored at 214 nm using the UV detector. Approximate molecular weights of hydrolysates were determined using Cytochrome C (12.384 kDa), Aprotinin (6.512 kDa), Vitamin B12 (1.855 kDa) and Glycine (0.075 kDa) as molecular weight standards. Peptide sizes of the samples were estimated from a plot of log MW (molecular weight) versus elution volume of the standard protein/amino acid.

4.2.7 Mass Spectrometry (MS) Analysis of Yellow Pea Protein Hydrolysates

The MS analysis of the protein hydrolysates was done according to a previously described method (Malomo and Aluko, 2016a) with slight modifications. Briefly, a 1 μg/μl aliquot of the hydrolysates (dissolved in deionised water that contained 0.1% formic acid) was directly infused into an Absciex QTRAP® 6500 mass spectrometer system (Absciex Ltd., Foster City, CA, USA) coupled with electrospray ionization source. The following parameters were used: ion spray voltage, 5.5 kV; temperature, 200 °C; ion source gas, 20; declustering potential, 90; entrance potential, 10 and a flow rate of 20 μL/min for 1.6 minutes in the positive ion mode. Potential amino acid sequences of the peptides were determined using the MS data and ExPASy Proteomics Server FindPept tool based on the primary structure of pea seed storage proteins (Swiss Institute of Bioinformatics, Switzerland).

4.2.8 Statistical Analysis

All assays were conducted in triplicate and data were analyzed as the mean values ± standard deviation (SD). The mean values were examined using analysis of variance (ANOVA) and then compared using Duncan's multiple range test with significant differences accepted at $p < 0.05$. All analyses were conducted using Statistical Package for the Social Science (SPSS) 16.0.

5. RESULTS AND DISCUSSION

5.1 Protein Yield (%) of yellow field pea protein hydrolysates and fractions.

The protein yield (expressed as a percentage) indicates the efficiency of the enzymatic hydrolysis process. Usually, a higher yield of peptides is the expected outcome for increased protein breakdown (Girgih et al., 2011b). From the data presented in Table 3, the results show that the yield (%) of the membrane fractions were significantly lower ($p<0.05$) in comparison to the unfractionated hydrolysates in all four enzymatic groups. The observed result is as expected, because the unfractionated hydrolysates consist of several peptides, resulting in a higher yield, while the fractionated peptides have been separated into specific molecular weights, thus their yield will be lower. The yield of the fractionated peptides and retentates were lower than that of the unfractionated hydrolysates because of loss during transfer after freeze-drying. Amongst the four enzymes used for hydrolysis, alcalase unfractionated hydrolysate had the highest yield (58.21 ± 3.78 %), while the trypsin unfractionated hydrolysate had the lowest yield (44.03 ± 1.66 %). This may be because of the broad and random specificity of the alcalase enzyme (Osman et al., 2016), producing various peptides of varying sizes and composition. Results also show that the retentates had higher yield compared to the peptide fractions in the chymotrypsin, pepsin and trypsin groups. This is because the retentates contained peptides of high molecular weight fractions (>10 kDa) and all other protein moieties retained in the samples. However, an exception was noted in the alcalase group, where the <1 kDa had the highest yield.

Table 3. Protein Yield (%) of yellow field pea protein hydrolysates and ultrafiltration fractions.

	Alcalase	**Chymotrypsin**	**Pepsin**	**Trypsin**
Hydrolysate	58.21 ± 3.78^{c}	48.18 ± 0.89^{c}	49.56 ± 2.14^{d}	44.03 ± 1.66^{c}
< 1 kDa	13.23 ± 0.94^{b}	6.41 ± 0.29^{a}	8.41 ± 0.04^{ab}	6.96 ± 0.18^{a}
1-3 kDa	12.14 ± 0.62^{ab}	8.66 ± 0.45^{a}	4.64 ± 0.6^{a}	5.50 ± 0.51^{a}
3-5 kDa	9.59 ± 1.03^{ab}	6.01 ± 0.34^{a}	4.93 ± 0.02^{a}	5.40 ± 0.67^{a}
5 -10 kDa	7.73 ± 0.96^{a}	9.81 ± 1.92^{a}	9.48 ± 0.64^{b}	8.04 ± 0.09^{a}
Retentate	10.19 ± 1.74^{ab}	13.90 ± 3.07^{b}	18.55 ± 3.31^{c}	14.34 ± 1.71^{b}

Results are presented as mean ± standard deviation (n=3). For each column, mean values that contain different letters are significantly different at $p < 0.05$.

5.2 Protein Content of yellow field pea protein isolate and fractions.

The protein content of the yellow field pea protein isolate was determined to be 70.35% ± 1.76, which is similar to the 74.55% reported by Barbana and Boye (2010). However, some studies have reported higher protein contents as up to 80-82% (Aluko et al., 2009; Adebiyi and Aluko, 2011; Aluko et al., 2015). The protein content of the hydrolysates and fractions after enzymatic hydrolysis is presented in Table 4.

The protein content of the hydrolysates and peptide fraction varied considerably. This is because the enzymes used in the hydrolysis are all endopeptidases, which hydrolyze the peptide bonds, usually at specific residues, producing large peptides, in turn resulting in an increased protein content (Silva et al., 2014). The results obtained for the unfractionated hydrolysates in this study are comparable to results reported in a previous work (Humiski and Aluko, 2007). The 3-5, 5-10 kDa fractions, and retentate had much higher protein contents than the <1 and 1-3 kDa fractions because LMW non-protein components such as salts and soluble sugars were removed during the previous ultrafiltration process, thus the protein contents were increased.

Table 4. Protein content (%) of yellow field pea protein hydrolysates and ultrafiltration fractions.

	Alcalase	**Chymotrypsin**	**Pepsin**	**Trypsin**
Hydrolysate	78.05 ± 0.01^{c}	78.52 ± 1.74^{b}	87.17 ± 1.02^{b}	71.54 ± 0.02^{c}
< 1 kDa	75.71 ± 0.1^{b}	59.59 ± 0.40^{a}	86.72 ± 0.17^{b}	59.35 ± 0.34^{a}
1-3 kDa	80.79 ± 0.15^{d}	85.14 ± 0.15^{c}	61.41 ± 0.42^{a}	65.01 ± 0.7^{b}
3-5 kDa	83.19 ± 0.14^{f}	93.25 ± 0.02^{d}	88.55 ± 1.68^{b}	75.90 ± 0.92^{d}
5 -10 kDa	81.31 ± 0.06^{e}	97.41 ± 1.03^{e}	93.44 ± 0.62^{c}	85.47 ± 0.75^{f}
Retentate	74.33 ± 0.31^{a}	95.11 ± 0.24^{d}	96.94 ± 3.30^{c}	82.07 ± 0.81^{e}

Results are presented as mean ± standard deviation (n=3). For each column, mean values that contain different letters are significantly different at $p < 0.05$.

5.3 Molecular Weight determination of pea protein isolate and hydrolysates

The molecular weight (MW) distributions of the yellow field pea protein isolate and its four enzymatic hydrolysates were determined by FPLC. Peptides with high molecular weight will elute early while those with low molecular weights will elute at a later time. This is because the low molecular weight peptides are able to penetrate the small pores present in the stationary phase, which reduces elution rate while the high molecular weight peptides cannot and are rapidly eluted from the column. Four known compounds were used as MW standards to calibrate the FPLC column and their chromatograms are shown in Figure 2. Determination of the molecular weight of hydrolysates is a key criterion in considering the potential bioactivities of peptides. This is because low molecular weight peptides have better chances of escaping structural degradation within the gastrointestinal tract and be absorbed into blood circulation than larger peptides. In addition, small-sized peptides tend to fit and bind tightly into an enzyme's active site and produce stronger inhibitory effects than larger peptides. As a result, one of the objectives of protein hydrolysate production is to ensure the abundance of small-sized peptides in order to enhance potency against metabolic targets (Malomo and Aluko, 2016b). Results of the individual size-exclusion chromatograms of the isolate and its four enzymatic hydrolysates are shown in Figure 3, while a comparative chromatogram is shown in Figure 4. The MW of the isolate ranged between 0.24-29.09 kDa, alcalase hydrolysate ranged between 0.85-4.98 kDa, chymotrypsin hydrolysate ranged between 0.41-9.14 kDa, pepsin hydrolysate ranged between 0.88-21.54 kDa and trypsin hydrolysate was determined to be between 0.85-13.57 kDa. The alcalase hydrolysate showed more low MW peaks, indicating greater proteolytic efficiency against the pea protein when compared to chymotrypsin, trypsin, and pepsin. However, protein

hydrolysate activity is also highly dependent on the type of peptides produced and not just the hydrolysis efficiency. More so, the peaks produced by the proteases were different in nature and this could have been the basis for their *in vitro* enzyme inhibitory activities. This is supported by a past study, which reported that the structure (size) and activity of peptides are largely dependent on its method of production, i.e., type of enzyme used (He et al., 2013). The results obtained in this study for the alcalase hydrolysate is in the range of 0.027-6.47 kDa that was reported for canola protein hydrolysates (Alashi et al., 2014). Alcalase produced smaller molecular weight peptides probably due to its broad specificity during protein digestion (Segura Campos et al., 2013; Malomo and Aluko, 2016b; Humiski and Aluko, 2007).

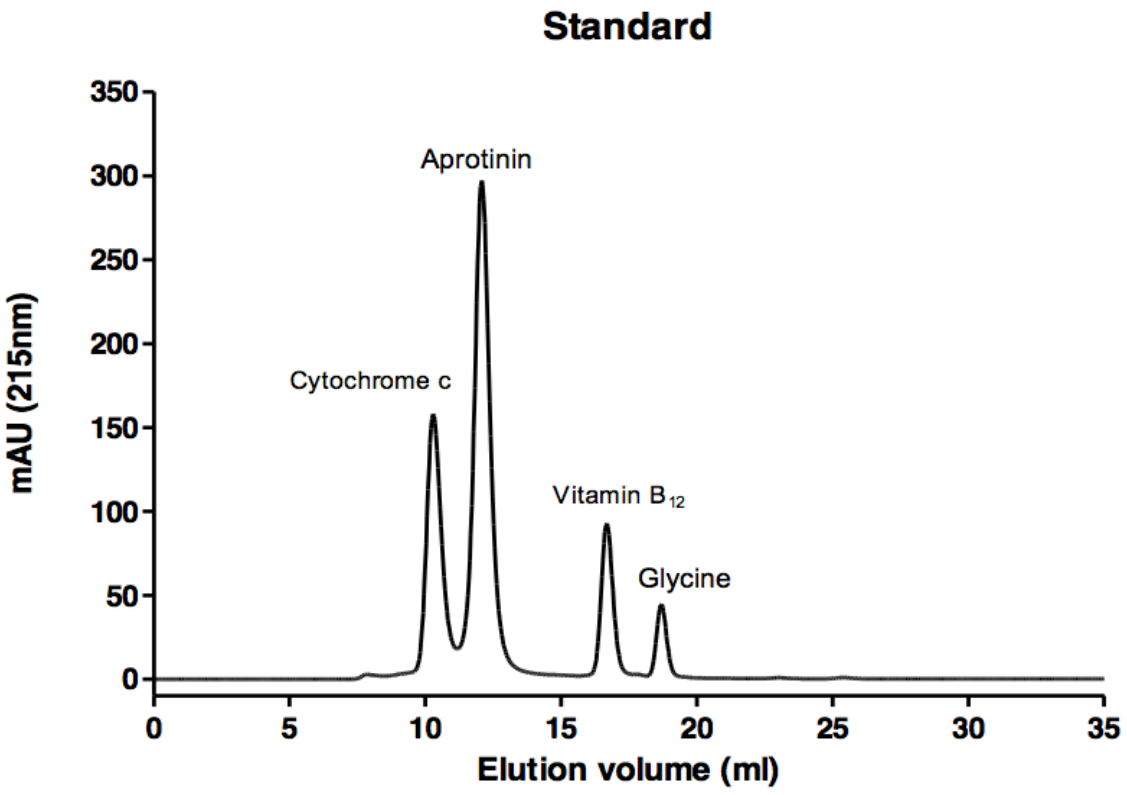

Figure 2. Chromatogram of standards used for the calibration of FPLC

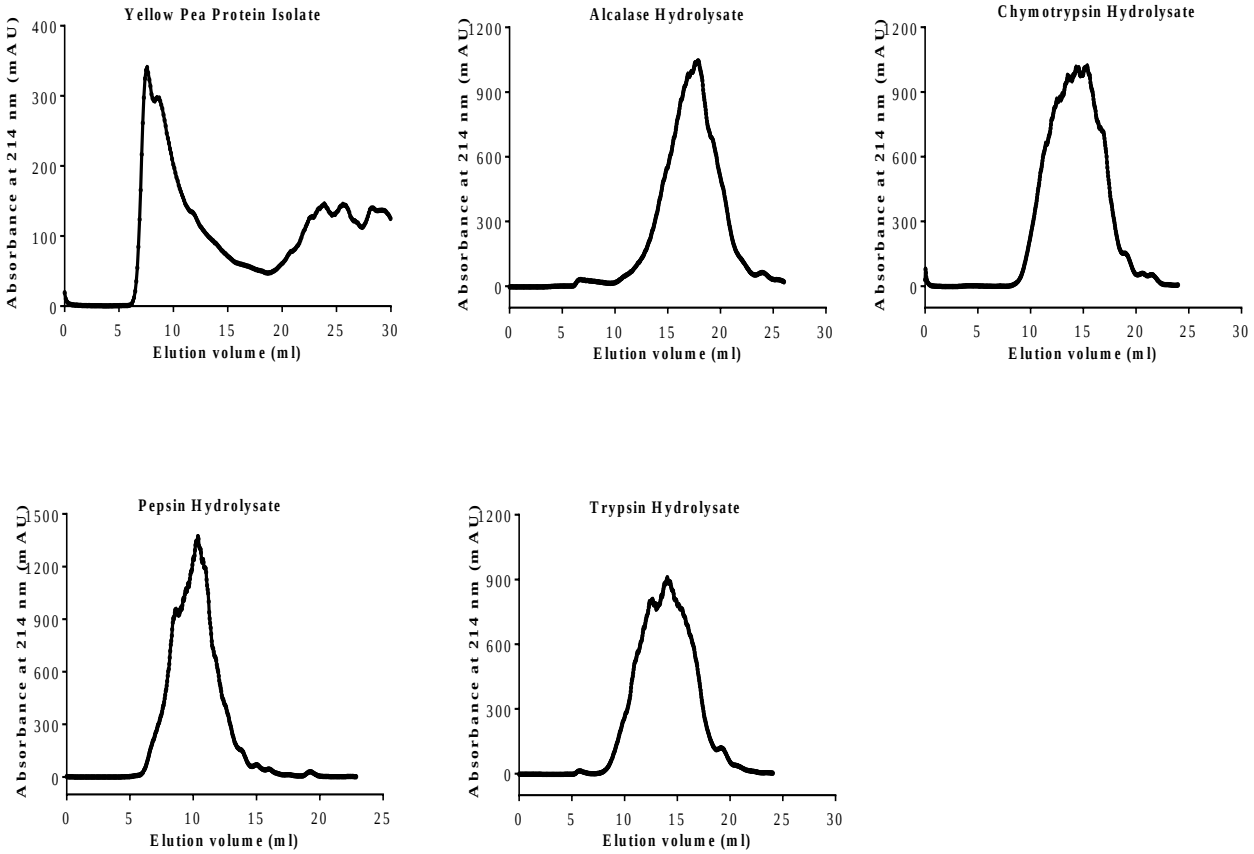

Figure 3. Individual size – exclusion chromatograms of yellow field pea protein isolate and enzymatic hydrolysates

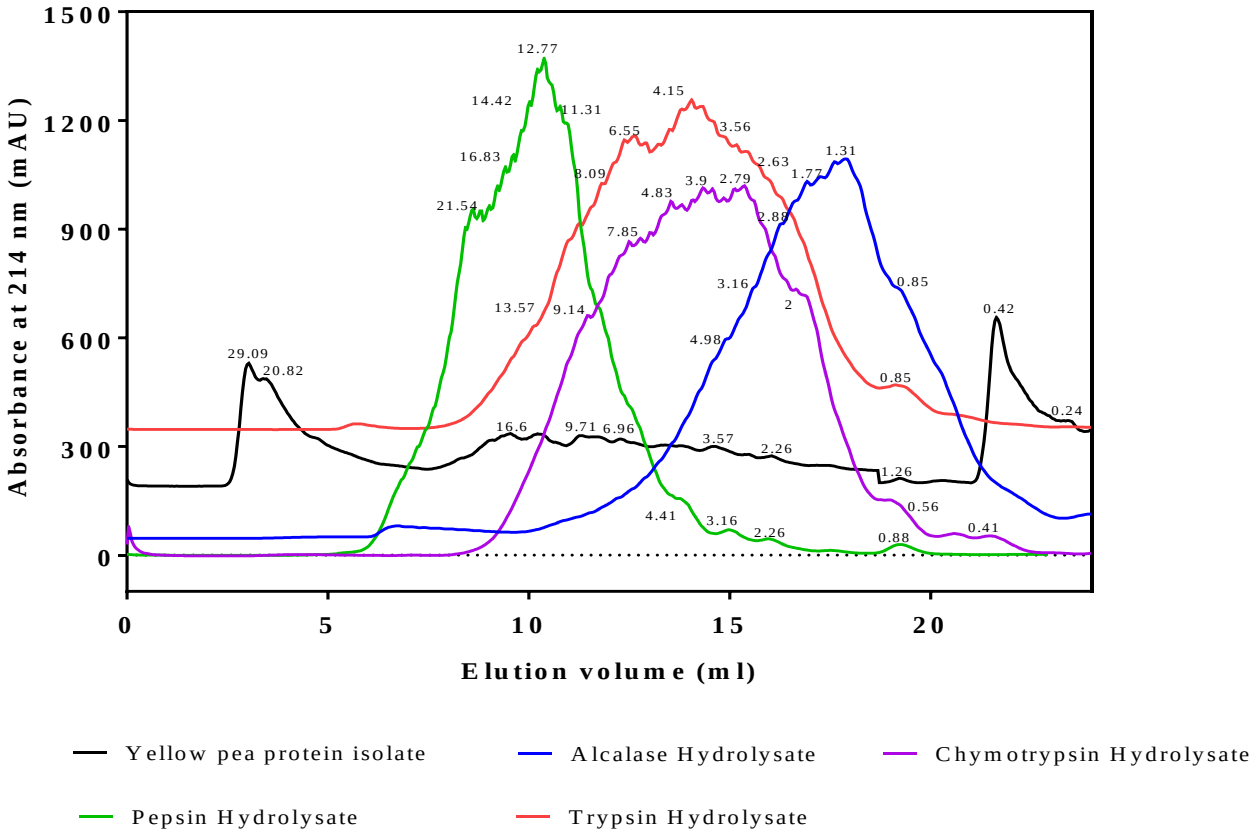

Figure 4. Comparative gel-permeation chromatograms of yellow field pea protein isolate and enzymatic hydrolysates after passage through a Superdex Peptide12 10/300 GL column.

5.4 *In vitro* Enzyme Inhibition Assays

5.4.1 α-Amylase Inhibition

α-amylase is one of the major enzymes involved in the digestion of dietary starch, releasing oligosaccharides that can be further broken down into glucose which is rapidly absorbed by the body. Therefore, α-amylase activity inhibition is regarded as an effective strategy for managing diabetes (Gropper and Smith, 2012). Results of the α-amylase inhibition activity by protein hydrolysates and peptide fractions are shown in Figure 5. The inhibitory activity of the hydrolysates and fractions were conducted at concentrations of 50, 100, 200 and 225 μg/ml and the concentration at which the samples had the highest inhibitory activity was 225 μg/ml. Inhibitory activity increased with increasing peptide concentration, indicating a dose-dependent effect. The inhibitory activity of the samples against α-amylase was much lower in comparison to the standard acarbose (acarbose = 73.65 ± 0.46% at 9 μg/ml). This is not surprising since acarbose is a purified synthetic inhibitor of α-amylase whereas the YFPP and its associated hydrolysates and peptide fractions are crude mixtures of proteins/peptides and probably non-protein components. Of all the samples at the highest tested concentration (225 μg/ml), the Chymotrypsin 1-3 kDa fraction (C 1-3 kDa) had the highest inhibitory activity of 30.52 ± 0.01%. With regards to each group, Alcalase 1-3 kDa (A 1-3 kDa) had the highest inhibitory activity of 27.65 ± 0.01%. Similarly, the Chymotrypsin 1-3 kDa fraction (C 1-3 kDa) was the most potent within the chymotrypsin group with an inhibitory activity of 30.52 ± 0.01%. However, for the pepsin group, the 3-5 kDa (P 3-5 kDa) fraction had the highest inhibitory activity of 29.07± 0.01%, while the unfractionated trypsin hydrolysate (YPTH) had the highest inhibitory activity of 30.39 ± 0.01% in the trypsin group. The mean inhibitory activities were calculated for the

protein samples by taking an average of the inhibition values at all the tested concentrations. The results are shown in Table 5. Based on the mean inhibitory activities of the sample, Alcalase 1-3 kDa (17.79 ± 0.05%) and Alcalase 5-10 kDa (17.81 ± 0.02%) fractions had the highest mean inhibitory activity within the alcalase group. In the chymotrypsin group, the Chymotrypsin 5-10 kDa fraction (18.07 ± 0.03%) had the highest mean inhibitory activity. Within the pepsin group, the 3-5 kDa fraction that had the highest value (16.19 ± 0.16%), while in the trypsin group, the trypsin < 1 kDa peptide fraction had the highest mean inhibitory value (17.12 ± 0.03%). The results indicate that fractionation improved the α-amylase inhibitory activity of the samples. This is because, in all the groups, the LMW peptides had better mean inhibitory activity against α-amylase in comparison to the unfractionated hydrolysates. A possible reason for this observation could be that low molecular weight peptides are smaller in size and therefore, can easily bind to the active site of the enzyme resulting in a higher inhibitory activity compared to the unfractionated hydrolysates which contained different and larger sizes of peptides, thus they are unable to bind tightly to the enzyme (Malomo and Aluko, 2016b). Another possible explanation could be due to the fact the unfractionated hydrolysates contain peptides which may have an antagonistic effect (Girgih et al., 2015a). However, when separated by ultrafiltration, there is a loss of the antagonistic effect, in other words, the use of ultrafiltration membrane helped to enrich specific peptide fractions that have potent inhibitory activity against α-amylase (Segura Campos et al., 2013). This is corroborated by some studies, which report that LMW peptides (specifically, < 1 and < 3 kDa) from pinto bean had the highest α-amylase inhibitory activity of 49.9 ± 1.4% and 62.1 ± 3.49%, respectively (Oseguera-Toledo et al., 2015; Ngoh and Gan, 2016).

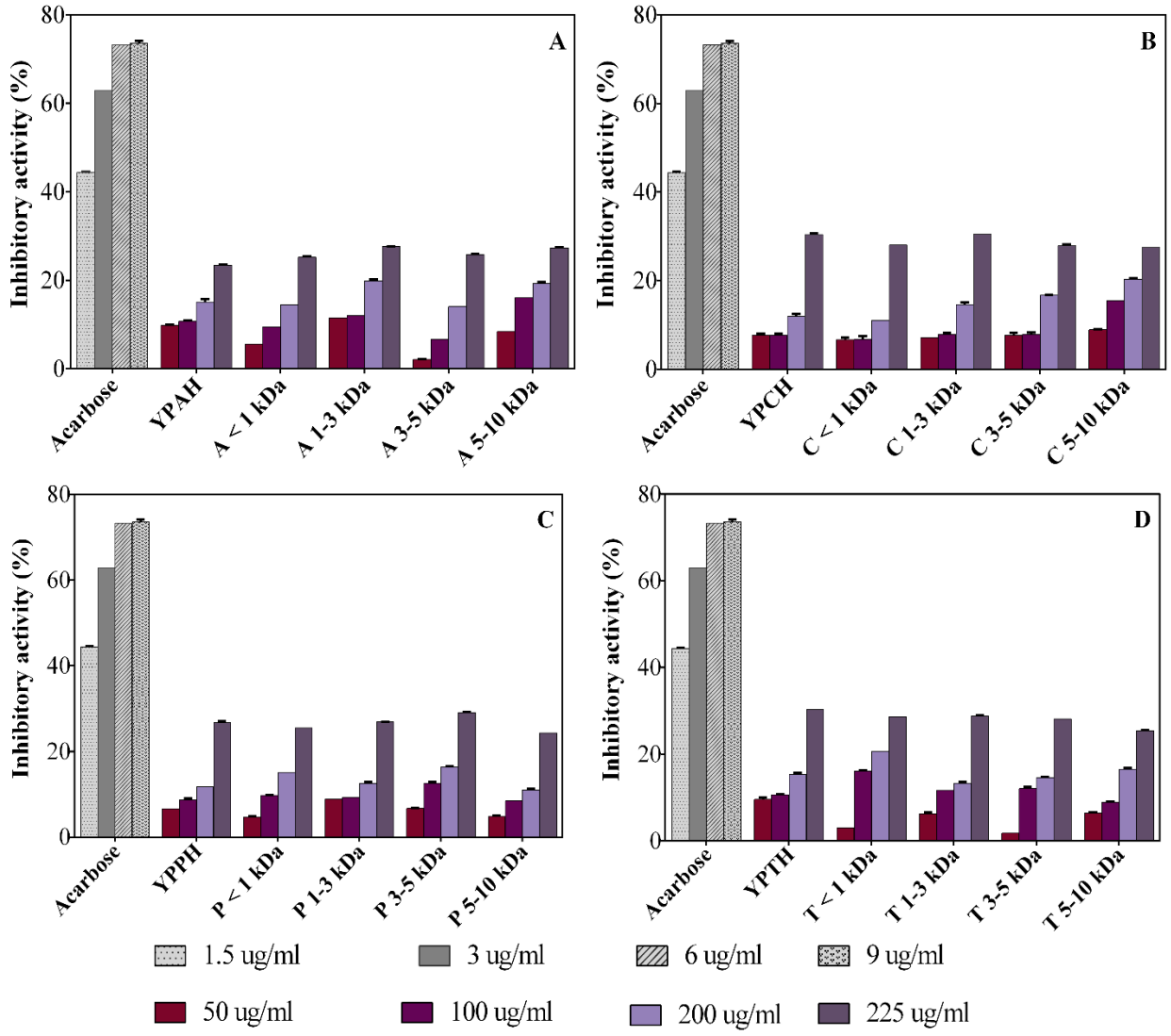

Figure 5. α-amylase inhibitory activity of yellow field pea protein hydrolysates and peptide fractions obtained with Alcalase (A), Chymotrypsin (B), Pepsin (C), and Trypsin (D). Results are presented as mean ± standard deviation (n=3).

Table 5. Mean α-amylase inhibitory activity (%) of yellow pea protein derived hydrolysates and fractions

	Alcalase	**Chymotrypsin**	**Pepsin**	**Trypsin**
Hydrolysate	14.77 ± 0.16^{c}	14.45 ± 0.39^{b}	13.47 ± 0.14^{b}	16.51 ± 0.21^{c}
< 1 kDa	13.72 ± 0.05^{b}	13.11 ± 0.35^{a}	13.76 ± 0.01^{b}	17.12 ± 0.03^{d}
1-3 kDa	17.79 ± 0.05^{d}	15.04 ± 0.2^{b}	14.37 ± 0.13^{c}	15.03 ± 0.07^{b}
3-5 kDa	12.21 ± 0.01^{a}	15.07 ± 0.35^{b}	16.19 ± 0.16^{d}	14.17 ± 0.13^{a}
5 -10 kDa	17.81 ± 0.02^{d}	18.07 ± 0.03^{c}	12.15 ± 0.02^{a}	14.34 ± 0.06^{a}

Results are presented as mean ± standard deviation (n=4). For each column, mean values that contain different letters are significantly different at $p < 0.05$.

5.4.2 α-Glucosidase Inhibition

α-glucosidase is another key membrane-bound enzyme located in the epithelium of the small intestine involved in starch digestion through the breakdown of oligo- and di-saccharides to produce glucose, which is then absorbed by the body. Therefore, inhibition of this enzyme is another effective strategy for lowering serum glucose level and ultimately managing disease symptoms associated with diabetes (Gropper and Smith, 2012). The result of the α-glucosidase inhibition activity by protein hydrolysates and fractions is shown in Figure 6. The inhibitory activity of the hydrolysates and fractions were conducted at concentrations of 5, 10 and 20 mg/ml and the concentration with highest inhibitory activity obtained was at 20 mg/ml. The inhibitory activities of the samples increased with increasing concentration, indicating a dose-dependent relationship. The inhibitory activity of peptide samples against α-glucosidase was comparable to that of the standard acarbose (acarbose = 66.05 ± 0.12% at 0.125 mg/ml). Based on the highest concentration tested (20 mg/ml), of all the samples, the chymotrypsin <1 kDa fraction (C < 1 kDa) had the highest inhibitory activity of 53.35 ± 2.78%. Results of a previous work have reported α-glucosidase inhibitory activity of 56% by brewer's spent grain protein hydrolysates, which is similar to the result obtained in this work (Lin et al., 2012). With regards to each group, Alcalase hydrolysate (YPAH) had the highest inhibitory activity of 38.39 ± 1.58% in the alcalase-derived hydrolysate and fractions group, whereas for the chymotrypsin group, the <1 kDa fraction (C < 1 kDa) was the most active with an inhibitory activity of 53.35 ± 2.78%. For the pepsin group, the 1-3 kDa (P 1-3 kDa) had the highest inhibitory activity of 44.74 ± 2.37% while in the trypsin group, trypsin hydrolysate (YPTH) was the most active with an inhibitory activity of 46.02 ± 3.67%.

However, based on the mean α-glucosidase inhibitory activity of the samples as shown in Table 6, the results indicate similar results as that of α-amylase, being that fractionation improved the α-glucosidase inhibitory activity of the samples. Results showed that in the alcalase group, the Alcalase 3-5 kDa fraction (27.91 ± 0.89%) had the highest mean inhibitory activity whereas for the other groups, the <1 kDa fraction had the highest mean inhibitory activity: chymotrypsin group (29.66 ± 0.85%), pepsin (33 ± 3.61%) and trypsin group (33.52 ± 0.09%). Similar to the α-amylase inhibition results, the results obtained from the α-glucosidase inhibition indicate that fractionated peptides (<1 and 3-5 kDa), which are of low molecular weights were more potent in inhibiting α-glucosidase in comparison to the unfractionated hydrolysates. This is due to the fact that the LMW peptides are smaller in size and therefore, can easily bind to the active site of the enzyme resulting in a greater inhibitory effect compared to the unfractionated hydrolysates which contain larger sizes of peptides and are unable to bind tightly to the enzyme (Malomo and Aluko, 2016b). Moreover, it has been noted that peptides of lower molecular weights tend to be better inhibitors of enzymes when compared to larger-sized peptides (Girgih et al., 2011a). These results are consistent with previous studies, which showed that LMW peptides are potent inhibitors of α-glucosidase, the <1 kDa peptide fraction derived from pinto Durango bean exhibited an inhibitory activity of 76.4 ± 0.5% against α-glucosidase (Oseguera-Toledo et al., 2015); while the <3 kDa peptide fraction from rice bran had an inhibitory activity of 47.9 % ± 2.6 against α-glucosidase (Uraipong and Zhao, 2016a).

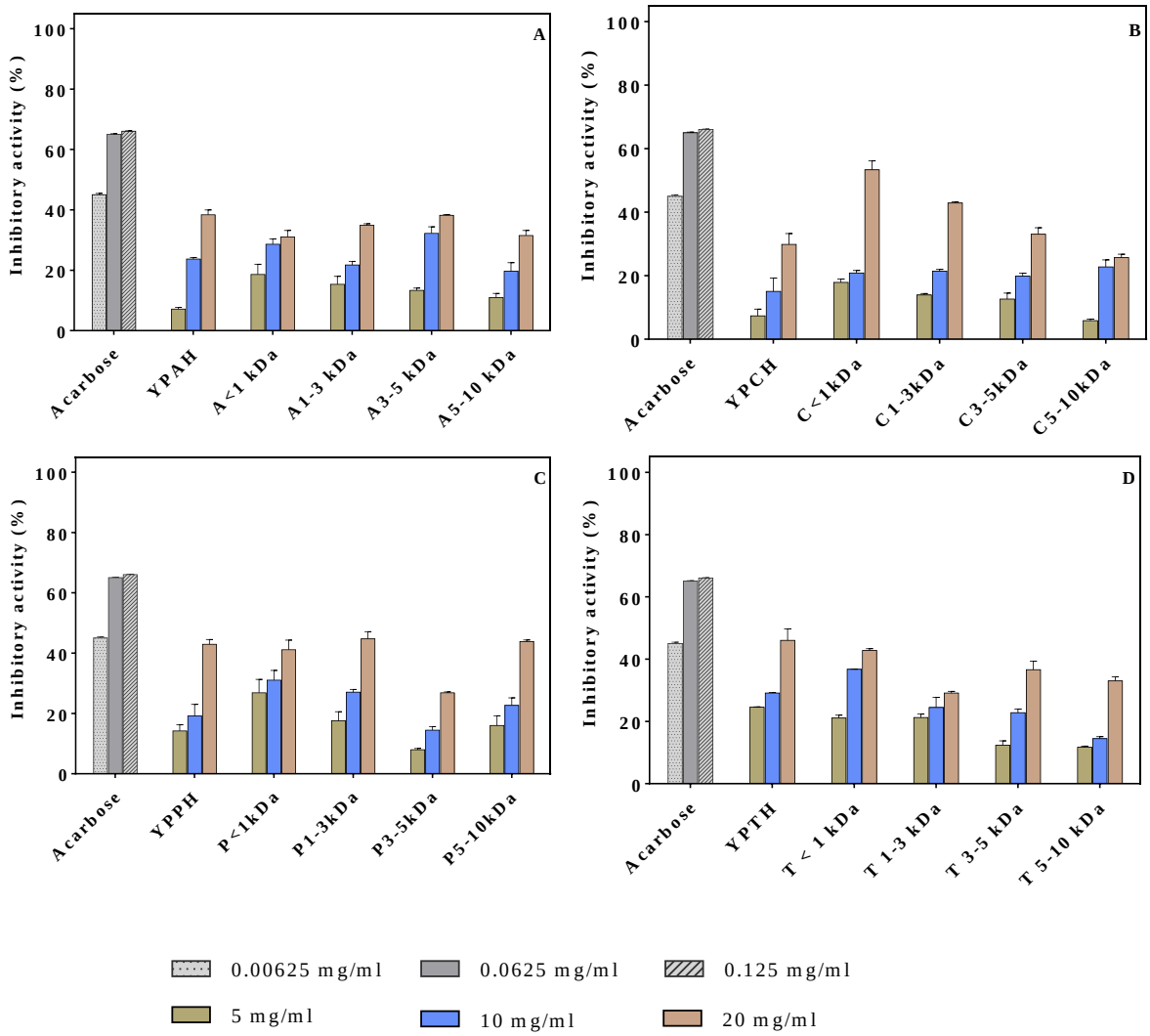

Figure 6. α-glucosidase inhibitory activity of yellow field pea protein hydrolysates and peptide fractions obtained with Alcalase (A), Chymotrypsin (B), Pepsin (C), and Trypsin (D). Results are presented as mean ± standard deviation (n=3).

Table 6. Mean α-glucosidase inhibitory activity (%) of yellow pea protein derived hydrolysates and fractions

	Alcalase	**Chymotrypsin**	**Pepsin**	**Trypsin**
Hydrolysate	23.07 ± 0.48^{b}	17.42 ± 3.2^{a}	25.43 ± 0.09^{b}	33.23 ± 1.19^{c}
< 1 kDa	26.09 ± 0.17^{c}	29.66 ± 0.85^{c}	33 ± 3.61^{c}	33.52 ± 0.09^{c}
1-3 kDa	23.99 ± 1.11^{b}	26.09 ± 0.22^{bc}	29.81 ± 0.5^{bc}	24.95 ± 0.87^{a}
3-5 kDa	27.91 ± 0.89^{c}	21.85 ± 1.62^{ab}	16.42 ± 0.45^{a}	23.66 ± 0.09^{a}
5 -10 kDa	20.69 ± 0.86^{a}	18.1 ± 0.55^{a}	27.51 ± 2.09^{b}	19.74 ± 0.52^{a}

Results are presented as mean ± standard deviation (n=3). For each column, mean values that contain different letters are significantly different at $p < 0.05$.

5.4.3 Trypsin Inhibition

Trypsin is one of the key human enzymes involved in protein digestion. It is an endopeptidase that is responsible for the cleavage of the interior peptide bonds of proteins and polypeptides (Erickson and Kim, 1990). Trypsin mainly cleaves peptide bonds on the carboxyl side of basic amino acids such as Arginine and Lysine (Erickson and Kim, 1990). Results of the IC_{50} (IC_{50} = the inhibitory concentration that reduces an enzyme's activity by 50%) values for the trypsin inhibition activity of pea protein hydrolysates and fractions are presented in Figure 7. It has been established that a lower IC_{50} value indicates a higher inhibitory activity. Generally, the results showed that the peptide fractions had lower IC_{50} values when compared to the unfractionated hydrolysates, except for pepsin-derived hydrolysate which had a lower IC_{50} in comparison to the other pepsin-derived peptide fractions. Overall, trypsin 5-10 kDa fraction had the lowest IC_{50} value of 2.14 ± 0.01 mg/ml. In the alcalase group, alcalase 5-10 kDa fraction had the lowest IC_{50} value of 3.14 ± 0.02 mg/ml whereas in the chymotrypsin group, the <1 kDa fraction had the lowest IC_{50} value of 2.63 ± 0.01 mg/ml. The unfractionated hydrolysate had the lowest IC_{50} value of 2.59 ± 0.04 mg/ml within the pepsin group, while in the trypsin group, the 5-10 kDa fraction had the lowest IC_{50} value of 2.14 ± 0.001 mg/ml. It was observed that the fractionated peptides (<1 and 5-10 kDa) were potent trypsin inhibitors. The results indicate that fractionation improved the inhibitory capacity of pea protein against trypsin activity. This may be because of the peptide antagonism within the unfractionated hydrolysates, which may have contributed to reduced trypsin inhibitory activity (Girgih et al., 2015a). Ultrafiltration separation led to reduced antagonistic effect in the fractionated peptides, hence the higher inhibitory effects of the peptide fractions. However, in comparison to the standard inhibitor of trypsin, AEBSF (IC_{50} = 0.003 ± 0 mg/ml), the IC_{50} values of pea protein hydrolysates and peptide fractions were significantly

($p<0.05$) higher, indicating that AEBSF is a more potent trypsin inhibitor than the unfractionated hydrolysates and peptide fractions. In comparison to this work, other studies have reported lower IC_{50} values of trypsin inhibitory peptides from other food sources. For instance, an isolated polypeptide from Chinese cabbage seeds inhibited trypsin with an IC_{50} value of 8.5 µM (Ngai and Ng, 2004). However, results from this work showed a much higher trypsin inhibitory activity than that reported in a study which identified 2 peptides with 50% trypsin inhibitory activity from *Opuntia joconostle* Weber (Aguirrezabala-Cámpano et al., 2013).

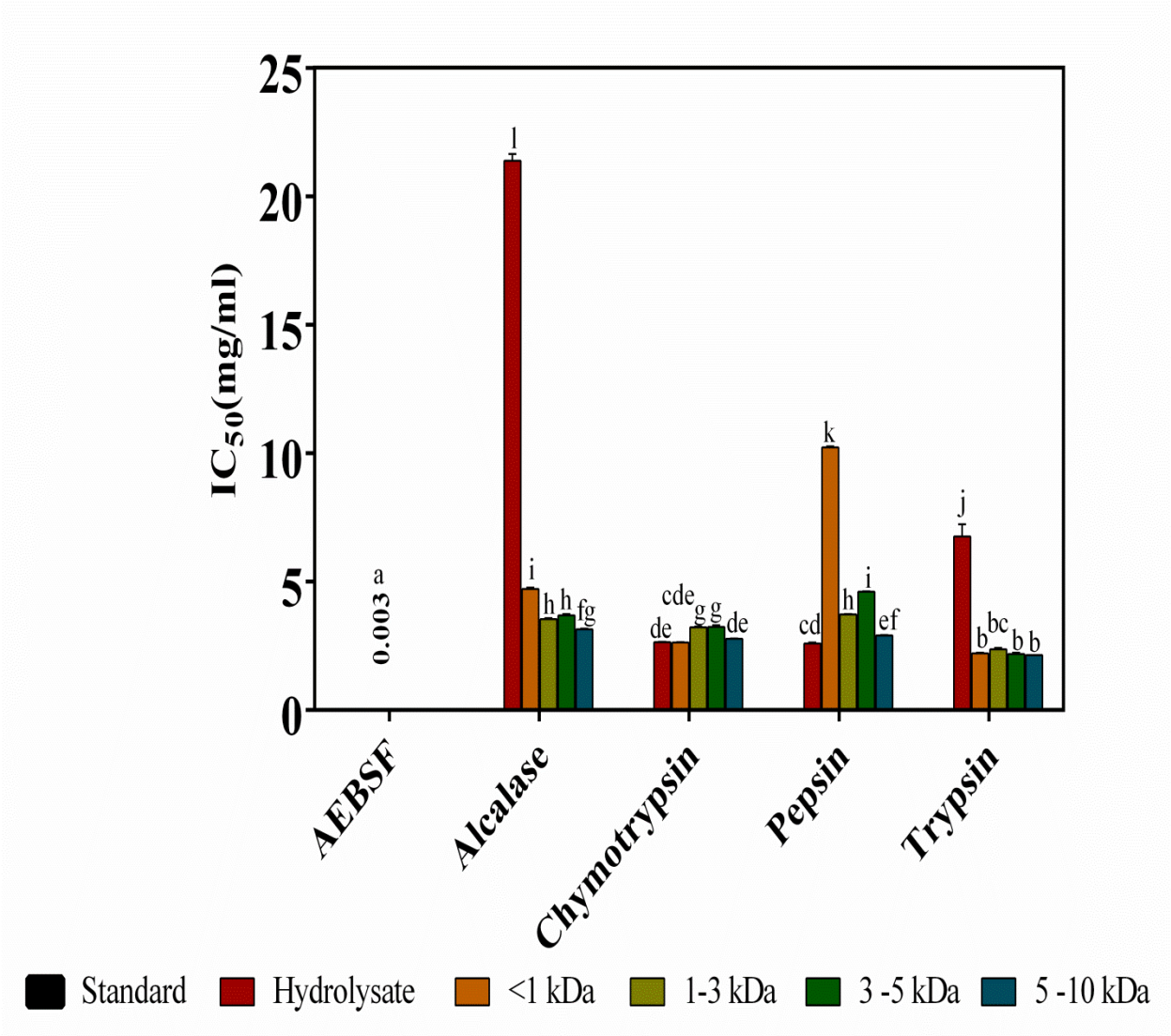

Figure 7. Inhibitory concentrations of yellow field pea protein hydrolysates and peptide fractions obtained with Alcalase, Chymotrypsin, Pepsin, and Trypsin that reduced 50% of trypsin's activity (IC_{50}). Results are presented as mean ± standard deviation (n=3). Samples with different letters are significantly different ($p < 0.05$).

5.4.4 Chymotrypsin Inhibition

Chymotrypsin is another key enzyme involved in protein digestion. Like its counterpart trypsin, it is also an endopeptidase that is responsible for the cleavage of the interior peptide bonds of proteins and polypeptides. However, its specificity differs from that of trypsin; it cleaves peptide bonds where the carbonyl group is an aromatic amino acid such as tyrosine, phenylalanine, and tryptophan (Erickson and Kim, 1990). Results of the chymotrypsin inhibition activity of pea protein hydrolysates and fractions are shown in Figure 8. The inhibitory activity of the hydrolysates and fractions were tested at concentrations of 2, 4 and 6 mg/ml; and the concentration at which the samples had the highest inhibitory activity was 6 mg/ml. The inhibitory activities of the samples increased with increasing peptide concentration, which indicates a dose-dependent relationship. Inhibitory activities of the samples against chymotrypsin were lower than that of the standard AEBSF (66.39 ± 0.5 % at 6 μg/ml). Based on the results of the highest concentration tested (6 mg/ml), of all the samples, the pepsin 5-10 kDa fraction (P 5-10 kDa) was noted to have the highest inhibitory activity of 48.13 ± 0.41 %. Concerning each group, Alcalase 3-5 kDa (A 3-5 kDa) had the highest chymotrypsin inhibitory activity of 29.68 ± 0.41 % within the alcalase group. In contrast, the 5-10 kDa fraction had the highest chymotrypsin inhibitory activity within the chymotrypsin group (38.90 ± 1.22%), pepsin group (48.13 ± 0.41%) and trypsin group (36.93 ± 1.04%). Some peptides such as the alcalase unfractionated hydrolysate (YPAH) and Pepsin <1 kDa fraction were noted to have stimulatory activities rather than inhibitory activities on the enzyme. This indicates that these two peptides did not inhibit the substrate binding to the enzyme, but facilitated the binding of the substrate to the enzyme.

Based on the mean chymotrypsin inhibitory activity shown in Table 7, the results showed that the fractionated and LMW peptides had better chymotrypsin inhibitory activity in comparison to

the unfractionated hydrolysates. In the alcalase, pepsin and trypsin group, the 5-10 kDa fraction had the highest mean inhibitory activity (alcalase: 21.05 ± 0.79%; pepsin group: 37.44 ± 0.26% and trypsin: 30.28 ± 0.56%). However, an exception was noted in the chymotrypsin group, the results showed that the chymotrypsin hydrolysate had the highest mean inhibitory activity (28.4 ± 0.47%), but this was closely followed by the Chymotrypsin 5-10 kDa (27.88 ± 0.59) and the Chymotrypsin 3-5 kDa (27.77 ± 0.6). The statistical analysis of these three samples showed no significant difference ($p = 0.247$). The results indicate that once again, fractionation improved the chymotrypsin inhibitory activity of the samples and that peptide fraction of 5-10 kDa were potent chymotrypsin inhibitors than the unfractionated hydrolysates.

Table 7. Mean chymotrypsin inhibitory activity (%) of yellow pea protein derived hydrolysates and fractions

	Alcalase	**Chymotrypsin**	**Pepsin**	**Trypsin**
Hydrolysate	-4.22 ± 0.07^{a}	28.4 ± 0.47^{c}	27.9 ± 0.29^{d}	17.53 ± 0.02^{b}
< 1 kDa	8.18 ± 0.61^{b}	21.33 ± 0.05^{b}	-2.14 ± 0.42^{a}	15.45 ± 0.13^{a}
1-3 kDa	14.27 ± 0.19^{c}	20 ± 0.39^{a}	14.46 ± 0.62^{b}	20.04 ± 0.14^{c}
3-5 kDa	19.78 ± 0.26^{d}	27.77 ± 0.6^{c}	20.25 ± 0.78^{c}	22.35 ± 0.49^{d}
5 -10 kDa	21.05 ± 0.79^{e}	27.88 ± 0.59^{c}	37.44 ± 0.26^{e}	30.28 ± 0.56^{e}

Results are presented as mean ± standard deviation (n=3). For each column, mean values that contain different letters are significantly different at $p < 0.05$.

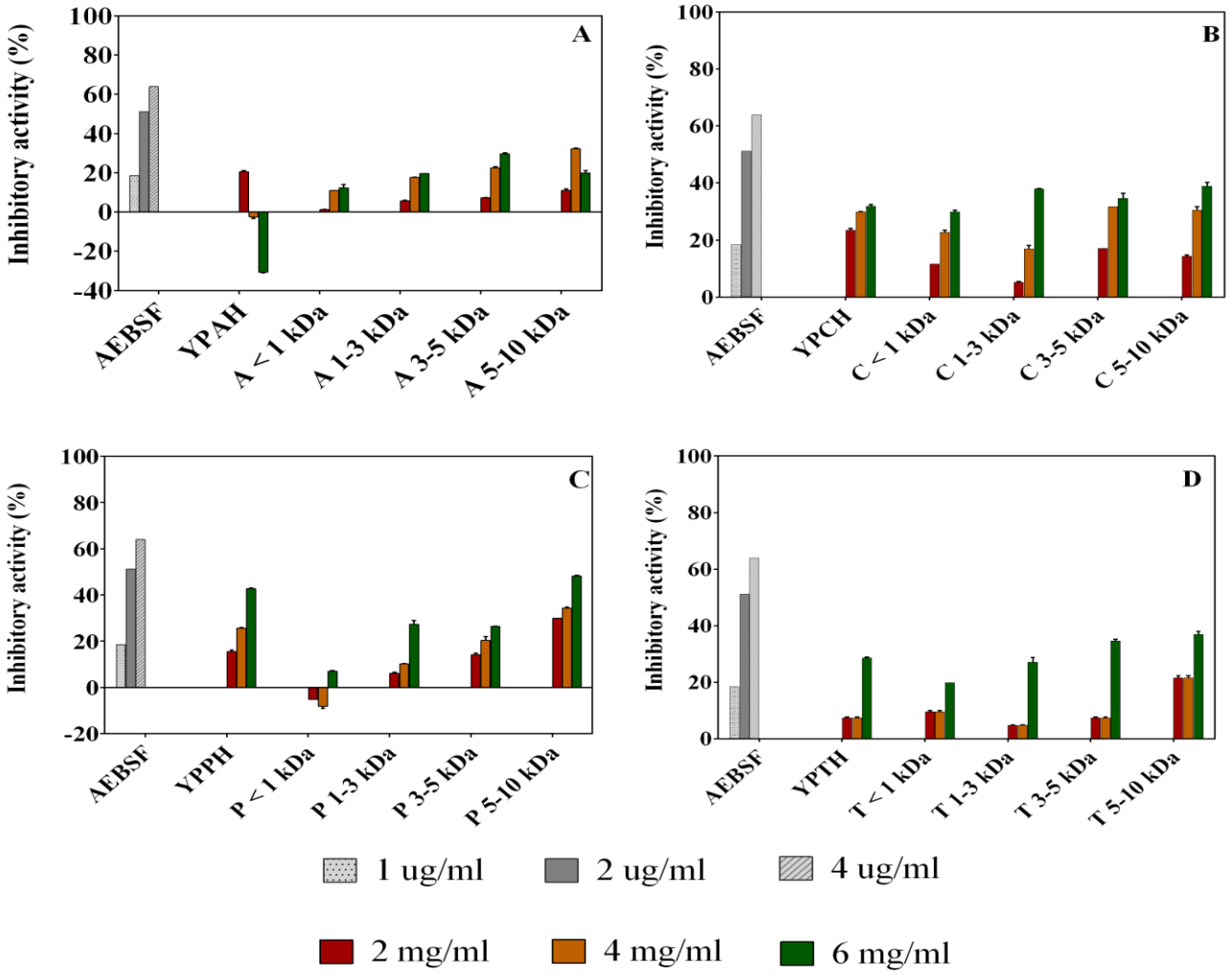

Figure 8. Chymotrypsin inhibitory activity of yellow field pea protein hydrolysates and peptide fractions obtained with Alcalase (A), Chymotrypsin (B), Pepsin (C), and Trypsin (D). Results are presented as mean ± standard deviation (n=3).

5.4.5 Pancreatic Lipase Inhibition

Pancreatic lipase is a key enzyme responsible for the intestinal digestion of dietary triacylglycerols, a major source of excess calorie intake. Inhibition of pancreatic lipase causes a reduction in the efficiency of fat absorption in the small intestine, in turn leading to modest long-term reductions in body weight (Lunder et al., 2005). Therefore, the suppression or delay of triacylglycerol digestion and absorption through the inhibition of lipase activity has been targeted for the development of anti-obesity agents (Lunagariya et al., 2014). The result of the IC_{50} (IC_{50}= the inhibitory concentration that reduces an enzyme's activity by 50%) values of the pancreatic lipase inhibition activity of yellow pea protein hydrolysates and fractions are presented in Figure 9. It has been established that the lower the IC_{50} of a sample is, the more active it is at inhibiting an enzyme. The results show that the unfractionated hydrolysates had lower IC_{50} values compared to the peptide fractions, meaning that the unfractionated hydrolysates were more potent pancreatic lipase inhibitors when compared to the fractionated peptides (Alcalase hydrolysate: 3.98 ±0.4 mg/ml; Chymotrypsin hydrolysate: 4.59 ± 0.06 mg/ml; Pepsin hydrolysate: 4.75 ± 0.38 mg/ml and Trypsin hydrolysate: 3.95 ± 0.04 mg/ml). It was observed that out of all the hydrolysates, the trypsin and alcalase-derived hydrolysates had the lowest IC_{50} values, indicating that they were the most potent in inhibiting pancreatic lipase. In other words, the results suggest that fractionation decreased the inhibitory capacity of yellow pea protein against pancreatic lipase activity. This may be because of the synergistic effect of the peptides contained in the unfractionated hydrolysates and it could be that this effect was reduced or lost during the fractionation process. However, in comparison to the standard inhibitor of pancreatic lipase, Orlistat (IC_{50} = 0.053 ± 0 mg/ml), the IC_{50} values of yellow field pea protein hydrolysates and peptide fractions were much higher, indicating that Orlistat is a more potent pancreatic lipase

inhibitor than the unfractionated hydrolysates and peptide fractions. The body of literature is very scarce on peptides derived from food sources that have inhibitory activity against pancreatic lipase; most of the research done on the inhibition of pancreatic lipase activity focus on other compounds derived from food such as polyphenols, tannins, isoflavonoids, saponins, and procyanidins. This makes this work the first study to show that peptides derived from a food source (yellow field pea protein) have the ability to inhibit pancreatic lipase activity. However, in the search of peptides that have inhibitory activity against pancreatic lipase, Є-polylysine (Є-PL, 25–30 lysine residues) was found to inhibit pancreatic lipase (93% inhibition against human pancreatic lipase and 80% against porcine pancreatic lipase at a maximum concentration of 100 mg/L) (Kido et al., 2003). It has been used as a food additive for 10 years in Japan, and its safety has been confirmed. In addition, a few studies have shown that synthesized peptides via phage display technique can inhibit the activity of pancreatic lipase. For example, a research study conducted synthesized a peptide called peptide D23 via phage display and showed that this peptide inhibited pancreatic lipase activity (Lunder et al., 2005). The IC_{50} value was reported to be < 50 μM and the amino acid sequence of this peptide was determined to be CQPHPGQTC.

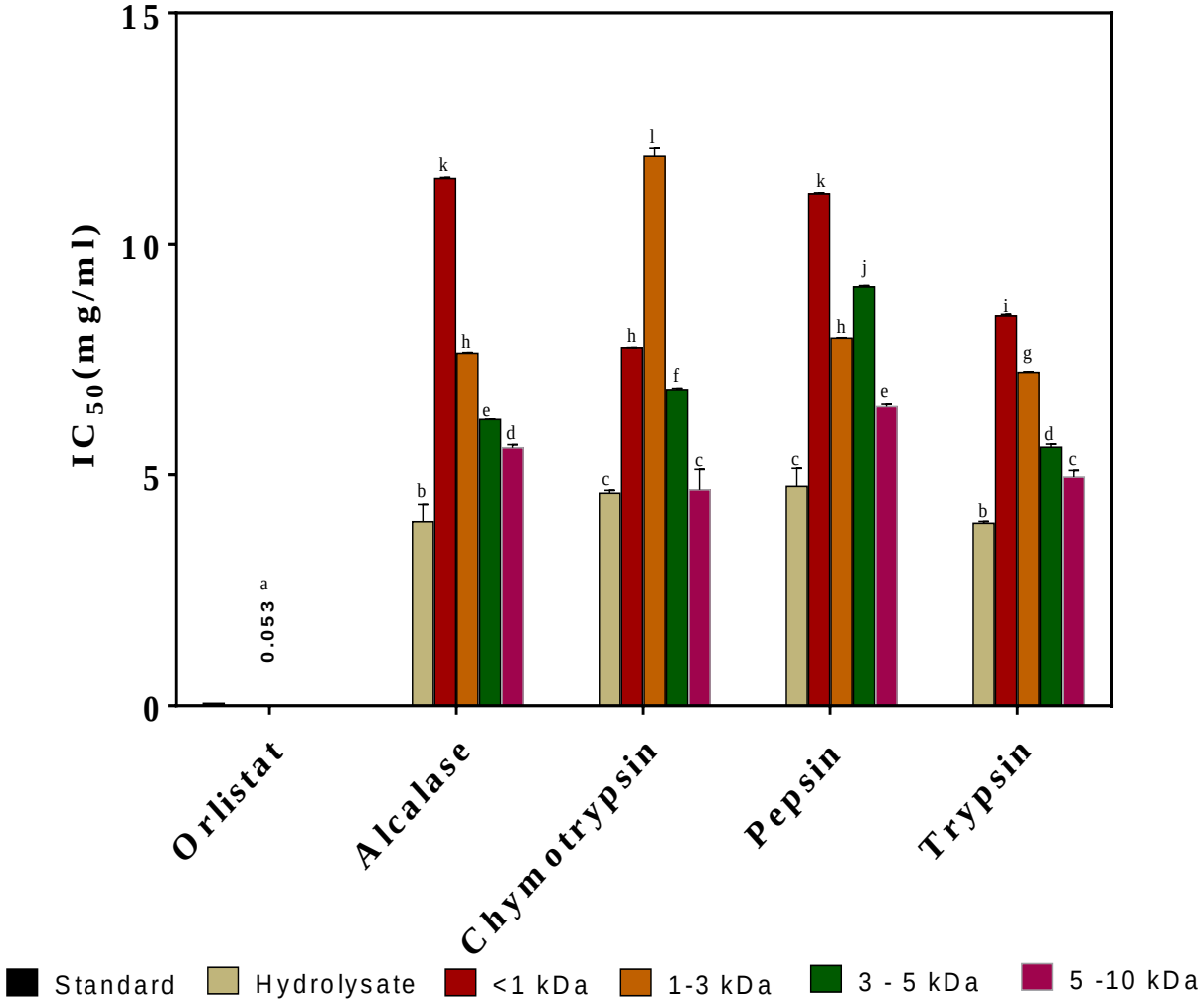

Figure 9. Peptide inhibitory concentrations of yellow field pea protein hydrolysates and peptide fractions obtained with Alcalase, Chymotrypsin, Pepsin, and Trypsin that reduced 50% of pancreatic lipase's activity (IC_{50}). Results are presented as mean ± standard deviation (n=3). Samples with different letters are significantly different ($p< 0.05$).

5.5 Kinetics of Enzyme Inhibition

Based on the inhibitory results in this study, the mode of inhibition of four enzyme activities (α-amylase, α-glucosidase, trypsin and chymotrypsin) were investigated via kinetic studies in the absence and presence of the enzymatic hydrolysates or the chosen peptide fractions. Table 8 lists the chosen samples used for the kinetics study. It is paramount to determine kinetic parameters as they are useful in interpreting the effectiveness of peptides in eliciting their inhibitory potential against the activities of enzymes. Furthermore, it is vital to determine the inhibitory kinetic properties of the unfractionated hydrolysates and their peptides fractions as this aims to reveal the possible mechanism(s) by which they are able to act as enzyme inhibitory agents (Girgih et al., 2015b). In addition, the approximate amount of substrate or peptides (inhibitor) required to accelerate the reaction or inhibit the activities of the enzymes is given by the kinetic plots; this is reflected by the affinity to bind to the active site of the enzyme (Girgih et al., 2011c). V_{max} is the maximum reaction velocity or rate at which an enzyme catalyzes a reaction, in other words, it is the rate of product formation when an enzyme is saturated with the substrate and it reflects how fast an enzyme can catalyze the reaction. K_m is the Michaelis-Menten constant and is defined as the substrate concentration at which the reaction speed is half of the V_{max}; it is a useful measure of how quickly the reaction rate increases with substrate concentration and a measure of an enzyme's affinity for its substrate. A lower K_m means only a small amount of substrate is needed to saturate the enzyme, indicating a high affinity for the substrate while a higher K_m means a lot of substrate must be present to saturate the enzyme, indicating a low affinity for the substrate. Ki is the enzyme-inhibitor dissociation constant and it defines the inhibitor's binding affinity to the enzyme to form the enzyme-inhibitor complex; a lower Ki value indicates a greater binding affinity to the enzyme, which in turn means a smaller amount of the inhibitor, is needed in order

to inhibit the activity of the enzyme. Lineweaver–Burk plots were used to determine the possible modes of inhibition of the four enzymes by the most active peptide fraction chosen alongside with its crude hydrolysate. Lineweaver-Burk plot is based on the reciprocal of the substrate on the horizontal axis and the reciprocal of velocity on the vertical axis, and the inhibition-type can be judged according to the location of the intersection. Competitive inhibitors are said to have the same y-intercept as uninhibited enzyme but different slopes and x-intercepts between the two datasets. Non-competitive inhibition produces a plot with the same x-intercept as the uninhibited enzyme, but different slopes and y-intercepts, while uncompetitive inhibition causes different intercepts on both the y- and x-axes (Howard, 2006).

Table 8. Chosen samples for the kinetics study of each enzyme

Enzyme kinetics	Chosen samples
α-amylase	Chymotrypsin hydrolysate (YPCH) and Chymotrypsin < 1 kDa fraction (C < 1 kDa)
α-glucosidase	Chymotrypsin hydrolysate (YPCH) and Chymotrypsin < 1 kDa fraction (C < 1 kDa)
Trypsin	Trypsin hydrolysate (YPTH) and Trypsin < 1 kDa fraction (T < 1 kDa)
Chymotrypsin	Chymotrypsin hydrolysate (YPCH) and Chymotrypsin 1-3 kDa fraction (C 1-3 kDa)

5.5.1 α-Amylase Kinetics of YPCH and C < 1 kDa peptide fraction

The Lineweaver-Burk plots of α-amylase catalyzed reactions in the absence and presence of the peptide inhibitors at two concentrations are shown in Figure 10 a and b for YPCH and C < 1 kDa peptide fraction, respectively. The results indicate a competitive type of inhibition. This is because the lines intersected at the y-axis at the same point. Competitive inhibitors are known to block the active site of the enzyme, thereby inhibiting the formation of the enzyme-substrate complex. However, when the enzyme-substrate complexes are formed, the inhibitor does not affect enzyme activity. Therefore, in competitive inhibition, the inhibitors do not affect the Vmax of the enzyme (i.e. Vmax is unchanged) but increases the Km. The results of the kinetic parameters are shown in Table 9. α-amylase inhibitory Vmax values for both YPCH and C < 1 kDa remained unchanged (0.0004), while the Km values increased. The inhibition constant (Ki), which indicates the binding ability to the α-amylase enzyme revealed that YPCH (248.8 μg/mL) binds stronger than the C < 1 kDa peptide fraction (649.1 μg/mL), suggesting that YPCH is a better inhibitor of α- amylase compared to its < 1 kDa peptide fraction. This also correlates with the results obtained from the mean inhibitory values, showing that YPCH (14.45 ± 0.39%) is a better inhibitor than the C < 1 kDa peptide fraction (13.11 ± 0.35%). Some studies have reported different modes of inhibition of α-amylase. For example, a non-competitive mode of inhibition by millet seed coat has been reported (Shobana et al., 2009), while a competitive mode of inhibition was reported for *Morinda lucida*, a Nigerian medicinal plant traditionally used in the treatment of type 2 diabetes (Kazeem et al., 2013). The competitive mode of inhibition exhibited by the peptides indicates that the peptides interacted with the active site of α-amylase and competed with the substrate for binding to the active site of α-amylase, thereby preventing the breaking down of starch into oligosaccharides, disaccharides and ultimately glucose.

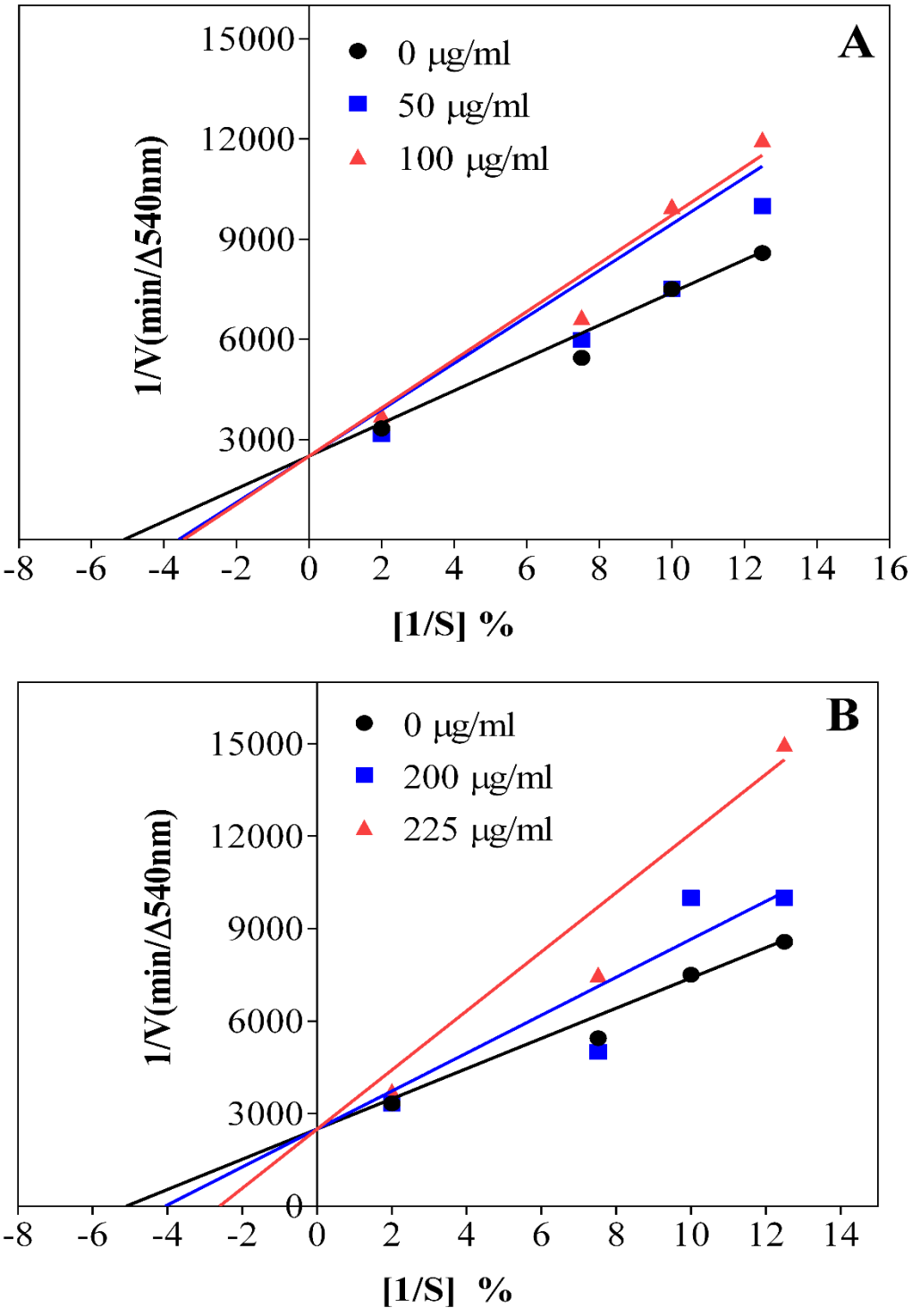

Figure 10. Lineweaver-Burk plots of α- amylase inhibition at different peptide concentrations: (a) yellow pea protein chymotrypsin-derived hydrolysate (YPCH) and (b) chymotrypsin < 1 kDa peptide fraction (C < 1 kDa).

Table 9. Kinetic parameters for α-amylase inhibition by YPCH and C < 1 kDa peptide fraction

Parameter	Control	YPCH		C < 1 kDa	
Peptides concentration (µg/ml)	0	50	100	200	225
V_{max}	0.0004 ± 3.27E-05	0.00049 ± 3.17E-05	0.0004 ± 4.95E-05	0.0004 ± 7.15E-05	0.0004 ± 4.01E-05
K_m	0.1961 ± 0.03	0.278 ± 0.03	0.2887 ± 0.06	0.2643 ± 0.08	0.3836 ± 0.06
K_i		248.8 ± 80.22		649.1 ± 293.4	

Results are presented as mean ± standard error. Km, Michaelis-Menten constant (%); Vmax, maximum reaction velocity; Ki, enzyme-inhibitor dissociation constant (µg/ml)

Table 10. Kinetic parameters for α-glucosidase inhibition by YPCH and C < 1 kDa peptide fraction

Parameter	Control	YPCH		C < 1 kDa	
Peptides concentration (mg/ml)	0	5	15	15	20
V_{max}	0.07 ± 0.001	0.05 ± 0.001	0.05 ± 0.001	0.04 ± 0.001	0.04 ± 0.001
K_m	0.71 ± 0.03	0.7 ± 0.03	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04
K_i		35.9 ± 3.2		28.76 ± 0.79	

Results are presented as mean ± standard error. Km, Michaelis-Menten constant (mM); Vmax, maximum reaction velocity; Ki, enzyme-inhibitor dissociation constant (mg/ml)

5.5.2 α-Glucosidase Kinetics of YPCH and C < 1 kDa peptide fraction

In contrast to α-amylase inhibition kinetics, the Lineweaver-Burk plots of α-glucosidase catalyzed reactions in the absence and presence of the peptide inhibitors at two concentrations are shown in Figure 11 a and b for YPCH and C < 1 kDa peptide fraction, respectively. The results indicate a non-competitive type of inhibition. This is because the lines intersect at the x-axis at the same point. Non-competitive inhibitors are known to block allosterically and alter the enzyme's conformation. They have equal affinity for free enzymes and enzyme-substrate complexes. Therefore, in non-competitive inhibition, the inhibitors do not affect the Km of the enzyme because the change in conformation does not alter the enzyme's affinity for the substrate (i.e. Km is unchanged). However, Vmax is reduced as the rate of catalysis is lowered. Non-competitive inhibition is typically exhibited with observed decreases in Vmax but with similar Km values; this pattern was also noted for the peptides as shown in Table 10. α-glucosidase inhibitory Km values for both YPCH and C < 1 kDa remained unchanged (0.7 mM), while the Vmax values decreased. The inhibition constant (Ki), which indicates the binding ability to the α-glucosidase enzyme revealed that C < 1 kDa peptide fraction (28.76 mg/mL) binds stronger than the YPCH (35.9 mg/mL), suggesting that C < 1 kDa peptide fraction is a better inhibitor of α- glucosidase compared to YPCH. These results are also consistent with the results obtained from the mean inhibitory values, indicating that the C < 1 kDa peptide fraction (29.66 ± 0.85%) is a more potent inhibitor than YPCH (17.42 ± 3.2%). Some studies have reported different modes of inhibition of α-glucosidase. For instance, a non-competitive mode of inhibition by millet seed coat, tea extracts derived with ethanol and wheat bran have been reported (Shobana et al., 2009; Hao et al., 2017; Tu et al., 2013). Likewise, a mixed non-competitive mode of inhibition was reported for *Morinda lucida,* a Nigerian medicinal plant traditionally used in the

treatment of type 2 diabetes (Kazeem et al., 2013) and tea extracts derived with water (Hao et al., 2017). The non-competitive mode of inhibition obtained from the Lineweaver-Burk plot point to the fact that the peptides did not compete with the substrate for binding to the active site, rather they are noted to bind to a separate site (non-active site) on the enzyme to retard the conversion of disaccharides to monosaccharides.

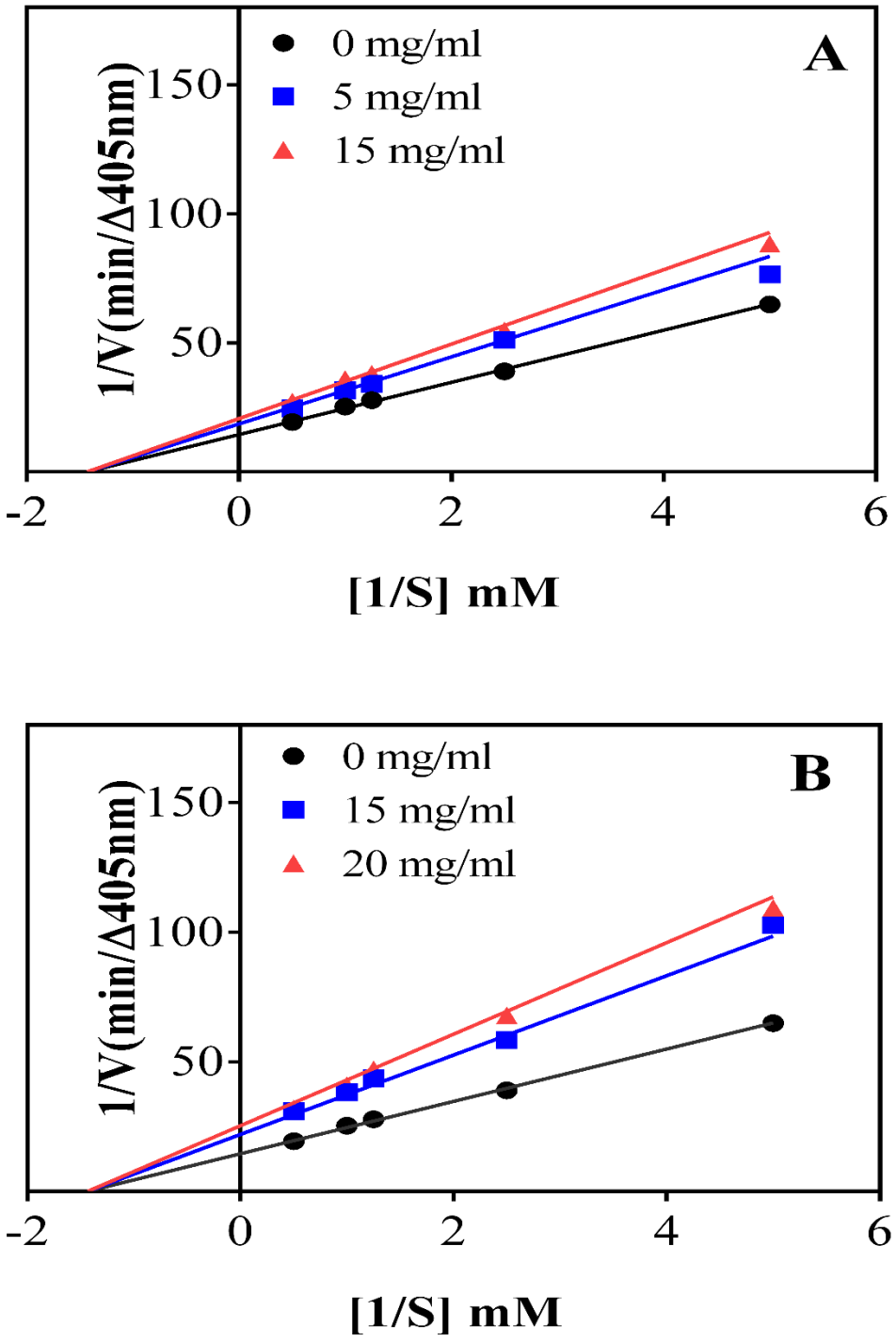

Figure 11. Lineweaver-Burk plots of α-glucosidase inhibition at different peptide concentrations: (a) yellow pea protein chymotrypsin-derived hydrolysate (YPCH) and (b) chymotrypsin < 1 kDa peptide fraction (C < 1 kDa).

5.5.3 Trypsin Kinetics of YPTH and T < 1 kDa peptide fraction

The Lineweaver-Burk plots of trypsin-catalyzed reactions in the absence and presence of the peptide inhibitors at two concentrations are shown in Figure 12 a and b for YPTH and T < 1 kDa peptide fraction, respectively. The results indicate a competitive type of inhibition. This is because the lines intersected at the y-axis at the same point. Therefore, in competitive inhibition, the inhibitors do not affect the Vmax of the enzyme (i.e. Vmax is unchanged) but increases the Km. The results of the kinetic parameters are shown in Table 11. Trypsin inhibitory Vmax values for both YPTH and T < 1 kDa remained unchanged (0.08), while the Km values increased. The inhibition constant (Ki), which indicates the binding ability to the trypsin enzyme revealed that T < 1 kDa peptide fraction (1.073 mg/mL) binds stronger than YPTH (1.172 mg/mL), suggesting that T < 1 kDa peptide fraction is a better inhibitor of trypsin compared to its hydrolysate. These results correspond with the results obtained from the IC_{50} values, indicating that the T < 1 kDa peptide fraction (2.21 ± 0.03 mg/ml) is a more potent inhibitor than YPTH (6.75 ± 0.49 mg/ml). Some studies have reported a similar mode of inhibition as our study from the fruits of *Solanum aculeatissimum Jacq*. (Meenu Krishnan and Murugan, 2015) and *Dolichos biflorus* (a legume grown in the tropics) (Kuhar et al., 2013). The competitive mode of inhibition exhibited indicate peptide binding to the active site of trypsin and competition with the substrate for binding to the active site of trypsin, thereby preventing the breaking down of the substrate.

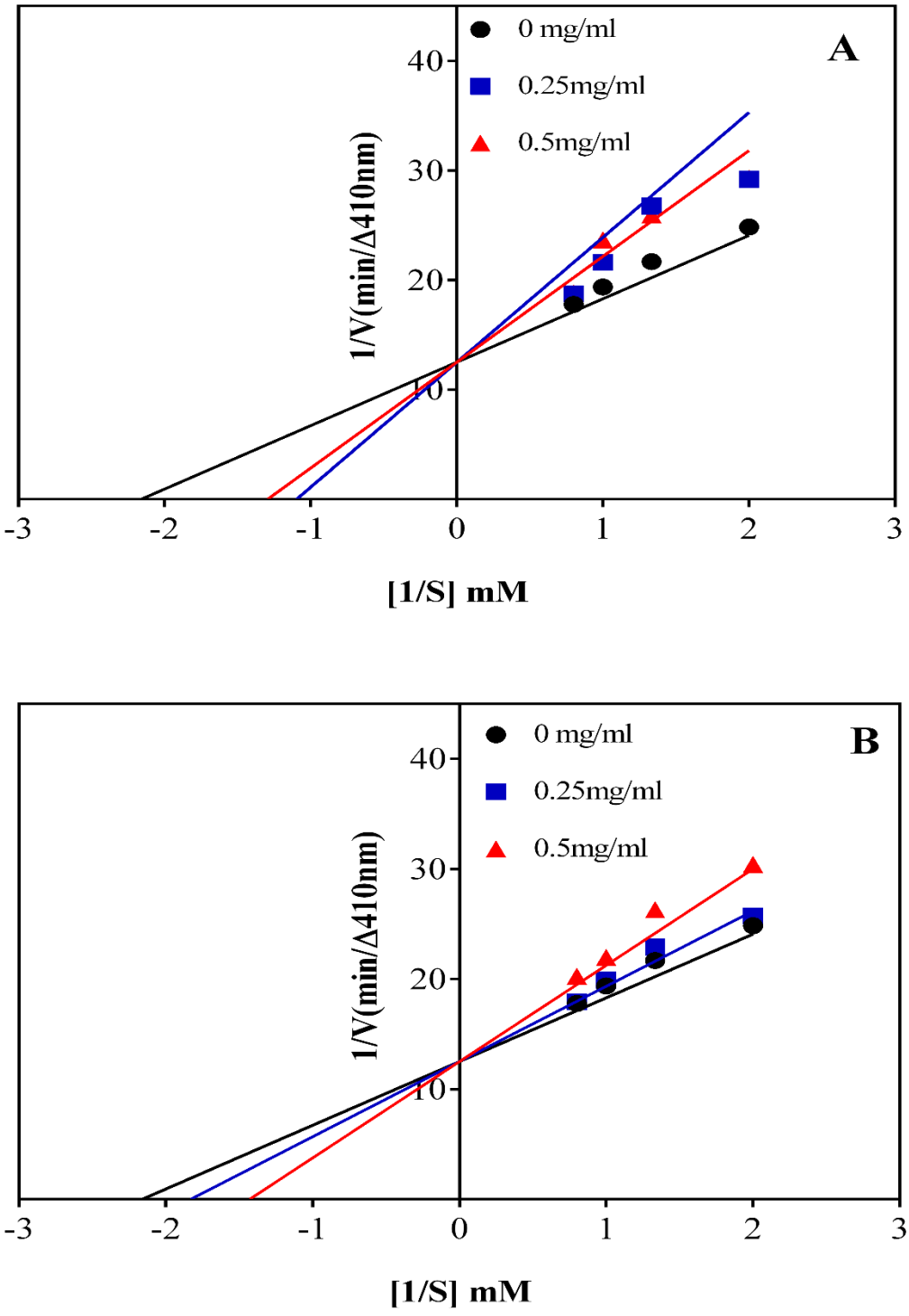

Figure 12. Lineweaver-Burk plots of trypsin inhibition at different peptide concentrations: (a) yellow pea protein trypsin-derived hydrolysate (YPTH) and (b) trypsin < 1 kDa peptide fraction (T < 1 kDa).

Table 11. Kinetic parameters for trypsin inhibition by YPTH and T < 1 kDa peptide fraction

Parameter	Control	YPTH		T < 1 kDa	
Peptides concentration (mg/ml)	0	0.25	5	0.25	5
V_{max}	0.08 ± 0.002	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.005	0.08 ± 0.006
K_m	0.46 ± 0.04	0.91 ± 0.26	0.77 ± 0.26	0.55 ± 0.09	0.7 ± 0.13
K_i		1.172 ± 0.28		1.073 ± 0.19	

Results are presented as mean ± standard error. Km, Michaelis-Menten constant (mM); Vmax, maximum reaction velocity; Ki, enzyme-inhibitor dissociation constant (mg/ml)

Table 12. Kinetic parameters for chymotrypsin inhibition by YPCH and C 1-3 kDa peptide fraction

Parameter	Control	YPCH		C 1-3 kDa	
Peptides concentration (mg/ml)	0	3	4	3	4
V_{max}	0.019 ± 0.001	0.019 ± 0.001	0.019 ± 0.002	0.019 ± 0.001	0.019 ± 0.001
K_m	0.011 ± 0.001	0.027 ± 0.004	0.078 ± 0.01	0.018 ± 0.004	0.025 ± 0.005
K_i		0.763 ± 0.1		1.897 ± 0.9	

Results are presented as mean ± standard error. Km, Michaelis-Menten constant (mM); Vmax, maximum reaction velocity; Ki, enzyme-inhibitor dissociation constant (mg/ml)

5.5.4 Chymotrypsin Kinetics of YPCH and C 1-3 kDa peptide fraction

Similar to its counterpart trypsin, the Lineweaver-Burk plots of chymotrypsin catalyzed reactions in the absence and presence of the peptide inhibitors at two concentrations are shown in Figure 13 a and b for YPCH and C 1-3 kDa peptide fraction, respectively. The results indicate a competitive type of inhibition. This is because the lines intersected at the y-axis at the same point. As stated previously, in competitive inhibition, the inhibitors do not affect the Vmax of the enzyme (i.e. Vmax is unchanged), but an increase in Km occurs. Chymotrypsin inhibitory Vmax values for both YPCH and C 1-3 kDa remained unchanged (0.019), while the Km values of the peptides increased as shown in Table 12. The inhibition constant (Ki), which indicates the binding ability to the chymotrypsin enzyme revealed that YPCH (0.763 mg/mL) binds stronger than the C 1- 3 kDa peptide fraction (1.897 mg/mL), suggesting that YPCH is a better inhibitor of chymotrypsin compared to C 1-3 kDa peptide fraction. These results obtained agree with the results obtained from the mean inhibitory values, indicating that YPCH (28.4 ± 0.47%) is a more potent inhibitor than the C 1-3 kDa peptide fraction (20 ± 0.39%). Similar modes of inhibition (competitive) have been observed from fruits of *Solanum aculeatissimum Jacq*. (Meenu Krishnan and Murugan, 2015) and *Dolichos biflorus* (Kuhar et al., 2013). Similar to that of trypsin, the competitive mode of inhibition exhibited by the peptides indicate that the peptides compete with the substrate for binding to the active site of chymotrypsin thereby preventing the breaking down of the substrate.

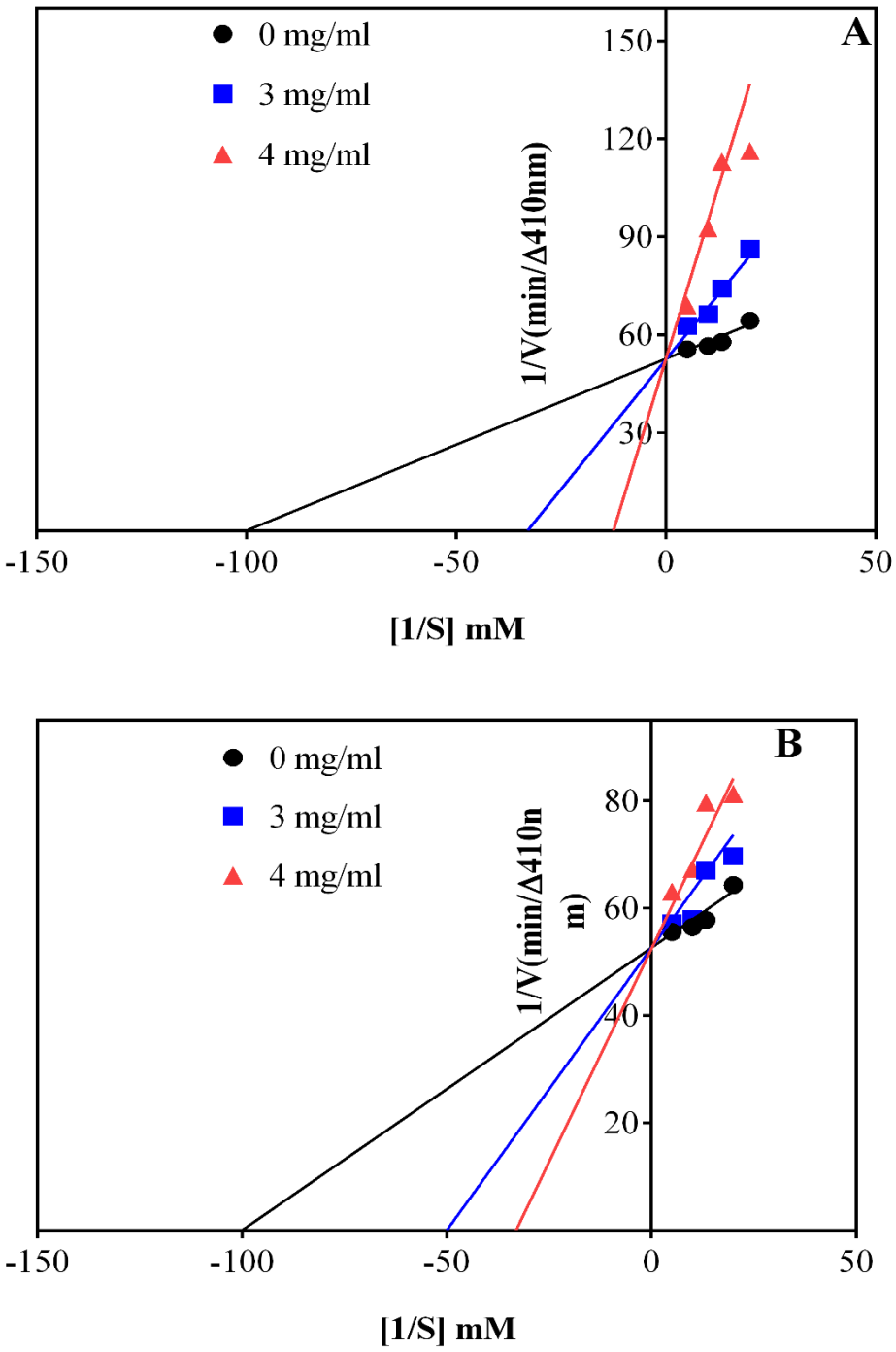

Figure 13. Lineweaver-Burk plots of chymotrypsin inhibition at different peptide concentrations: (a) yellow pea protein chymotrypsin-derived hydrolysate (YPCH) and (b) chymotrypsin 1-3 kDa peptide fraction (C 1-3 kDa).

5.6 Mass Spectrometry (MS) Analysis

The potential peptide masses present in all four enzymatic hydrolysates are shown in Figures 14-17. Results of the MS analysis showed that there were several molecular species present in all four enzymatic hydrolysates. In terms of potential amino acid sequences, all the four enzymatic hydrolysates consisted of peptides with different sizes ranging from a dipeptide to a polypeptide (alcalase hydrolysate: polypeptides of 11 amino acids; chymotrypsin hydrolysate: polypeptides of 12 amino acids; pepsin and trypsin hydrolysate: polypeptides of 13 amino acids). The potential amino acid sequences of peptides present in the four enzymatic hydrolysates are given in Tables 13-16. The total pea proteins can be divided into two major groups: albumins and globulins; the major pea storage proteins are often referred to as legumin (11S), vicilin (7S), and convicilin composing the globulin fraction (Rubio et al., 2014; Reinkensmeier et al., 2015). The parent proteins for the four enzymatic hydrolysates were identified to be pea vicilin, convicilin, and legumin, confirming that these peptides are encrypted within the primary structure of pea proteins. Generally, the hydrolysates were dominated by LMW (120-1523 Da) peptides as shown in the MS scans. The results agree with previous works that have shown that low molecular weight peptides tend to have better bioactivities and act as strong enzyme inhibitors because of their ability to bind tightly and interact with the enzyme's active site (Aluko et al., 2015). It should be noted that the current MS data does not provide conclusive information since definite peptide sequences can only be derived from MS/MS analysis. In other words, these results give an overview of potential peptides that dominate the sample, and further purification coupled with MS/MS analysis will be paramount in providing the actual amino acid sequences that are responsible for each MS ion signal.

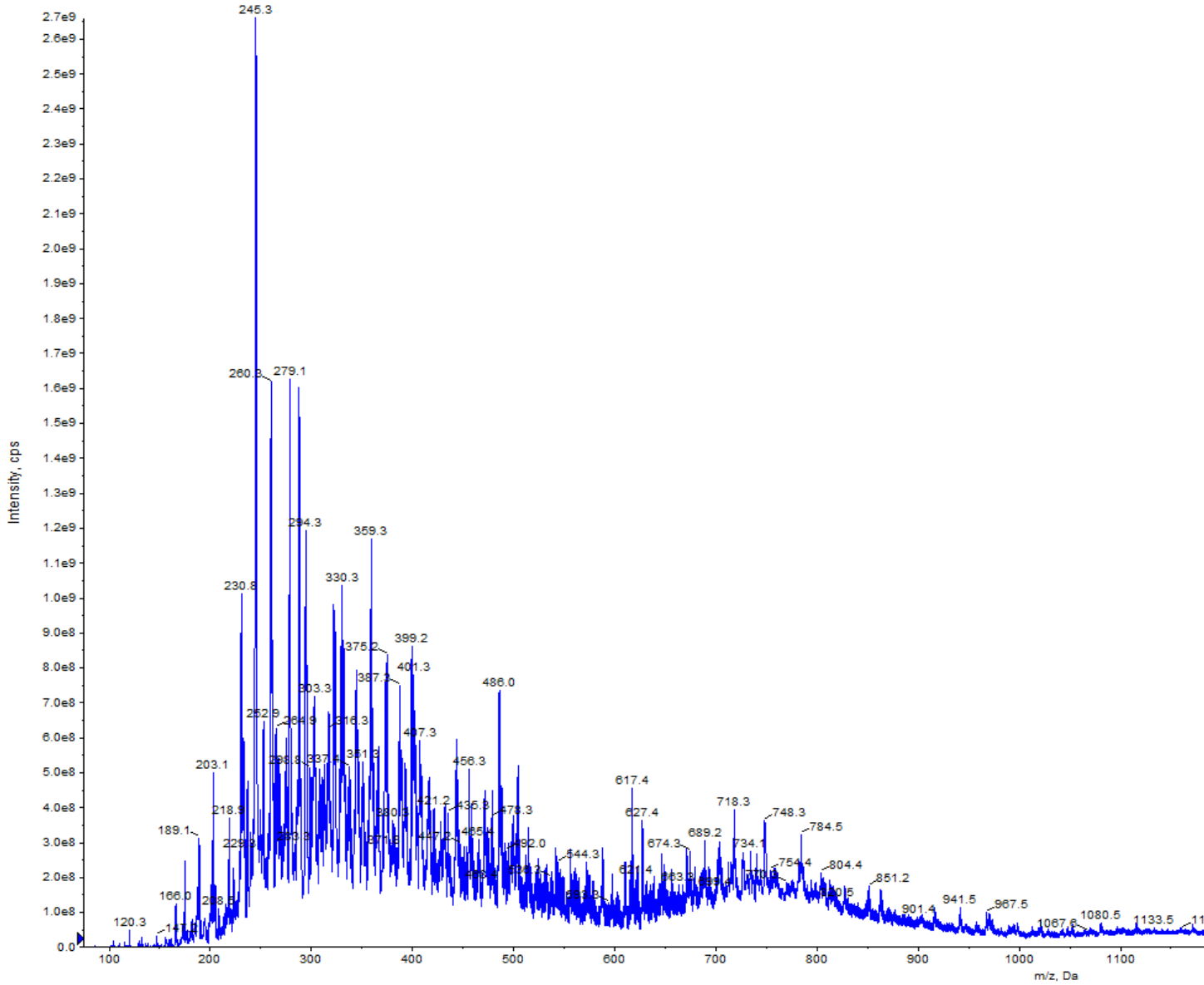

Figure 14. Electrospray ionization mass spectra of Alcalase hydrolysate

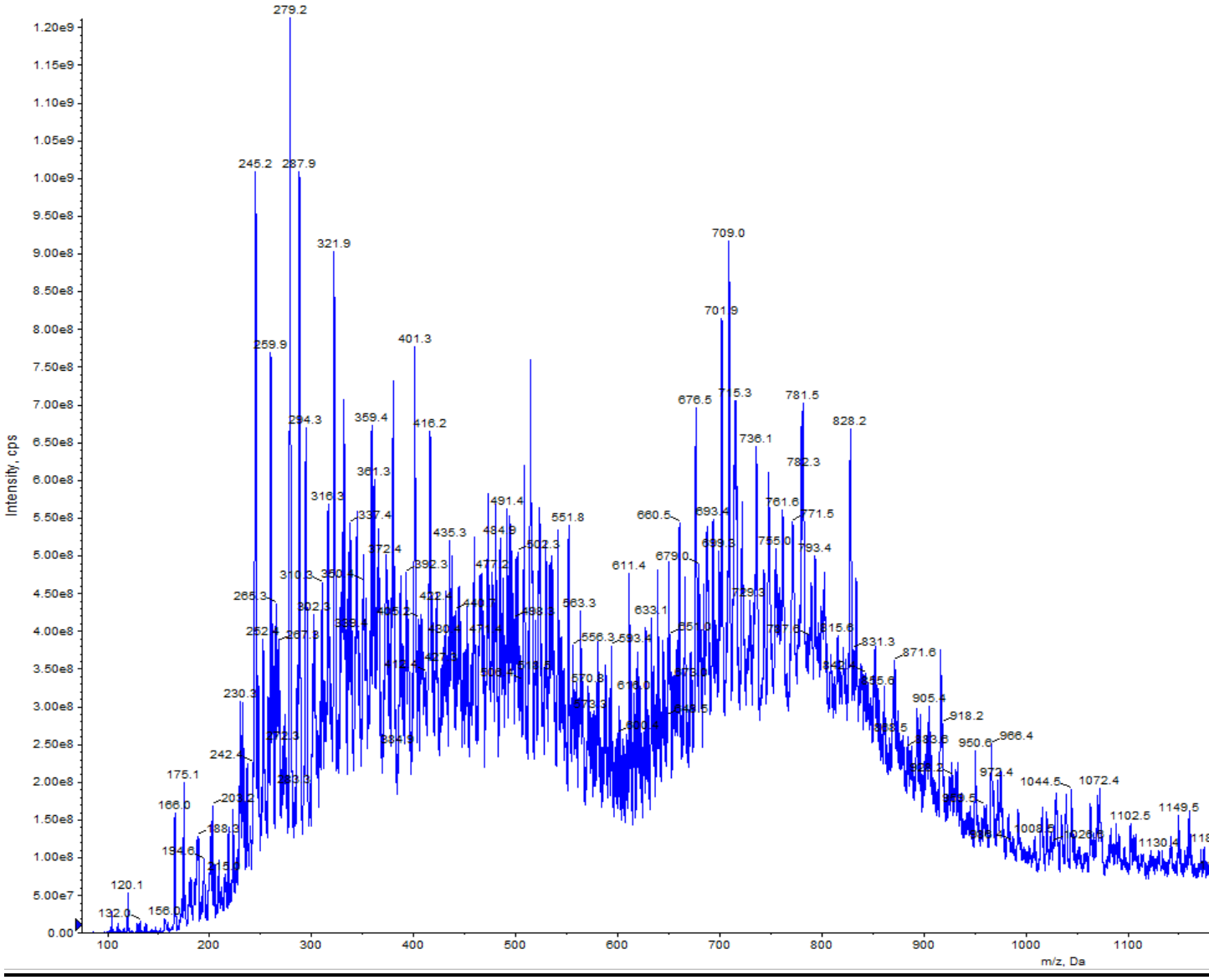

Figure 15. Electrospray ionization mass spectra of Chymotrypsin hydrolysate

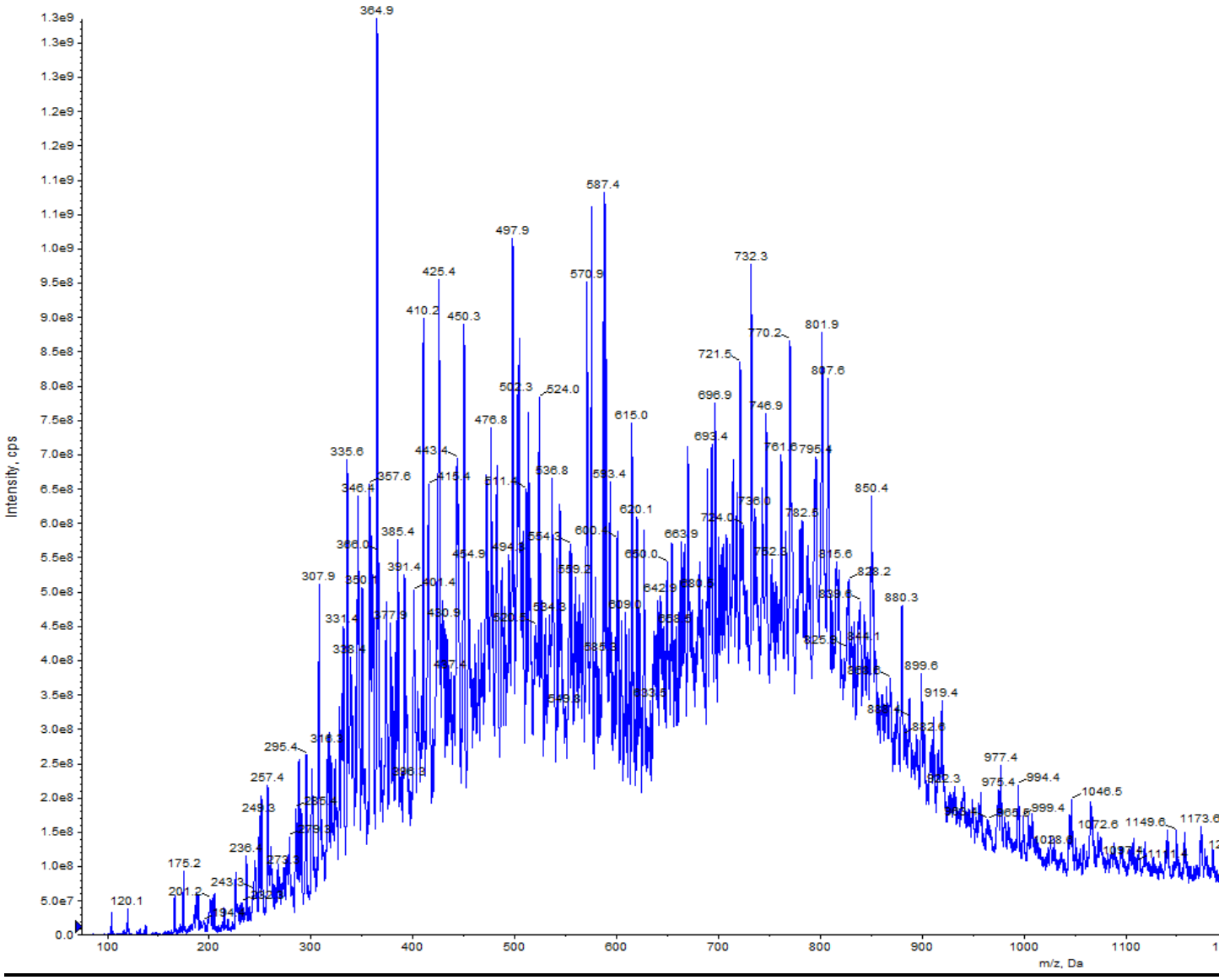

Figure 16. Electrospray ionization mass spectra of Pepsin hydrolysate

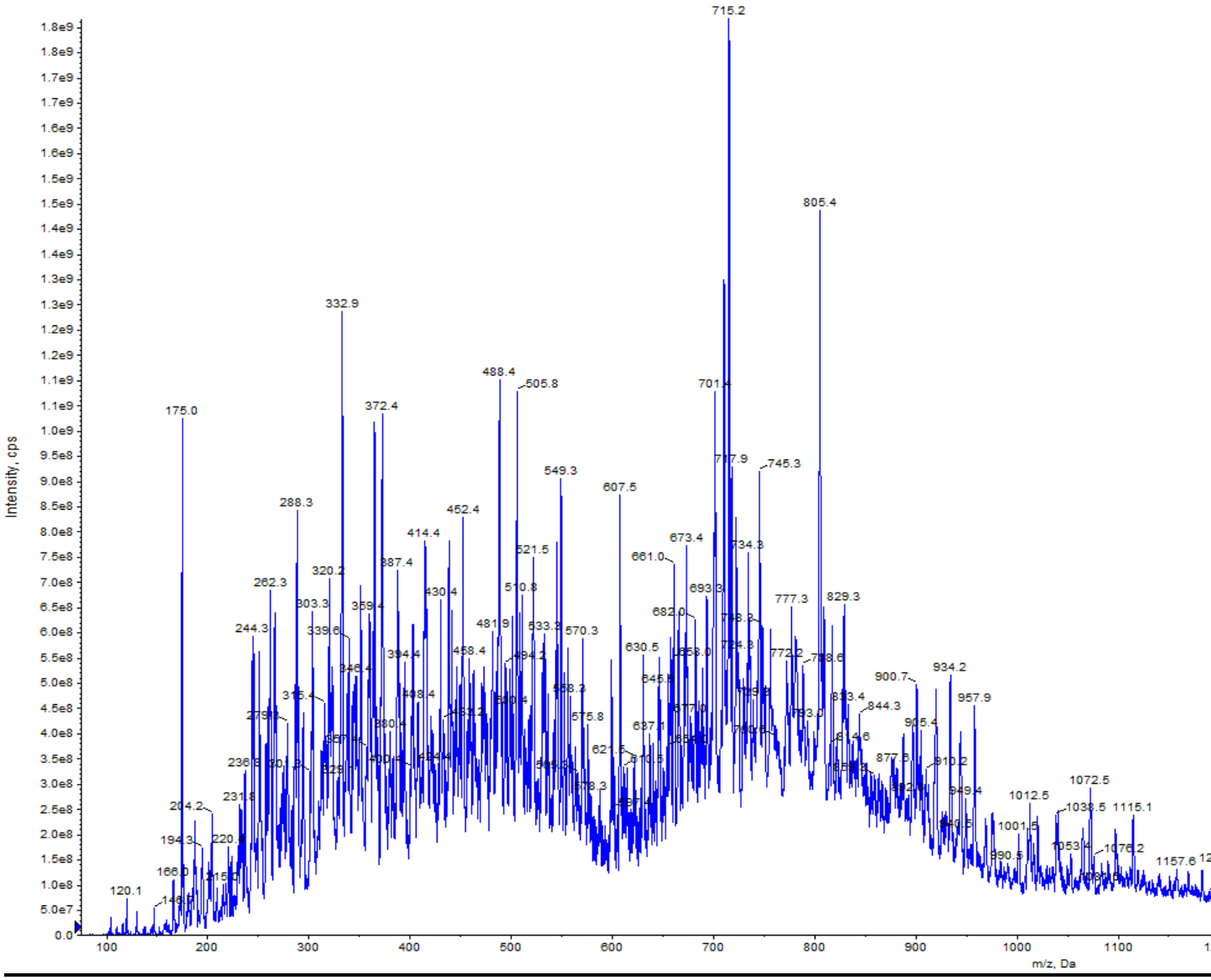

Figure 17. Electrospray ionization mass spectra of Trypsin hydrolysate

Table 13. Potential amino acid sequences of peptides present in alcalase-derived yellow field pea protein hydrolysate.

Observed mass (Da)	Calculated mass (Da)	Potential sequence	Location	Protein type
175.100	175.108	VG	f323-324	Vicilin
			f211-212	Legumin MS
		GV	f67-68	Legumin A2
			f68-69 f100-101	Legumin MS
189.100	189.123	LG	f15-16 f440-441 f491-492	Convicilin
			f384-385	Vicilin
			f17-18	Legumin A2
			f102-103 f184-185 f487-488	Legumin MS
		GI	f16-17 f494-495	Convicilin
			f17-18 f387-388	Vicilin
			f466-467	Legumin A2
			f147-148	Legumin A2, Legumin MS
			f271-272 f378-379 f447-448	Legumin MS
		GL	f282-283 f441-442	Convicilin
			f51-52 f253-254 f336-337	Legumin A2
			f52-53 f80-81 f103-104 f390-391 f542-543	Legumin MS

		VA	f480-481	Convicilin
			f373-374	Vicilin
			f68-69 f445-446 f457-458 f485-486	Legumin A2
			f161-162 f499-500 f548-549	Legumin MS
		AV	f374-375	Vicilin
			f143-144 f162-163 f444-445	Legumin A2
			f105-106 f521-522	Legumin MS
		IG	f350-351	Legumin A2
203.100	203.103	PS	f61-62 f364-365	Convicilin
			f352-353	Legumin A2
			f407-408	Legumin MS
		SP	f229-230 f467-468 f561-562	Convicilin
			f258-259 f355-356	Legumin A2
			f89-90 f257-258 f276-277	Legumin MS
	203.139	LA	f20-21 f354-355	Convicilin
			f21-22	Vicilin, Legumin MS
			f149-150 f398-399 f416-417	Vicilin
			f6-7 f184-185 f470-471	Legumin A2
			f104-105 f241-242 f537-538	Legumin MS

		AI	f223-224 f339-340 f424-425 f481-482	Convicilin
			f104-105 f150-151 f307-308	Vicilin
			f246-247	Legumin A2
			f143-144 f162-163	Legumin MS
		AL	f414-415	Convicilin
			f7-8 f22-23 f39-40 f69-70 f79-80 f409-410 f436-437	Legumin A2
			f40-41	Legumin MS
		IA	f132-133	Vicilin
			f142-143	Legumin A2, Legumin MS
			f161-162 f467-468	Legumin A2
			f404-405	Legumin MS
279.100	279.134	YP	f395-396	Convicilin
		PY	f83-84	Legumin A2
			f149-150	Legumin MS
	279.170	LF	f13-14 f156-157 f165-166 f185-186 f386-387	Convicilin
			f46-47	Vicilin
			f15-16	Legumin MS
		FL	f14-15	Convicilin, Legumin A2

			f19-20 f155-156 f205-206 f504-505	Convicilin
			f162-163	Convicilin, Vicilin
			f20-21 f86-87 f397-398	Vicilin
			f192-193 f220-221 f507-508	Legumin A2
			f5-6 f13-14 f209-210 f240-241	Legumin MS
		IF	f18-19 f204-205	Convicilin
			f37-38 f66-67 f85-86 f286-287	Vicilin
			f92-93 f212-213	Legumin A2
			f93-94	Legumin MS
		FI	f36-37	Vicilin
			f93-94	Legumin A2
			f94-95	Legumin MS
303.300	303.203	VKG	f250-252	Legumin A2
		KGV	f99-101	Legumin MS
	303.214	KR	f54-55 f130-131 f181-182 f329-330 f559-560	Convicilin
			f214-215	Vicilin
			f217-218	Legumin A2
			f253-254	Legumin MS

		RK	f328-329 f352-353 f452-453 f558-559	Convicilin
			f334-335	Vicilin
			f290-291 f303-304 f326-327 f346-347 f387-388	Legumin MS
330.300	330.202	ITP	f389-391	Convicilin
			f273-275	Vicilin
		TLP	f427-429	Legumin MS
	330.239	LVV	f216-218	Convicilin
			f97-99	Vicilin
			f490-492	Legumin MS
		VVL	f217-219 f426-428	Convicilin
			f98-100	Vicilin
		IVV	f425-427	Convicilin
		VVI	f471-473	Convicilin
		VIV	f223-225 f309-311	Vicilin
359.300	359.204	RSP	f560-562	Convicilin
			f256-258	Legumin MS
		RPS	f60-62	Convicilin
			f406-408	Legumin MS
		PSR	f407-409	Legumin MS
		PRS	f116-118 f339-341	Legumin MS
	359.229	NIL	f297-299	Convicilin

			f180-182	Vicilin
		NLL	f489-491	Convicilin
		LNL	f488-490	Convicilin
		LNI	f348-350	Legumin A2
		LQV	f416-418	Legumin A2
		QIV	f264-266	Legumin MS
		QLV	f489-491	Legumin MS
		LVQ	f560-562	Legumin MS
		GLAV	f103-106	Legumin MS
		GGLI	f50-53	Legumin A2
		GVAL	f67-70	Legumin A2
		GAIV	f245-248	Legumin A2
		VVAA	f484-487	Legumin A2
		LLGG	f16-19	Legumin A2
	359.240	ARL	f120-122 f464-466	Convicilin
			f414-416 f468-470	Legumin A2
		RAI	f423-425	Convicilin
			f306-308	Vicilin
		ALR	f22-24 f79-81	Legumin A2
		RAL	f435-437	Legumin A2
		RLA	f469-471	Legumin A2
		IAR	f467-469	Legumin A2
			f404-406	Legumin MS
	359.265	IKV	f341-343	Convicilin

		KVL	f312-314	Convicilin
			f195-197	Vicilin
		VLK	f108-110	Vicilin
		VKL	f225-227	Vicilin
		IVK	f224-226	Vicilin
			f247-249	Legumin A2
			f265-267	Legumin MS
366.200	366.166	FEA	f278-280	Convicilin
		EAF	f279-281	Convicilin
		PSY	f61-63	Convicilin
			f84-86	Legumin MS
		YSP	f89-88	Legumin MS
	366.202	FLS	f504-506	Convicilin
			f5-7	Legumin MS
		ISF	f18-20	Vicilin
		SFL	f19-21 f161-163	Vicilin
			f208-210	Legumin MS
		IAY	f132-134	Vicilin
		AYL	f133-135	Vicilin
		LSF	f10-12	Legumin A2
		YLA	f183-185	Legumin A2
		IYA	f407-409	Legumin A2
			f448-450	Legumin MS
		YAL	f408-410	Legumin A2

		TVF	f425-427	Legumin A2
		IFS	f212-214	Legumin A2
		FSL	f9-11	Legumin MS
	366.214	LPH	f417-419	Convicilin
			f300-302	Vicilin
		HLP	f82-84	Legumin MS
375.200	375.151	SPGD	f467-470	Convicilin
	375.162	NNQ	f499-501	Convicilin
			f392-394	Vicilin
			f172-174	Legumin A2
		NQN	f167-169	Vicilin
		QGNG	f96-99	Legumin A2
	357.170	QMP	f177-179	Legumin A2
		VPGC	f106-109	Legumin MS
	375.187	QEV	f530-532	Convicilin
			f423-425	Vicilin
		QVE	f102-104	Convicilin
		NLE	f237-239 f436-438	Convicilin
			f118-120	Vicilin
		EIN	f409-411	Convicilin
			f347-349	Vicilin
		IEN	f516-518	Convicilin
		EVQ	f86-88 f458-460	Convicilin
		EQV	f101-103	Convicilin

		ENL	f187-189	Convicilin
			f68-70	Vicilin
		LQD	f398-400	Convicilin
			f281-283	Vicilin
		QDL	f399-401	Convicilin
			f282-284	Vicilin
		LAGD	f398-401	Vicilin
		LEN	f429-431	Vicilin
		EGGL	f49-52	Legumin A2
		QLD	f174-176	Legumin A2, Legumin MS
			f34-36	Legumin MS
		LDQ	f175-177	Legumin A2
		ENI	f402-404	Legumin MS
	375.199	ERA	f118-120	Convicilin
		GKGN	f433-436	Convicilin
		EAR	f463-465	Convicilin
			f495-497	Legumin A2
		NKN	f286-288	Convicilin
		KNN	f498-500	Convicilin
		NNK	f59-61	Legumin A2
	375.224	KEV	f85-87	Convicilin
		EKV	f311-313	Convicilin
			f194-196	Vicilin
		VKE	f520-522	Convicilin
			f413-415	Vicilin

		LKD	f211-213	Vicilin
		VLSG	f99-102	Vicilin
			f232-235 f485-488	Legumin MS
		KVE	f267-269	Legumin MS
		GVSL	f68-71	Legumin MS
	375.235	TRV	f36-38	Convicilin
		LRS	f372-374	Convicilin
			f256-258	Vicilin
			f255-257	Legumin MS
		RLS	f465-467	Convicilin
			f455-457	Vicilin
			f435-437	Legumin MS
		RSL	f209-211	Vicilin
		LSR	f227-229	Vicilin
			f70-72	Legumin A2
		IRS	f168-170	Legumin A2
		RIS	f419-421	Legumin MS
399.200	399.162	NHE	f187-189	Legumin A2
	399.210	RSH	f373-375	Convicilin
		RHS	f544-546	Legumin MS
	399.260	PVAI	f479-482	Convicilin
		AIPV	f150-153	Vicilin
		VIPA	f366-369	Vicilin
		PVIA	f159-162	Legumin A2

		LVPA	f508-511	Legumin A2
		IAVP	f142-145	Legumin A2
		PLVA	f159-162	Legumin MS
		LAVP	f104-107	Legumin MS
407.300	407.214	TVTS	f368-371	Legumin A2
	407.229	AFGL	f280-283	Convicilin
			f540-543	Legumin MS
		KYP	f394-396	Convicilin
		FLAG	f397-400	Vicilin
		FIQ	f93-95	Legumin A2
	407.265	KLF	f385-387	Convicilin
		LFK	f156-158	Convicilin
		KFL	f161-163	Convicilin
			f506-508	Legumin A2
		KIF	f65-67	Vicilin
		IFK	f37-39	Vicilin
421.200	421.156	EGSE	f32-35	Convicilin
		ESEG	f47-50	Legumin A2
		TDAD	f91-94	Vicilin
	421.168	SSNN	f170-173	Legumin A2
	421.193	SLSD	f449-452	Legumin A2
		VVSE	f247-250	Vicilin
	421.208	TFPG	f524-527	Convicilin
		ASFP	f8-11	Vicilin
	421.212	VCVT	f23-26	Convicilin
		LVSC	f404-407	Convicilin

	421.256	RVF	f180-182	Legumin MS
		VFR	f528-530	Legumin MS
429.400	429.307	LLAL	f5-8	Legumin A2
		LVVV	f490-493	Legumin MS
456.300	456.220	SHVN	523-526	Legumin MS
	456.256	KHTA	350-353	Legumin MS
	456.282	NLPL	f479-482	Legumin A2
		VIPAG	f366-370	Vicilin
		GLAVP	f103-107	Legumin MS
		LAVPG	f104-108	Legumin MS
		PLVQ	f559-562	Legumin MS
		LVQP	f560-563	Legumin MS
	456.293	IARP	f404-407	Legumin MS
	456.318	VLKP	f108-111	Vicilin
478.300	478.214	LSSGD	f359-363	Vicilin
		LSGSD	f505-509	Convicilin
	478.226	ERSS	f143-146	Convicilin
		RSSE	f144-147	Convicilin
	478.230	AFPGS	f417-421	Vicilin
		FPGSA	f418-422	Vicilin
	478.233	ASVCV	f22-26	Vicilin
			f21-25	Convicilin
	478.241	PAGHP	f475-479	Convicilin
		PAGHP	f368-372	Vicilin
	478.266	NFVV	f496-499	Legumin MS

	478.288	RRF	f134-136	Legumin MS
			f180-182	Legumin A2
		RFR	f135-137	Legumin A2
544.300	544.261	ALEPD	f39-43	Legumin A2
			f40-44	Legumin MS
	544.298	DTPVI	f157-161	Legumin A2
		SLDLP	f371-375	Legumin A2
	544.309	VVAQQ	f498-502	Legumin MS
	544.316	ALMLP	f414-418	Convicilin
	544.320	IQRQ	f379-382	Legumin MS
		QQLR	f323-326	Convicilin
	544.331	VGRRG	f211-215	Legumin MS
	544.334	LDLAI	f147-151	Vicilin
		ELLGL	f438-442	Convicilin
		ADLIL	f212-216	Convicilin
		LELLG	f437-441	Convicilin
	544.343	RRRG	f308-311	Legumin A2
	544.345	LGLKN	f440-444	Convicilin
		GQIVK	f263-267	Legumin MS
	544.357	QKIR	f131-134	Legumin MS
		ALKGR	f409-413	Legumin A2
		LKGRA	f410-414	Legumin A2
		AGRIK	f363-367	Legumin A2
		RQLK	f497-500	Legumin A2
	544.370	IVIVT	f308-312	Vicilin

595.300	595.236	NEEGF	f505-509	Legumin MS
		EDAFN	f222-226	Legumin A2
	595.257	SVSSES	f246-251	Vicilin
	595.272	KGDFE	f317-321	Vicilin
		NTVFD	f424-428	Legumin A2
	595.283	YQRE	f77-80	Convicilin
		QRYE	f460-463	Convicilin
	595.309	SYLVN	f252-256	Convicilin
		AGTIAY	f129-134	Vicilin
	595.320	LPQHT	f87-91	Vicilin
	595.356	KPHTI	f81-85	Vicilin
			f200-204	Convicilin
	595.367	GHIRL	f52-56	Vicilin
617.400	617.300	QRNEA	f492-496	Legumin A2
		NEARQ	f494-498	Legumin A2
	617.304	WNINA	f453-457	Legumin MS
	617.314	EGGLIE	f49-54	Legumin A2
		EGDIIA	f138-143	Legumin A2
	617.325	EGKGNL	f432-437	Convicilin
		GKGNLE	f433-438	Convicilin
	617.350	EIEKV	f192-196	Vicilin
		VEIKE	f291-295	Vicilin
	617.362	LTDIR	f165-169	Legumin A2
	617.366	IFLPQ	f85-89	Vicilin
			f204-208	Convicilin

		PQLIF	f90-94	Legumin MS
	617.388	RKWK	f346-349	Legumin MS
663.300	663.247	QEEEE	f292-296	Legumin A2
			f280-284	Legumin MS
		EEEEQ	f281-285 f294-298	Legumin MS
			f339-343	Vicilin
		EEEQE	f295-299	Legumin MS
	663.283	KEEEE	f291-295 f371-375	Legumin MS
		EKEEE	f330-334 f370-374	Legumin MS
	663.294	SSQEVN	f528-533	Convicilin
	663.306	SENRAS	f514-519	Legumin A2
	663.335	LPSYSP	f83-88	Legumin MS
		ELTFPG	f522-527	Convicilin
	663.357	NQVFR	f526-530	Legumin MS
	663.382	KKYPQ	f393-397	Convicilin
	663.394	KVNRF	f132-136	Legumin A2
674.300	674.299	DQDTPV	f155-160	Legumin A2
	674.310	INAENN	f388-393	Vicilin
	674.322	QQQRD	f124-128	Legumin MS
	674.326	YLAGNH	f183-188	Legumin A2
		EWRPS	f58-62	Convicilin
	674.347	ELEAGR	f430-435	Legumin A2
		DSTPRV	f176-181	Legumin MS
		REQIE	f345-349	Convicilin

	674.358	NSGAGRI	f414-420	Legumin MS
		DKRQQ	f213-217	Vicilin
	674.369	QSRRQ	f119-123	Legumin MS
		SRRQQ	f120-124	Legumin MS
	674.372	VEIKEG	f291-296	Vicilin
		IVTVNE	f310-315	Vicilin
		SNLDLL	f379-384	Vicilin
	674.383	EELRK	f349-353	Convicilin
	674.394	KDKRQ	f212-216	Vicilin
689.200	689.274	NDQDTP	f154-159	Legumin A2
	689.285	NAENNQ	f389-394	Vicilin
	689.289	HEQEF	f188-192	Legumin A2
718.300	718.289	EEPQES	f113-118	Legumin A2
	718.289	EPQESE	f114-119	Legumin A2
	718.300	GQQQEE	f216-221	Legumin MS
	718.311	QGRNED	f236-241	Legumin A2
	718.337	KQEQE	f201-206	Legumin A2
		VNEGKGD	f313-319	Vicilin
	718.341	PDIYNP	f356-361	Legumin A2
	718.348	RTQQGE	f330-335	Convicilin
	718.359	SRRQGD	f329-334	Legumin A2
	718.362	EGGLIET	f49-55	Legumin A2
		QIEELS	f231-236	Vicilin
	718.373	ASSNLNL	f484-490	Convicilin
	718.377	GLIETW	f51-56	Legumin A2

	718.398	AVSLTD	f161-167	Legumin A2
	718.398	AVSLTDI	f162-168	Legumin A2
		IEELSK	f232-237	Vicilin
		ETIEKV	f308-313	Convicilin
	718.399	NRPGKF	f273-278	Convicilin
744.300	744.316	EQPEQN	f25-30	Legumin A2
		QPEQNE	f26-31	Legumin A2
	744.341	PELQEE	f277-282	Legumin MS
	744.363	KPDDR	f110-115	Vicilin
748.300	748.300	EDEEQV	f98-103	Convicilin
		DEEQVE	f99-104	Convicilin
	748.311	REGEEE	f137-142	Convicilin
		GEEEER	f139-144	Convicilin
		QQSQEE	f216-221	Vicilin
		RGEEEE	f310-315	Legumin A2
	748.326	WEREE	f94-98	Convicilin
		EEEWR	f104-108	Convicilin
		SHFASAE	f542-548	Convicilin
	748.329	NQLDQM	f173-178	Legumin A2
	748.333	VFPGCPE	f104-110	Legumin A2
	748.351	NPEIEF	f187-192	Legumin MS
	748.384	VAISASSN	f480-487	Convicilin
		AGTSSVIN	f471-478	Legumin A2
	748.391	DILVSCV	f402-408	Convicilin
	748.395	SGAGRIST	f415-422	Legumin MS

834.400	834.336	GEEEEEL	f363-369	Legumin MS
		EEEEEGI	f373-379	Legumin MS
	834.348	GDNGLEET	f333-340	Legumin A2
	834.359	GSRQEEE	f289-295	Legumin A2
	834.363	HTDADYI	f90-96	Vicilin
	834.363	YEKEEH	f63-68	Convicilin
	834.399	FPGSAQEV	f418-425	Vicilin
	834.406	ERGSRET	f446-452	Vicilin
	834.436	EIFIQQG	f91-97	Legumin A2
		LTVPQNY	f437-443	Legumin A2
	834.440	QMPRRF	f177-182	Legumin A2
	834.443	TRDRLSS	f452-458	Vicilin
	834.447	KSNNPFK	f500-506	Legumin A2
		PNGLHLPS	f78-85	Legumin MS
		PNHPELK	f59-65	Legumin MS
	834.458	RLQRFD	f175-180	Convicilin
	834.472	VVAATFNL	f484-491	Legumin A2
	834.483	VLLYRNG	f441-447	Legumin MS
		TVKSRFP	f4-10	Convicilin
	834.494	SFLVGRR	f208-214	Legumin MS
	834.497	DYILVVL	f94-100	Vicilin
901.400	901.390	EEHEKET	f199-205	Vicilin
	901.426	NDQDTPVI	f154-161	Legumin A2
	901.460	QRNEARQ	f492-498	Legumin A2
		GALMLPHY	f413-420	Convicilin

	901.464	NAFGLRHS	f539-546	Legumin MS
	901.474	TVLSPNDR	f226-233	Convicilin
941.500	941.436	PEYSNKFG	f377-384	Convicilin
	941.459	EKRHGEW	f53-59	Convicilin
	941.484	QQRYSFL	f204-210	Legumin MS
	941.491	QPQQRER	f441-447	Vicilin
	941.494	PQLQDLDI	f279-286	Vicilin
			f396-403	Convicilin
	941.505	KLSAEHGSL	f381-389	Legumin A2
		ERLNALEP	f35-42	Legumin A2
		LKLSAEHGS	f380-388	Legumin A2
	941.516	QKEVQPGR	f84-91	Convicilin
	941.530	IENPVKEL	f516-523	Convicilin
	941.557	FVIPVNRP	f268-275	Convicilin
	941.578	QGKGVLGLAV	f97-106	Legumin MS
967.500	967.430	VNCNGNTVF	f419-427	Legumin A2
	967.448	YNLERGDT	f236-243	Convicilin
	967.463	RPYYSNAP	f82-89	Legumin A2
	967.507	RSYETRR	f340-346	Legumin MS
	967.521	NYKAKLSSG	f354-362	Vicilin
	967.529	ARHQRGSR	f264-271	Legumin A2
	967.546	SPGDVVIIPA	f467-476	Convicilin
	967.593	GKGVLGLAVPG	f98-108	Legumin MS
1080.500	1080.444	EEERSSESQ	f141-149	Convicilin
		EERSSESQE	f142-150	Convicilin

	1080.451	DNGLEETVCT	f334-343	Legumin A2
	1080.459	ENEGNNIFSG	f206-215	Legumin A2
	1080.484	GNTVFDGELE	f423-432	Legumin A2
	1080.532	YNLERGDTI	f236-244	Convicilin
	1080.541	HKNAMFVPH	f390-398	Legumin A2
	1080.591	RAAVSHVNQV	f519-528	Legumin MS

Table 14. Potential amino acid sequences of peptides present in chymotrypsin-derived yellow field pea protein hydrolysate.

Observed mass (Da)	Calculated mass (Da)	Potential sequence	Location	Protein type
175.100	175.108	GV	f68-69 f100-101	Legumin MS
		VG	f211-212	Legumin MS
203.200	203.103	PS	f61-62 f364-365	Convicilin
			f352-353	Legumin A2
			f84-85 f88-89 f407-408	Legumin MS
		SP	f229-230 f467-468 f561-562	Convicilin
			f87-88 f89-90 f257-258 f276-277	Legumin MS
			f258-259 f355-356	Legumin A2
203.200	203.139	LA	f20-21 f354-355	Convicilin
			f149-150 f398-399 f416-417	Vicilin
			f21-22	Vicilin, Legumin MS
			f241-242 f104-105 f537-538	Legumin MS
			f6-7 f184-185	Legumin A2

			f470-471	
		AI	f223-224 f339-340 f424-425 f481-482	Convicilin
			f104-105 f150-151 f307-308	Vicilin
			f246-247	Legumin A2
			f143-144 f162-163	Legumin MS
		AL	f414-415	Convicilin
			f7-8 f22-23 f39-40 f69-70 f79-80 f409-410 f436-437	Legumin A2
			f40-41	Legumin MS
		IA	f132-133	Vicilin
			f142-143	Legumin A2, Legumin MS
			f161-162 f467-468	Legumin A2
			f404-405	Legumin MS
245.200	245.113	PE	f116-117 f377-378 f391-392 f549-550	Convicilin
			f275-276	Vicilin
			f27-28 f109-110	Legumin A2

			f260-261 f361-362	
		EP	f368-369 f548-549	Convicilin
			f252-253	Vicilin
			f41-42 f114-115	Legumin A2
			f115-116 f158-159 f201-202 f338-339	Legumin MS
	245.186	LL	f11-12 f314-315 f428-429 f439-440 f490-491	Convicilin
			f12-13	Convicilin, Vicilin
			f56-57 f75-76 f163-164 f197-198 f298-299 f299-300 f383-384	Vicilin
			f5-6 f15-16 f16-17	Legumin A2
			f14-15 f165-166 f442-443 f460-461	Legumin MS
		II	f17-18 f340-341 f473-474	Convicilin
			f95-96 f274-275	Legumin MS

			f141-142 f256-257 f406-407	Legumin A2
		LI	f71-72 f92-93	Legumin MS
			f214-215 f535-536	Convicilin
			f52-53	Legumin A2
		IL	f215-216 f224-225 f298-299 f403-404	Convicilin
			f96-97 f105-106 f181-182 f428-429	Vicilin
			f430-431	Legumin MS
279.200	279.134	YP	f395-396	Convicilin
		PY	f149-150	Legumin MS
	279.170	LF	f13-14 f156-157 f165-166 f185-186 f386-387	Convicilin
			f46-47	Vicilin
			f15-16	Legumin MS
		FL	f19-20 f155-156 f205-206 f504-505	Convicilin
			f14-15	Convicilin, Legumin A2
			f162-163	Convicilin, Vicilin

			f192-193 f220-221 f507-508	Legumin A2
			f20-21 f86-87 f397-398	Vicilin
			f5-6 f13-14 f209-210 f240-241	Legumin MS
		IF	f18-19 f204-205	Convicilin
			f37-38 f66-67 f85-86 f286-287	Vicilin
			f92-93 f212-213	Legumin A2
			f93-94	Legumin MS
		FI	f36-37	Vicilin
			f93-94	Legumin A2
			f94-95	Legumin MS
302.300	302.207	LGI	f15-17	Convicilin
		GII	f16-18	Convicilin
		AIV	f424-426	Convicilin
			f307-309	Vicilin
			f246-248	Legumin A2
		LLG	f439-441 f490-492	Convicilin
			f383-385	Vicilin
			f16-18	Legumin A2

		LGL	f440-442	Convicilin
			f102-104	Legumin MS
		VAI	f480-482	Convicilin
			f161-163	Legumin MS
		VIA	f160-162	Legumin A2
			f141-143	Legumin MS
		GLI	f51-53	Legumin A2
		VAL	f68-70	Legumin A2
		IAV	f142-144 f161-163	Legumin A2
		VLA	f536-538	Legumin MS
		LAV	f104-106	Legumin MS
		LVA	f160-162	Legumin MS
316.300	316.223	AIL	f223-225	Convicilin
			f104-106	Vicilin
		AII	f339-341	Convicilin
		LAI	f149-151	Vicilin
		LAL	f6-8	Legumin A2
		LLA	f5-7	Legumin A2
		IIA	f141-143	Legumin A2
		IAI	f142-144	Legumin MS
		VVV	f491-493	Legumin MS
361.300	361.208	AISA	f481-484	Convicilin
		AGTI	f129-132	Vicilin
		GTIA	f130-133	Vicilin

		QTL	f44-46	Vicilin
		LAGT	f470-473	Legumin A2
		VAAT	f485-488	Legumin A2
		TLQ	f74-76	Legumin A2
		AGLT	f51-54	Legumin MS
		DKV	f483-485	Legumin MS
	361.219	VSR	f343-345	Convicilin
	361.245	TIK	f243-245	Convicilin
			f124-126	Vicilin
		TKL	f3-5	Legumin A2
		IKT	f366-368	Legumin A2
401.300	401.203	PGEV	f533-536	Legumin MS
	401.214	EPR	f115-117 f201-203 f338-340	Legumin MS
	401.276	AIVV	f424-427	Convicilin
		VIAV	f160-163	Legumin A2
		VLGL	f101-104	Legumin MS
	401.287	RLI	f534-536	Convicilin
		IRL	f54-56	Vicilin
		RLL	f55-57 f74-76	Vicilin
		RIL	f427-429	Vicilin
		LRL	f346-348	Legumin A2
			f434-436	Legumin MS
		LIR	f71-73	Legumin MS

		ILR	f430-432	Legumin MS
416.200	416.164	FCF	f12-14	Legumin A2
	416.178	GNPE	f186-189	Legumin MS
	416.182	SFY	f569-571	Convicilin
		FSY	f454-456	Legumin A2
		YSF	f207-209	Legumin MS
	416.193	PHY	f418-420	Convicilin
			f397-399	Legumin A2
		WNP	f56-58	Legumin A2
			f57-59	Legumin MS
	416.225	SRGP	f258-261	Vicilin
		PSRG	f407-410	Legumin MS
	416.229	HFL	f4-6	Legumin MS
	416.250	NLLG	f489-492	Convicilin
		LVGQ	f322-325	Vicilin
		LNIG	f348-351	Legumin A2
		GQIV	f263-266	Legumin MS
		GQLV	f488-491	Legumin MS
		VVAQ	f498-501	Legumin MS
		VLAN	f536-539	Legumin MS
	416.262	QLR	f324-326	Convicilin
		RLQ	f175-177	Convicilin
			f234-236 f415-417	Legumin A2
		LQR	f176-178	Convicilin

			f491-493	Legumin A2
		IQR	f409-411	Vicilin
			f379-381	Legumin MS
		RQL	f497-499	Legumin A2
		RAGI	f464-467	Legumin A2
		GIAR	f466-469	Legumin A2
		RLAG	f469-472	Legumin A2
		AGRI	f363-366	Legumin A2
			f417-420	Legumin MS
		RAAV	f519-522	Legumin MS
		GRAL	f434-437	Legumin A2
	416.287	VAVK	f373-376	Vicilin
		KGVL	f99-102	Legumin MS
	416.298	LRK	f351-353	Convicilin
		RKL	f352-354	Convicilin
		KLR	f345-347	Legumin A2
		RIK	f365-367	Legumin A2
		KIR	f132-134 f399-401	Legumin MS
		KRL	f253-255	Legumin MS
418.300	418.204	ERN	f454-456	Convicilin
		RNE	f326-328	Vicilin
			f238-240 f493-495	Legumin A2
		ENR	f515-517	Legumin A2
		DQR	f61-63	Vicilin

		QRD	f126-128	Legumin MS
		REN	f401-403	Legumin MS
		GEGR	f121-124	Legumin A2
			f466-469	Legumin MS
		DRAG	f463-466	Legumin A2
		RGEG	f465-468	Legumin MS
	418.220	WRG	f107-109	Convicilin
	418.230	VNSV	f288-291	Vicilin
		KGDV	f138-141	Legumin MS
		TINA	f37-40	Legumin MS
	418.241	KRD	f130-132	Convicilin
			f217-219	Legumin A2
		DRK	f327-329 f451-453	Convicilin
		DKR	f180-182	Convicilin
			f213-215	Vicilin
	418.252	RRS	f208-210	Vicilin
		RSR	f257-259	Vicilin
		SRR	f329-331	Legumin A2
			f120-122	Legumin MS
	418.266	LAKS	f354-357	Convicilin
		AKLS	f357-360	Vicilin
		KLSA	f381-384	Legumin A2
		AKSL	f447-450	Legumin A2
		SAKI	f397-400	Legumin MS

461.400	461.308	KKSV	f244-247	Vicilin
510.400	510.329	IAIPP	f142-146	Legumin MS
525.200	525.219	ETFE	f110-113	Legumin A2
		TFEE	f111-114	Legumin A2
	525.255	YETI	f307-310	Convicilin
	525.267	NTKY	f304-307	Convicilin
		SGFSK	f175-179	Vicilin
	525.289	VNRH	f227-230	Legumin A2
530.200	530.225	YANY	f27-30	Convicilin
	530.236	PHYN	f418-421	Convicilin
			f301-304	Vicilin
			f397-400	Legumin A2
		WNPN	f56-59	Legumin A2
			f57-60	Legumin MS
	530.257	PEER	f116-119	Convicilin
		EEPR	f114-117	Legumin MS
	530.283	ARHF	f2-5	Legumin MS
563.300	563.267	KEDTA	f248-252	Legumin MS
		DDKVS	f482-486	Legumin MS
		EDTAK	f249-253	Legumin MS
		QLDST	f174-178	Legumin MS
	563.286	ELKCA	f63-67	Legumin MS
	563.303	GTSSVI	f472-477	Legumin A2
	563.319	NLLGF	f489-493	Convicilin
		FVVAQ	f497-501	Legumin MS

	563.322	MATKL	f1-5	Legumin A2
	563.330	LQRF	f176-179	Convicilin
		AFGLR	f540-544	Legumin MS
588.400	588.310	NLERG	f237-241	Convicilin
			f118-122	Vicilin
		DRLQG	f233-237	Legumin A2
	588.321	QRRE	f111-114	Convicilin
		QRER	f41-44	Convicilin
			f444-447	Vicilin
		REQR	f321-324	Legumin MS
	588.324	DLDIL	f400-404	Convicilin
	588.335	ISQIQ	f406-410	Vicilin
		TLNQL	f171-175	Legumin MS
	588.346	ARLAGT	f468-473	Legumin A2
		AGRALT	f433-438	Legumin A2
		RATLQ	f72-76	Legumin A2
		RIVNS	f471-475	Legumin MS
	588.358	RKER	f452-455	Convicilin
		LRSRG	f256-260	Vicilin
		KERR	f319-322	Legumin A2
	588.372	LSGKAI	f100-105	Vicilin
		SGKAIL	f101-106	Vicilin
		VAAKSL	f445-450	Legumin A2
	588.383	TAKLR	f343-347	Legumin A2
		TAKRL	f251-255	Legumin MS

611.400	611.355	FGKLF	f383-387	Convicilin
	611.413	VVIIPA	f471-476	Convicilin
	611.424	LPILR	f428-432	Legumin MS
660.500	660.404	KKEQK	f81-85	Convicilin
		LSKNAK	f235-240	Vicilin
		LSRATL	f70-75	Legumin A2
670.500	670.403	PVLRW	f375-379	Legumin A2
	670.413	IIAVPTG	f141-147	Legumin A2
		IAVPTGI	f142-148	Legumin A2
	670.450	ILVVLN	f215-220	Convicilin
		LTVLKP	f106-111	Vicilin
	670.461	RAIVVL	f423-428	Convicilin
		RAIVIV	f306-311	Vicilin
676.500	676.403	LNLLGF	f488-493	Convicilin
	676.406	MATKLL	f1-6	Legumin A2
693.400	693.312	LEETVC	f337-342	Legumin A2
	693.320	EGNNIF	f208-213	Legumin A2
		YEEPR	f113-117	Legumin MS
	693.331	LAHHSE	f21-26	Legumin MS
	693.357	ATFNLQ	f487-492	Legumin A2
		EGNKVF	f476-481	Legumin MS
	693.360	AATTMKA	f2-8	Vicilin
	693.368	QRFDK	f177-181	Convicilin
		RNFLSG	f502-507	Convicilin
		NQYLR	f288-292	Convicilin

		QNYRL	f190-194	Convicilin
		LQNYR	f189-193	Convicilin
		KFDQR	f59-63	Vicilin
		QNYRL	f71-75	Vicilin
		LQNYR	f70-74	Vicilin
		RNGIYA	f445-450	Legumin MS
	693.382	ADYILV	f93-98	Vicilin
	693.390	RQPRH	f282-286	Legumin A2
		PRHQR	f284-288	Legumin A2
		RQPRH	f302-306	Legumin A2
		PRHQR	f304-308	Legumin A2
	693.393	YRLLE	f73-77	Vicilin
		RLLEY	f74-78	Vicilin
	693.415	RRFSK	f134-138	Legumin MS
715.300	715.301	EQSHSQ	f284-289	Legumin MS
	715.326	GGNPEIE	f185-191	Legumin MS
	715.341	YSNKFG	f379-384	Convicilin
	715.362	ENPVKE	f517-522	Convicilin
	715.373	ARLSPGD	f464-470	Convicilin
		RTIDPN	f74-79	Legumin MS
		LRSPQD	f255-260	Legumin MS
	715.377	PQNPFI	f32-37	Vicilin
		VEFLAH	f238-243	Legumin MS
	715.396	RRQQQ	f121-125	Legumin MS
	715.398	GNLELLG	f435-441	Convicilin

729.300	729.324	PYWTY	f149-153	Legumin MS
	729.364	LSFCFL	f10-15	Legumin A2
		SFCFLL	f11-16	Legumin A2
		FSLCFL	f9-14	Legumin MS
	729.378	TIDPNGL	f75-81	Legumin MS
	729.389	EARLSPG	f463-469	Convicilin
		RATPGEV	f530-536	Legumin MS
	729.393	LHLPSY	f81-86	Legumin MS
	729.396	LGLAVPGC	f102-109	Legumin MS
781.500	781.420	HPVAISAS	f478-485	Convicilin
	781.443	RHQKVN	f129-134	Legumin A2
		HQKVNR	f130-135	Legumin A2
	781.445	GDVIAIPP	f139-146	Legumin MS
		DVIAIPPG	f140-147	Legumin MS
815.600	815.510	TAKLRLN	f343-349	Legumin A2
	815.524	TIEKVLL	f309-315	Convicilin
	815.546	LRKLAKS	f351-357	Convicilin
845.300	845.327	EEEQSHS	f282-288	Legumin MS
	845.364	EDPEERA	f114-120	Convicilin
	845.368	VTYANYD	f25-31	Convicilin
	845.379	SHFASAEP	f542-549	Convicilin
	845.382	NQLDQMP	f173-179	Legumin A2
	845.386	GQRNENQ	f324-330	Vicilin
		NAENNQR	f389-395	Vicilin
		AENNQRN	f390-396	Vicilin

	845.390	HYNLNAN	f398-404	Legumin A2
867.300	867.359	DDRNSFN	f112-118	Vicilin
	867.367	FNQCQLD	f30-36	Legumin MS
	867.370	HHSESDR	f23-29	Legumin MS
	867.378	ACLAHHSE	f19-26	Legumin MS
878.600	878.509	LVEYRAK	f194-200	Convicilin
905.400	905.373	ELQEEEE	f278-284	Legumin MS
	905.396	SSNNQLDQ	f170-177	Legumin A2
	905.410	EEEELEK	f365-371	Legumin MS
		EEELEKE	f366-372	Legumin MS
		EELEKEE	f367-373	Legumin MS
		ELEKEEE	f368-374	Legumin MS
		LEKEEEE	f369-375	Legumin MS
	905.425	SLPSEFEP	f362-369	Convicilin
	905.432	KEEESQR	f552-558	Convicilin
		EEESQRK	f553-559	Convicilin
		SQRKEEE	f288-294	Legumin MS
	905.436	AFPGSAQEV	f417-425	Vicilin
		SNAPQEIF	f86-93	Legumin A2
	905.459	NNQRNFL	f499-505	Convicilin
			f392-398	Vicilin
	905.469	LTDIRSSN	f165-172	Legumin A2
	905.473	QEIFIQQ	f90-96	Legumin A2
	905.473	ALTVPQNY	f436-443	Legumin A2
	905.473	LTVPQNYA	f437-444	Legumin A2

959.500	959.418	RSSESQEH	f144-151	Convicilin
	959.418	SSESQEHR	f145-152	Convicilin
	959.422	LFENENGH	f165-172	Convicilin
			f46-53	Vicilin
		FENENGHI	f166-173	Convicilin
			f47-54	Vicilin
	959.425	NNQLDQMP	f172-179	Legumin A2
	959.429	NAENNQRN	f389-396	Vicilin
	959.432	AEPEQKEE	f547-554	Convicilin
	959.443	EPDNRIES	f41-48	Legumin A2
		PDNRIESE	f42-49	Legumin A2
	959.447	GGNPEIEFP	f185-193	Legumin MS
	959.469	NQKQSHFA	f538-545	Convicilin
			f431-438	Vicilin
	959.479	VNEGKGNLE	f430-438	Convicilin
	959.502	RGRQEGEK	f44-51	Convicilin
	959.506	HYNSRAIV	f419-426	Convicilin
			f302-309	Vicilin
	959.513	LHKNAMFV	f389-396	Legumin A2
	959.516	ILENQKQS	f428-435	Vicilin
		ELEAGRALT	f430-438	Legumin A2
		IVNSEGNKV	f472-480	Legumin MS
	959.531	DFVIPVNR	f267-274	Convicilin
	959.549	RDRKRTQ	f326-332	Convicilin
	959.567	VIPAGHPVAV	f366-375	Vicilin

		RPVKELAF	f411-418	Vicilin
966.400	966.405	TFEEPQES	f111-118	Legumin A2
	966.420	PSEFEPFN	f364-371	Convicilin
	966.432	EWRPSYE	f58-64	Convicilin
	966.443	YNHGHEPL	f153-160	Legumin MS
	966.453	TVNEGKGDF	f312-320	Vicilin
984.400	984.442	EGNNIFSGF	f208-216	Legumin A2
	984.478	EQEFLRY	f189-195	Legumin A2
		DSTPRVFY	f176-183	Legumin MS
		HLPSYSPSP	f82-90	Legumin MS
1044.500	1044.474	AGNHEQEFL	f185-193	Legumin A2
		LAGNHEQEF	f184-192	Legumin A2
	1044.496	VVAQQAGNEE	f498-507	Legumin MS
		HSLNTKEDT	f243-251	Legumin MS
	1044.500	SYVAFKTND	f455-463	Legumin A2
	1044.518	ERNNEVQR	f454-461	Convicilin
		RRQGDNGLE	f330-338	Legumin A2
	1044.521	DQDTPVIAVS	f155-164	Legumin A2
	1044.543	LKNEQQER	f442-449	Convicilin
		SLCFLLFTT	f10-18	Legumin MS
	1044.547	WNINANSLL	f453-461	Legumin MS
	1044.554	RKERNNEV	f452-459	Convicilin
		QIKSNGNRG	f549-558	Legumin MS
	1044.568	EQIEELRK	f346-353	Convicilin
		LSRGQIEEL	f227-235	Vicilin

	1044.572	NPVKELTFP	f518-526	Convicilin
		PVKELAFPGS	f412-421	Vicilin
	1044.594	ISTVNSLTLP	f420-429	Legumin MS
		STVNSLTLPI	f421-430	Legumin MS
1091.400	1091.437	QGEEEEELE	f362-370	Legumin MS
		EEEEEEGIQ	f372-380	Legumin MS
	1091.474	GEEEEELEK	f363-371	Legumin MS
		KEEEEEEGI	f371-379	Legumin MS
1140.500	1140.486	WTYNHGHEP	f151-159	Legumin MS
	1140.505	NTDYEEIEK	f187-195	Vicilin
	1140.568	NKFQTLFEN	f41-49	Vicilin
		FKRDFLEDA	f216-224	Legumin A2
		KRDFLEDAF	f217-225	Legumin A2
	1140.579	LMGISFLASVC	f15-25	Vicilin
	1140.594	KFLTLFENE	f161-169	Convicilin
1160.600	1160.512	HGEWRPSYE	f56-64	Convicilin
	1160.529	NRDDNEELR	f137-145	Vicilin
	1160.543	AGDEDNVISQI	f399-409	Vicilin
		LAGDEDNVISQ	f398-408	Vicilin
		DEDNVISQIQ	f401-410	Vicilin
	1160.554	RNEDEEKGAI	f238-247	Legumin A2
	1160.569	SRSDPQNPFI	f28-37	Vicilin
	1160.573	LQVVNCNGNTV	f416-426	Legumin A2
	1160.606	FLVPARQSEN	f507-516	Legumin A2
	1160.616	GDTIKIPAGTTS	f241-252	Convicilin

	1160.642	NLLGFGINAKN	f489-499	Convicilin
		LLGFGINAKNN	f490-500	Convicilin
		IYNPEAGRIK	f358-367	Legumin A2
	1160.663	EELRKLAKSS	f349-358	Convicilin
		AGRIKTVTSLD	f363-373	Legumin A2
		GRISTVNSLTL	f418-428	Legumin MS
	1160.679	IRLLQKFDQ	f54-62	Vicilin
1235.600	1235.554	PETYEEPRSQ	f110-119	Legumin MS
	1235.561	GLAVPGCPETYE	f103-114	Legumin MS
	1235.568	ASVCVSSRSDPQ	f22-33	Vicilin
	1235.580	NHEQEFLRY	f187-195	Legumin A2
	1235.594	YLGGNPEIEFP	f183-193	Legumin MS
	1235.601	RSDLFENLQN	f182-191	Convicilin
		QSFLLSGNQNQ	f160-170	Vicilin
		SFLLSGNQNQQ	f161-171	Vicilin
	1235.605	PSEFEPFNLR	f364-373	Convicilin
	1235.613	ARPSRGDLYNS	f405-415	Legumin MS
	1235.617	TWNPNHPELK	f56-65	Legumin MS
	1235.631	PSYSPSPQLIF	f84-94	Legumin MS
	1235.635	EERQPRHQR	f280-288	Legumin A2
			f300-308	Legumin A2
	1235.653	NRPGKFEAFGL	f273-283	Convicilin
	1235.663	FSKNILEASLN	f294-304	Convicilin
		KVFDDKVSLGQ	f479-489	Legumin MS
		GLHIISPELQE	f271-281	Legumin MS
		ISASSNLNLLGF	f482-493	Convicilin
	1235.697	NVNRHIVDRL	f226-235	Legumin A2

	1235.699	QHIDADLILVV	f208-218	Convicilin
		EIKEGSLLLPH	f292-302	Vicilin
1397.600	1397.534	EEEDEDEPRSY	f332-342	Legumin MS
		EEDEDEPRSYE	f333-343	Legumin MS
	1397.652	EEEEREQRHR	f317-326	Legumin MS
	1397.663	RHQRGSRQEED	f265-275	Legumin A2
	1397.677	EEEGIQRQHSKG	f375-386	Legumin MS
	1397.679	EASLNTKYETIE	f300-311	Convicilin
	1397.691	TPEKNPQLQDLD	f274-285	Vicilin
	1397.692	AVPTGIVFWMYN	f143-154	Legumin A2

Table 15. Potential amino acid sequences of peptides present in pepsin-derived yellow field pea protein hydrolysate.

Observed mass (Da)	Calculated mass (Da)	Potential sequence	Location	Protein type
175.200	175.108	VG	f323-324	Vicilin
			f211-212	Legumin MS
		GV	f67-68	Legumin A2
			f68-69 f100-101	Legumin MS
316.300	316.223	AIL	f104-106	Vicilin
			f223-225	Convicilin
		LAI	f149-151	Vicilin
		AII	f339-341	Convicilin
		LAL	f6-8	Legumin A2
		LLA	f5-7	Legumin A2
		IIA	f141-143	Legumin A2
		IAI	f142-144	Legumin MS
		VVV	f491-493	Legumin MS
429.400	429.307	LLAL	f5-8	Legumin A2
		LVVV	f490-493	Legumin MS
	450.219	GTSSV	f472-476	Legumin A2
450.300	450.235	FGIN	f386-389	Vicilin
			f493-496	Convicilin
		AFNV	f224-227	Legumin A2
	450.238	TMKA	f5-8	Vicilin
		MATK	f1-4	Legumin A2

	450.246	QRF	f177-179	Convicilin
		QFR	f62-64	Legumin A2
	450.282	FKR	f216-218	Legumin A2
502.300	502.214	EPEQ	f548-551	Convicilin
		EQPE	f25-28	Legumin A2
		EEPQ	f113-116	Legumin A2
		EPQE	f114-117	Legumin A2
	502.226	PDDR	f111-114	Vicilin
	502.251	TPGEV	f532-536	Legumin MS
	502.273	NVNR	f226-229	Legumin A2
	502.284	GRRGG	f212-216	Legumin MS
	502.287	GDVVI	f469-473	Convicilin
		PLSSV	f562-566	Convicilin
	502.298	RVLD	f145-148	Vicilin
		VDRI	f425-428	Vicilin
		INKQ	f348-351	Vicilin
		KQVQ	f350-353	Vicilin
		LNGKA	f219-223	Convicilin
		NGKAI	f220-224	Convicilin
		DLRV	f262-265	Convicilin
		NKGAL	f411-415	Convicilin
		GINAK	f494-498	Convicilin
		INKGA	f410-414	Convicilin
		IKNQ	f536-539	Convicilin
		VDRL	f232-235	Legumin A2

		IVDR	f231-234	Legumin A2
		IQGKG	f96-100	Legumin MS
	502.310	KQAR	f262-265	Legumin A2
	502.323	GSLLL	f296-300	Vicilin
		IAVSL	f161-165	Legumin A2
		GLSII	f253-257	Legumin A2
579.300	579.226	SESQE	f146-150	Convicilin
	579.244	CQLDT	f33-37	Legumin MS
	579.281	ASVCVT	f21-26	Convicilin
		TTACLA	f17-22	Legumin MS
	579.314	AYLVN	f133-137	Vicilin
		IFVNS	f286-290	Vicilin
	579.325	GHPVAV	f370-375	Vicilin
		LRGFS	f291-295	Convicilin
		QYLR	f289-292	Convicilin
		LRYQ	f193-196	Legumin A2
587.400	587.303	LKPDD	f109-113	Vicilin
	587.337	RRQQ	f121-124	Legumin MS
		RQQR	f203-206	Legumin MS
	587.340	ITPEK	f273-277	Vicilin
			f389-393	Convicilin
		NLDLL	f380-384	Vicilin
		LLVNE	f428-432	Convicilin
		GDIIAV	f139-144	Legumin A2
		GDVIAI	f139-144	Legumin MS

		LDVVAA	f482-487	Legumin A2
	587.351	RDAII	f337-341	Convicilin
	587.362	RKNGL	f387-391	Legumin MS
	587.366	LRWL	f377-380	Legumin A2
	587.374	QRKR	f557-560	Convicilin
		KGRAR	f411-415	Legumin A2
	587.376	LVVLSG	f97-102	Vicilin
		IVKVE	f265-269	Legumin MS
	587.387	VKVKGG	f248-253	Legumin A2
		VSLIR	f69-73	Legumin MS
600.400	600.303	FLSSF	f5-9	Legumin MS
	600.314	IYAPH	f448-452	Legumin MS
	600.335	KEVQP	f85-89	Convicilin
		LNIGPS	f348-353	Legumin A2
	600.346	ARLSPG	f464-469	Convicilin
		LRSPQ	f255-259	Legumin MS
	600.383	KRSPL	f559-563	Convicilin
		AGIARL	f465-470	Legumin A2
		GIARLA	f466-471	Legumin A2
		IARLAG	f467-472	Legumin A2
	600.383	VRIVN	f470-474	Legumin MS
	600.394	GRVRI	f468-472	Legumin MS
	600.394	LVGRR	f210-214	Legumin MS
	600.419	AKLRL	f344-348	Legumin A2
		LRKLA	f351-355	Convicilin

670.200	670.250	MYNDQ	f152-156	Legumin A2
	670.257	ETYEE	f111-115	Legumin MS
	670.286	CVTYAN	f24-29	Convicilin
693.400	693.312	LEETVC	f337-342	Legumin A2
	693.316	RSSESQ	f144-149	Convicilin
	693.320	EGNNIF	f208-213	Legumin A2
	693.331	LAHHSE	f21-26	Legumin MS
	693.357	ATFNLQ	f487-492	Legumin A2
		EGNKVF	f476-481	Legumin MS
	693.360	AATTMKA	f2-8	Vicilin
	693.368	KFDQR	f59-63	Vicilin
		QRFDK	f177-181	Convicilin
		RNFLSG	f502-507	Convicilin
		NQYLR	f288-292	Convicilin
		QNYRL	f71-75	Vicilin
			f190-194	Convicilin
		LQNYR	f70-74	Vicilin
			f189-193	Convicilin
		RNGIYA	f445-450	Legumin MS
	693.382	ADYILV	f93-98	Vicilin
	693.390	RQPRH	f282-286 f302-306	Legumin A2
		PRHQR	f284-288 f304-308	Legumin A2
	693.393	YRLLE	f73-77	Vicilin
		RLLEY	f74-78	Vicilin

	693.415	RRFSK	f134-138	Legumin MS
732.300	732.316	QGDNGLE	f332-338	Legumin A2
		EDEPRS	f336-341	Legumin MS
	732.327	VNRDDN	f136-141	Vicilin
		QDERGQ	f259-264	Legumin MS
	732.352	EERDAI	f335-340	Convicilin
		NEGNSVL	f227-233	Legumin MS
		IVNSEGN	f472-478	Legumin MS
	732.363	SQEVNR	f529-534	Convicilin
		NDRAAVS	f517-523	Legumin MS
	732.372	PGIPYW	f146-151	Legumin MS
	732.375	GSQRRE	f109-114	Convicilin
		QRERGS	f444-449	Vicilin
		ERRGSQ	f320-325	Legumin A2
		RGSRQE	f268-273 f288-293	Legumin A2
		KSNGNRG	f552-558	Legumin MS
	732.400	KNQKQS	f537-542	Convicilin
770.200	770.284	NTDYEE	f187-192	Vicilin
787.600	787.504	VKVKGGLS	f248-255	Legumin A2
795.400	795.352	SSPDIYN	f354-360	Legumin A2
	795.357	RFNQCQ	f29-34	Legumin MS
	795.363	PQHIDAD	f207-213	Convicilin
		ENKNQY	f285-290	Convicilin
		YNLNANS	f399-405	Legumin A2

		NALEPDH	f39-45	Legumin MS
	795.382	GCFALRE	f19-25	Legumin A2
	795.393	NKQFRC	f60-65	Legumin A2
	795.404	PGKFEAF	f275-281	Convicilin
	795.433	QARHQR	f263-268	Legumin A2
	795.458	RHIVDR	f229-234	Legumin A2
839.600	839.524	VLDLAIPV	f146-153	Vicilin
	839.560	PLVAITLL	f159-166	Legumin MS
850.400	850.369	PDDRNSF	f111-117	Vicilin
	850.372	CVSSRSDP	f25-32	Vicilin
	850.379	SKKEDED	f306-312	Legumin MS
	850.394	FPGSSQEV	f525-532	Convicilin
	850.405	FNLERGD	f117-123	Vicilin
	850.442	AYLVNRD	f133-139	Vicilin
		QVQNYKA	f351-357	Vicilin
	850.467	GTIAYLVN	f130-137	Vicilin
		SKIFENL	f64-70	Vicilin
		FSKNILE	f177-183	Vicilin
			f294-300	Convicilin
		KVFDDKV	f479-485	Legumin MS
	850.478	VQNYKAK	f352-358	Vicilin
860.500	860.401	YFGMVFP	f100-106	Legumin A2
	860.404	PEIEFPE	f188-194	Legumin MS
	860.411	EEEGIQR	f375-381	Legumin MS

		QNEGNSVL	f226-233	Legumin MS
		NEGKGNLE	f431-438	Convicilin
	860.415	LTETWNP	f53-59	Legumin MS
	860.419	GFEYVVF	f508-514	Legumin MS
	860.433	QQRERGS	f443-449	Vicilin
		ARQSENR	f511-517	Legumin A2
		RQSENRA	f512-518	Legumin A2
		QRGSRQE	f267-273 f287-293	Legumin A2
	860.436	DEEKGAIV	f241-248	Legumin A2
	860.437	HYNSRAI	f302-308	Vicilin
			f419-425	Convicilin
	860.445	LHKNAMF	f389-395	Legumin A2
	860.447	VSREQIE	f343-349	Convicilin
		RIESEGGL	f45-52	Legumin A2
		RVESEAGL	f46-53	Legumin MS
		IVNSEGNK	f472-479	Legumin MS
	860.462	FALREQP	f21-27	Legumin A2
		YKSKPHT	f78-84	Vicilin
	860.470	KERRGSQ	f319-325	Legumin A2
		ERRGSQK	f320-326	Legumin A2
	860.472	ILEASLNT	f298-305	Convicilin
	860.484	SQVAQIKS	f546-553	Legumin MS
	860.488	VKELAFPG	f413-420	Vicilin
	860.499	VIPAGHPVA	f366-374	Vicilin

		IPAGHPVAVK	f367-375	Vicilin
	860.506	KKERRGS	f318-324	Legumin A2
	860.527	MATKLLAL	f1-8	Legumin A2
	860.535	KSRFPLL	f6-12	Convicilin
880.300	880.269	EEDEDED	f273-279	Legumin A2
		EDEDEDE	f274-280	Legumin A2
			f309-315	Legumin MS
		DEDEDEE	f275-281	Legumin A2
			f310-316	Legumin MS
	880.368	NFLAGDED	f396-403	Vicilin
		FLAGDEDN	f397-404	Vicilin
888.400	888.394	EPEQKEE	f548-554	Convicilin
		PEQKEEE	f549-555	Convicilin
	888.417	ERNNEVQ	f454-460	Convicilin
		RQGDNGLE	f331-338	Legumin A2
	888.425	NEFGKFF	f265-271	Vicilin
	888.432	NQKQSHF	f431-437	Vicilin
			f538-544	Convicilin
		LKNEQQE	f442-448	Convicilin
	888.453	KERNNEV	f453-459	Convicilin
		RIVNSEGN	f471-478	Legumin MS
	888.457	EYKSKPH	f77-83	Vicilin
	888.467	LDTINALE	f35-42	Legumin MS
		LLEEQEK	f314-320	Convicilin
	888.476	RGSQRRE	f108-114	Convicilin

		QRERGSR	f444-450	Vicilin
	888.478	KEQKEVQ	f82-88	Convicilin
		ASSNLNLLG	f484-492	Convicilin
	888.483	SPSPQLIF	f87-94	Legumin MS
899.600	899.520	SKGDVIAIP	f137-145	Legumin MS
	899.531	RGDTIKLP	f121-128	Vicilin
		RGDTIKIP	f240-247	Convicilin
	899.556	NLELLGLK	f436-443	Convicilin
		LELLGLKN	f437-444	Convicilin
	899.578	RLIKNQK	f534-540	Convicilin
	899.592	ILVVLSGKA	f96-104	Vicilin
		LVVLSGKAI	f97-105	Vicilin
		VVLSGKAIL	f98-106	Vicilin
	899.604	KGAIVKVKG	f244-252	Legumin A2
919.400	919.400	AGDEDNVIS	f399-407	Vicilin
		DEDNVISQ	f401-408	Vicilin
	919.412	EQRKEDD	f332-338	Vicilin
		RNEDEEK	f238-244	Legumin A2
		DNRIESEG	f43-50	Legumin A2
		QRKEDDE	f333-339	Vicilin
	919.430	VVNCNGNTV	f418-426	Legumin A2
	919.437	LSGSDDNVI	f505-513	Convicilin
	919.452	ELAFPGSAQ	f415-423	Vicilin
		LAFPGSAQE	f416-424	Vicilin

	919.463	IYNPEAGR	f358-365	Legumin A2
		YNPEAGRI	f359-366	Legumin A2
	919.470	SRETRDR	f449-455	Vicilin
	919.474	NQRNFLAG	f393-400	Vicilin
	919.488	DLLGFGINA	f382-390	Vicilin
		KELAFPGSA	f414-422	Vicilin
	919.492	ELKCAGVSL	f63-71	Legumin MS
	919.500	PAGHPVAISA	f475-484	Convicilin
940.600	940.525	SNKFGKLF	f380-387	Convicilin
		YVLLYRN	f440-446	Legumin MS
	940.550	IAIPPGIPY	f142-150	Legumin MS
	940.557	QRPVKELA	f410-417	Vicilin
		ISQIQRPV	f406-413	Vicilin
		VISQIQRP	f405-412	Vicilin
963.400	963.354	VNQDDEED	f255-262	Convicilin
	963.379	GEEEEELE	f363-370	Legumin MS
		EEEEEEGI	f372-379	Legumin MS
	963.401	GSRQEEEE	f289-296	Legumin A2
	963.405	YEKEEHE	f63-69	Convicilin
	963.472	CFALREQP	f20-27	Legumin A2
	963.478	QDLDIFVN	f282-289	Vicilin
	963.485	ETRDRLSS	f451-458	Vicilin
977.400	977.369	NQDDEEDL	f256-263	Convicilin
	977.473	DPQNPFIF	f31-38	Vicilin
	977.494	FEITPEKN	f271-278	Vicilin

	977.495	RFYLAGNH	f181-188	Legumin A2
994.400	994.385	SEKEEEDE	f329-336	Legumin MS
	994.396	EEEERSSE	f140-147	Convicilin
	994.400	FEEPQESE	f112-119	Legumin A2
	994.411	NFLAGDEDN	f396-404	Vicilin
	994.419	VPGCPETYE	f106-114	Legumin MS
	994.438	ETWNPNHP	f55-62	Legumin MS
		TWNPNHPE	f56-63	Legumin MS
	994.448	VNEGKGDFE	f313-321	Vicilin
		QTLFENEN	f44-51	Vicilin
	994.459	DRNSFNLE	f113-120	Vicilin
		QNQQNYLS	f168-175	Vicilin
	994.470	EQGEGRRY	f119-126	Legumin A2
	994.484	EAFGLSENK	f279-287	Convicilin
	994.489	HGSLHKNAM	f386-394	Legumin A2
	994.495	LEPDHRVE	f41-48	Legumin MS
1149.600	1149.506	SSPDIYNPEAG	f354-364	Legumin A2
	1149.517	VAQQAGNEEGF	f499-509	Legumin MS
	1149.536	GCFALREQPE	f19-28	Legumin A2
	1149.542	NIGPSSSPDIY	f349-359	Legumin A2
		IGPSSSPDIYN	f350-360	Legumin A2
	1149.569	HKPEYSNKF	f375-383	Convicilin
	1149.579	QDLDIFVNSV	f282-291	Vicilin
	1149.593	EETVCTAKLR	f338-347	Legumin A2
		EETICSAKIR	f392-401	Legumin MS

		ETICSAKIRE	f393-402	Legumin MS
	1149.624	PEKQARHQR	f260-268	Legumin A2
	1149.637	QKFDQRSKI	f58-66	Vicilin
		LQKFDQRSK	f57-65	Vicilin
	1149.651	KIPAGTTSYLV	f245-255	Convicilin
	1149.671	NGHIRRLQRF	f170-178	Convicilin
1173.600	1173.513	DEDEERQPR	f277-285	Convicilin
			f297-305	Legumin A2
	1173.524	QRNENQQEQ	f325-333	Vicilin
	1173.553	TLFENENGHI	f45-54	Vicilin
			f164-173	Convicilin
		LTLFENENGH	f163-172	Convicilin
	1173.561	NENQQEQRK	f327-335	Vicilin
		RRQGDNGLEE	f330-339	Legumin A2
	1173.575	QGEEEINKQV	f343-352	Vicilin
		GEEEINKQVQ	f344-353	Vicilin
		QQSQEENVIV	f216-225	Vicilin
	1173.586	LKNEQQERE	f442-450	Convicilin
	1173.590	AKSLSDRFSY	f447-456	Legumin A2
		PQEIFIQQGN	f89-98	Legumin A2
	1173.597	NEARQLKSNN	f494-503	Legumin A2
	1173.611	QSQEENVIVK	f217-226	Vicilin
	1173.615	ENPVKELTFP	f517-526	Convicilin
	1173.620	RSQSRRQQQ	f117-125	Legumin MS
	1173.622	RGQIEELSKN	f229-238	Vicilin

	1173.659	REKKEQKEV	f79-87	Convicilin
		SSQEVNRLIK	f528-537	Convicilin
	1173.663	GDVVIIPAGHPV	f469-480	Convicilin
	1173.681	KKERRGSQKG	f318-327	Legumin A2
		KERRGSQKGK	f319-328	Legumin A2
	1173.684	VIAVSLTDIRS	f160-170	Legumin A2
	1173.699	VIIPAGHPVAIS	f472-483	Convicilin
1201.700	1201.614	SRRQQQQRD	f120-128	Legumin MS
		RRQQQQRDS	f121-129	Legumin MS
	1201.615	MLPHYNSRAI	f416-425	Convicilin
		ALMLPHYNSR	f414-423	Convicilin
		LMLPHYNSRA	f415-424	Convicilin
	1201.617	EDNVISQIQR	f402-411	Vicilin
	1201.621	KSLSDRFSYV	f448-457	Legumin A2
	1201.646	LPSYSPSPQLI	f83-93	Legumin MS
	1201.667	TIEKVLLEEQ	f309-318	Convicilin
	1201.679	VAISASSNLNLL	f480-491	Convicilin
	1201.683	GDIIAVPTGIVF	f139-150	Legumin A2
	1201.690	IARLAGTSSVIN	f467-478	Legumin A2
1229.600	1229.562	RSSESQEHRN	f144-153	Convicilin
	1229.580	EGGLIETWNPN	f49-59	Legumin A2
	1229.591	QKQSHFASAEP	f539-549	Convicilin
	1229.598	RGSQRREDPE	f108-117	Convicilin
	1229.613	RYQHQQGGKQ	f194-203	Legumin A2
	1229.616	EGSLLLPHYNS	f295-305	Vicilin

	1229.637	GTSSVINNLPLD	f472-483	Legumin A2
	1229.648	ERGQIVKVEDG	f261-271	Legumin MS
	1229.653	GRARLQVVNCN	f412-422	Legumin A2
		RARLQVVNCNG	f413-423	Legumin A2
	1229.660	EESQRKRSPL	f554-563	Convicilin
	1229.662	IEKVLLEEQE	f310-319	Convicilin
	1229.668	IFSGFKRDFL	f212-221	Legumin A2
	1229.671	QRNEARQLKS	f492-501	Legumin A2
	1229.685	RGDTIKIPAGTT	f240-251	Convicilin
1300.600	1300.576	HSQRKEEEEE	f287-296	Legumin MS
	1300.628	MGISFLASVCVSS	f16-28	Vicilin
	1300.639	HFLSSFSLCFL	f4-14	Legumin MS
	1300.661	IFLASVCVTYAN	f18-29	Convicilin
	1300.674	ELLGLKNEQQE	f438-448	Convicilin
		AGTSSVINNLPLD	f471-483	Legumin A2
	1300.683	MLPHYNSRAIV	f416-426	Convicilin
	1300.686	QDERGQIVKVE	f259-269	Legumin MS
	1300.697	TAKRLRSPQDE	f251-261	Legumin MS
		EDTAKRLRSPQ	f249-259	Legumin MS

Table 16. Potential amino acid sequences of peptides present in trypsin-derived yellow field pea protein hydrolysate.

Observed mass (Da)	Calculated mass (Da)	Potential sequence	Location	Protein type
204.200	204.134	GK	f102-103 f268-269 f316-317	Vicilin
			f221-222 f276-277 f384-385 f433-434	Convicilin
			f327-328	Legumin A2
			f98-99	Legumin MS
		KG	f317-318	Vicilin
			f412-413 f434-435	Convicilin
			f244-245 f251-252 f326-327 f411-412	Legumin A2
			f99-100 f138-139 f385-386	Legumin MS
288.300	288.203	IR	f54-55	Vicilin
			f173-174	Convicilin
			f168-169	Legumin A2
			f72-73 f133-134 f400-401 f464-465	Legumin MS
		RL	f254-255 f435-436	Legumin MS

			f55-56 f74-75 f455-456	Vicilin
			f121-122 f175-176 f193-194 f465-466 f534-535	Convicilin
			f36-37 f234-235 f347-348 f415-416 f469-470	Legumin A2
		RI	f427-428	Vicilin
			f45-46 f365-366	Legumin A2
			f419-420 f471-472	Legumin MS
		LR	f144-145 f256-257	Vicilin
			f122-123 f263-264 f291-292 f325-326 f351-352 f372-373	Convicilin
			f23-24 f80-81 f193-194 f346-347 f377-378	Legumin A2
			f255-256 f431-432 f434-435 f543-544	Legumin MS

320.200	320.109	DAD	f92-94	Vicilin
		EGD	f138-140	Legumin A2
		DGE	f428-430	Legumin A2
		GDE	f400-402	Vicilin
		EDG	f269-271	Legumin MS
	320.145	IST	f420-422	Legumin MS
	320.160	FPG	f418-420	Vicilin
			f525-527	Convicilin
			f105-107	Legumin A2
	320.164	MGI	f16-18	Vicilin
		VCV	f24-26	Vicilin
			f23-25	Convicilin
		LMG	f15-17	Vicilin
	320.182	TTV	f3-5	Convicilin
		SLT	f164-166	Legumin A2
			f425-427	Legumin MS
		TVT	f368-370	Legumin A2
		TSL	f370-372	Legumin A2
403.300	403.205	RNN	f455-457	Convicilin
		GNRG	f555-558	Legumin MS
	403.212	MPR	f178-180	Legumin A2
	403.219	DVVA	f483-486	Legumin A2
		GDVI	f139-142	Legumin MS
		SLPS	f362-365	Convicilin
		SPLS	f561-564	Convicilin

		PLSS	f562-565	Convicilin
	403.230	DRI	f426-428	Vicilin
			f454-456	Vicilin
			f233-235	Legumin A2
		DLR	f262-264	Convicilin
		QQK	f196-198	Legumin MS
		LRD	f325-327	Convicilin
		QKQ	f539-541	Convicilin
			f197-199	Legumin MS
			f432-434	Vicilin
		DIR	f167-169	Legumin A2
		RVE	f46-48	Legumin MS
	403.255	VAIT	f161-164	Legumin MS
		LALS	f6-9	Legumin A2
		ALSL	f7-10	Legumin A2
		ALTV	f436-439	Legumin A2
	403.266	KQK	f72-74	Convicilin
430.400	430.302	LGLK	f440-443	Convicilin
		AIVK	f246-249	Legumin A2
488.400	488.308	IEKV	f193-196	Vicilin
			f310-313	Convicilin
		EKVL	f194-197	Vicilin
			f311-314	Convicilin
		VEIK	f291-294	Vicilin
		VKEL	f413-416	Vicilin

			f520-523	Convicilin
		GVSLI	f68-72	Legumin MS
		VIAVS	f160-164	Legumin A2
	488.319	SLIR	f70-73	Legumin MS
		LRLS	f434-437	Legumin MS
		KVKGG	f249-253	Legumin A2
	488.330	KGRK	f385-388	Legumin MS
549.300	549.215	SDDNV	f508-512	Convicilin
		SEQGE	f118-122	Legumin A2
		ESEQG	f117-121	Legumin A2
	549.226	SSNNQ	f170-174	Legumin A2
	549.230	ETWN	f54-57	Legumin A2
	549.251	LDTSN	f166-170	Legumin MS
		SSQEV	f528-532	Convicilin
	549.263	GSRET	f448-452	Vicilin
		GSETR	f33-37	Convicilin
	549.267	AFPGSA	f417-422	Vicilin
	549.270	CAGVSL	f66-71	Legumin MS
	549.288	KSLSD	f448-452	Legumin A2
	549.303	KASFP	f7-11	Vicilin
		NFVVA	f496-500	Legumin MS
	549.314	FNLR	f370-373	Convicilin
			f254-257	Vicilin
		RNFL	f502-505	Convicilin
			f395-398	Vicilin

		QVFR	f527-530	Legumin MS
558.300	558.215	HTDA	f90-94	Vicilin
	558.252	DAQPQ	f439-443	Vicilin
	558.252	NAPQE	f87-91	Legumin A2
	558.288	TVPQN	f438-442	Legumin A2
		VQPQS	f561-565	Legumin MS
	558.299	PARQS	f510-514	Legumin A2
	558.313	IISPE	f274-278	Legumin MS
		ISPEL	f275-279	Legumin MS
	558.325	QVAQI	f547-551	Legumin MS
	558.347	RNLR	f432-435	Legumin MS
	558.350	DVVII	f470-474	Convicilin
	558.361	IIQGK	f95-99	Legumin MS
		VAQIK	f548-552	Legumin MS
	558.386	ILTVL	f105-109	Vicilin
		ILTVL	f224-228	Convicilin
570.300	570.204	YDEGS	f30-34	Convicilin
	570.227	NENGH	f49-53	Vicilin
			f168-172	Convicilin
	570.252	HIDAD	f209-213	Convicilin
		DPQNP	f31-35	Vicilin
		EDGLH	f269-273	Legumin MS
	570.292	KFFE	f269-272	Vicilin
		FYLAG	f182-186	Legumin A2
		IFSGF	f212-216	Legumin A2

	570.256	FEAFG	f278-282	Convicilin
	570.263	HREE	f124-127	Convicilin
	570.325	INNLP	f477-481	Legumin A2
		NNLPL	f478-482	Legumin A2
	570.336	RPGQL	f155-159	Vicilin
		VPARQ	f509-513	Legumin A2
		NIARP	f403-407	Legumin MS
601.400	601.330	RTIDP	f74-78	Legumin MS
	601.338	GALMLP	f413-418	Convicilin
	601.342	GIQRQ	f378-382	Legumin MS
	601.353	VGRRGG	f211-216	Legumin MS
	601.356	NLELL	f436-440	Convicilin
	601.367	INKQV	f348-352	Vicilin
		NGKA	f218-223	Convicilin
		DLRVV	f262-266	Convicilin
		LRVVD	f263-267	Convicilin
		IQGKGV	f96-101	Legumin MS
		QGKGVL	f97-102	Legumin MS
	601.392	IEKVL	f310-314	Convicilin
			f193-197	Vicilin
		EKVLL	f311-315	Convicilin
			f194-198	Vicilin
		KVLLE	f312-316	Convicilin
			f195-199	Vicilin
		VIAVSL	f160-165	Legumin A2

	601.403	KVKGGL	f249-254	Legumin A2
630.500	630.418	AIIKVS	f339-344	Convicilin
673.400	673.301	GYFGMV	f99-104	Legumin A2
	673.309	YAPHW	f449-453	Legumin MS
	673.319	YLSGFS	f173-178	Vicilin
	673.330	HKPEY	f375-379	Convicilin
	673.338	RGGQQQ	f214-219	Legumin MS
	673.351	GEVLANA	f534-540	Legumin MS
	673.363	INAKNN	f495-500	Convicilin
	673.374	RGEGRV	f465-470	Legumin MS
		GEGRVR	f466-471	Legumin MS
	673.385	RERGR	f42-46	Convicilin
		RRRGE	f308-312	Legumin A2
	673.388	NVISQI	f404-409	Vicilin
			f511-516	Convicilin
		KGNLEL	f434-439	Convicilin
		LGLKNE	f440-445	Convicilin
		SNLNLL	f486-491	Convicilin
	673.399	LSRGQI	f227-232	Vicilin
		AGVALSR	f66-72	Legumin A2
		GVALSRA	f67-73	Legumin A2
		EAGRIK	f362-367	Legumin A2
		NLRLSA	433-438	Legumin MS
	673.424	VKGGLSI	f250-256	Legumin A2
		VVLSGKA	f98-104	Vicilin

	673.436	GRIKTV	f364-369	Legumin A2
693.300	693.269	SGSDDNV	f506-512	Convicilin
	693.312	LEETVC	f337-342	Legumin A2
	693.316	RSSESQ	f144-149	Convicilin
	693.320	EGNNIF	f208-213	Legumin A2
		YEEPR	f113-117	Legumin MS
	693.331	LAHHSE	f21-26	Legumin MS
	693.357	ATFNLQ	f487-492	Legumin A2
		EGNKVF	f476-481	Legumin MS
	693.360	AATTMKA	f2-8	Vicilin
	693.368	KFDQR	f59-63	Vicilin
		QNYRL	f71-75	Vicilin
		LQNYR	f70-74	Vicilin
	693.368	QRFDK	f177-181	Convicilin
		RNFLSG	f502-507	Convicilin
		NQYLR	f288-292	Convicilin
		QNYRL	f190-194	Convicilin
		LQNYR	f189-193	Convicilin
		RNGIYA	f445-450	Legumin MS
	693.382	ADYILV	f93-98	Vicilin
	693.390	RQPRH	f282-286 f302-306	Legumin A2
		PRHQR	f284-288 f304-308	Legumin A2
	693.393	YRLLE	f73-77	Vicilin
		RLLEY	f74-78	Vicilin

701.400	701.321	PQDERG	f258-263	Legumin MS
	701.333	SNGNRGP	f553-559	Legumin MS
	701.344	AMFVPH	f393-398	Legumin A2
	701.346	AEPEQK	f547-552	Convicilin
	701.358	LSPNDR	f228-233	Convicilin
	701.380	RRGGQQ	f213-218	Legumin MS
	701.383	ITPEKN	f273-278	Vicilin
	701.394	ELVGQR	f321-326	Vicilin
		ERGQIV	f261-266	Legumin MS
	701.419	ENVIVK	f221-226	Vicilin
	701.430	IARLAGT	f467-473	Legumin A2
	701.456	LSGKAIL	f100-106	Vicilin
		GKAILTV	f102-108	Vicilin
			f221-227	Convicilin
	701.467	TAKLRL	f343-348	Legumin A2
704.400	704.310	QGEEEI	f343-348	Vicilin
		DEDNVI	f401-406	Vicilin
		EEEGIQ	f375-380	Legumin MS
	704.332	EREDR	f448-452	Convicilin
		ARQSEN	f511-516	Legumin A2
		QSENRA	f513-518	Legumin A2
	704.346	IESEGGL	f46-52	Legumin A2
		ESEGGLI	f47-53	Legumin A2
		SEGGLIE	f48-54	Legumin A2
		VESEAGL	f47-53	Legumin MS

	704.357	TVNEGKG	f312-318	Vicilin
		LSENKN	f283-288	Convicilin
	704.361	APQEIF	f88-93	Legumin A2
	704.369	KTNDRA	f515-520	Legumin MS
			f460-465	Legumin A2
	704.382	SVEIKE	f290-295	Vicilin
	704.394	LTDIRS	f165-170	Legumin A2
		SLTDIR	f164-169	Legumin A2
	704.398	SPQLIF	f89-94	Legumin MS
		KELAFP	f414-419	Vicilin
	704.409	IIPAGHP	f473-479	Convicilin
	704.420	LRRPY	f80-84	Legumin A2
724.300	724.278	NEEGFE	f505-510	Legumin MS
	724.300	SVSSESE	f246-252	Vicilin
	724.315	SDLFEN	f183-188	Convicilin
	724.337	YNSGAGR	f413-419	Legumin MS
	724.351	LSSGDVF	f359-365	Vicilin
	724.362	SNKFQT	f40-45	Vicilin
		SLSDRF	f449-454	Legumin A2
		LSDRFS	f450-455	Legumin A2
	724.374	YETRR	f342-346	Legumin MS
	724.396	ARHQRG	f264-269	Legumin A2
734.300	734.284	DDNEEL	f139-144	Vicilin
	734.295	QSQEEN	f217-222	Vicilin

	734.314	QNECQL	f29-34	Legumin A2
	734.320	VSSESEP	f247-253	Vicilin
	734.336	PSYSPSP	f84-90	Legumin MS
	734.343	SENRASA	f514-520	Legumin A2
	734.368	TSSVINN	f473-479	Legumin A2
		KASSNLD	f376-382	Vicilin
	734.394	NQVFRA	f526-531	Legumin MS
739.300	739.326	DLYNSGA	f411-417	Legumin MS
		NFLSGSD	f503-509	Convicilin
		NSYNLE	f234-239	Convicilin
	739.398	VLLEEH	f196-201	Vicilin
745.300	745.297	GNGYFGM	f97-103	Legumin A2
	745.322	NQNQQN	f167-172	Vicilin
	745.326	TWNPNN	f55-60	Legumin A2
	745.338	VFWMY	f149-153	Legumin A2
	745.347	EEPRSQ	f114-119	Legumin MS
	745.370	RRQGDN	f330-335	Legumin A2
	745.373	TVLSPND	f226-232	Convicilin
	745.384	GSETRVP	f33-39	Convicilin
		SETRVPG	f34-40	Convicilin
		LLSGNQN	f163-169	Vicilin
		ELEAGRA	f430-436	Legumin A2
		NLNANSI	f400-406	Legumin A2
	803.401	GRQEGEK	f45-51	Convicilin
803.500	803.426	LERGDTI	f119-125	Vicilin

			f238-244	Convicilin
		VTVNEGKG	f311-318	Vicilin
	803.430	DFVIPVN	f267-273	Convicilin
	803.448	DRKRTQ	f327-332	Convicilin
		ESQRKR	f555-560	Convicilin
	803.462	VSLTDIR	f163-169	Legumin A2
	803.466	PVKELAF	f412-418	Vicilin
		VKELAFP	f413-419	Vicilin
		AVPTGIVF	f143-150	Legumin A2
	803.477	VIIPAGHP	f472-479	Convicilin
		IIPAGHPV	f473-480	Convicilin
	803.503	LIFIIQG	f92-98	Legumin MS
805.400	805.332	RQEEDE	f271-276	Legumin A2
		EDEERQ	f278-283 f298-303	Legumin A2
	805.347	EEEWRG	f104-109	Convicilin
	805.373	SPDIYNP	f355-361	Legumin A2
		GNPEIEF	f186-192	Legumin MS
	805.380	DIRSSNN	f167-173	Legumin A2
	805.394	SEGGLIET	f48-55	Legumin A2
		VESEAGLT	f47-54	Legumin MS
	805.405	ISASSNLN	f482-489	Convicilin
		SASSNLNL	f483-490	Convicilin
	805.420	QNFVVAQ	f495-501	Legumin MS
		NFVVAQQ	f496-502	Legumin MS

	805.431	QRNFLAG	f394-400	Vicilin
	805.441	KAKLSSGD	f356-363	Vicilin
	805.445	PAGTIAYL	f128-135	Vicilin
		LPAGTIAY	f127-134	Vicilin
	805.493	VVIIPAGH	f471-478	Convicilin
814.600	814.503	ENVIVKL	f221-227	Vicilin
		VSLGQLVV	f485-492	Legumin MS
		SLGQLVVV	f486-493	Legumin MS
	814.514	RDAIIKV	f337-343	Convicilin
	814.530	VLRWLK	f376-381	Legumin A2
	814.540	GKAILTVL	f102-109	Vicilin
			f221-228	Convicilin
	814.551	VIVKLSR	f223-229	Vicilin
829.300	829.373	IYSNEFG	f262-268	Vicilin
	829.380	PQDERGQ	f258-264	Legumin MS
844.300	844.384	EYSNKFG	f378-384	Convicilin
	844.384	YSNEFGK	f263-269	Vicilin
	844.366	GNGYFGMV	f97-104	Legumin A2
	844.343	EEQSHSQ	f283-289	Legumin MS
852.400	852.337	AGNEEGFE	f503-510	Legumin MS
		YNDQDTP	f153-159	Legumin A2
	852.373	TFPGSSQE	f524-531	Convicilin
	852.377	PSEFEPF	f364-370	Convicilin
	852.388	VCVSSRSD	f24-31	Vicilin
	852.394	KSVSSESE	f245-252	Vicilin

	852.403	GGCFALRE	f18-25	Legumin A2
	852.425	PGKFEAFG	f275-282	Convicilin
	852.446	KLSSGDVF	f358-365	Vicilin
	852.455	QARHQRG	f263-269	Legumin A2
	852.457	KSNKFQT	f39-45	Vicilin
		KSLSDRF	f448-454	Legumin A2
	852.461	PFIFKSN	f35-41	Vicilin
		NPFIFKS	f34-40	Vicilin
		PFLFKSN	f154-160	Convicilin
		NPFLFKS	f153-159	Convicilin
	852.469	VNRHIVD	f227-233	Legumin A2
		YETRRK	f342-347	Legumin MS
929.300	929.385	EKEEHEE	f64-70	Convicilin
		KEEHEEE	f65-71	Convicilin
		EEHEEEK	f66-72	Convicilin
990.500	990.412	VNRDDNEE	f136-143	Vicilin
	990.437	DDEEDLRV	f258-265	Convicilin
	990.460	RGSRQEEE	f288-295	Legumin A2
	990.474	SDDNVISQI	f508-516	Convicilin
	990.492	CQLDTINAL	f33-41	Legumin MS
	990.508	ERGSRETR	f446-453	Vicilin
		RERGSRET	f445-452	Vicilin
	990.510	LLDTSNTLN	f165-173	Legumin MS
	990.518	LDILVSCVE	f401-409	Convicilin
		DILVSCVEI	f402-410	Convicilin

	990.525	TPEKKYPQL	f390-397	Convicilin
		GEVLANAFGL	f534-543	Legumin MS
	990.534	EQRHRKH	f299-305 f322-328	Legumin MS
	990.541	PQNPFIFK	f32-39	Vicilin
	990.558	RISTVNSLT	f419-427	Legumin MS
	990.559	RRLQRFD	f174-180	Convicilin
999.400	999.449	DHRVESEAG	f44-52	Legumin MS
	999.482	SFLASVCVSS	f19-28	Vicilin
	999.485	HTAEKERE	f351-358	Legumin MS
	999.497	EQSHSQRK	f284-291	Legumin MS
		QSHSQRKE	f285-292	Legumin MS
1001.500	1001.415	QQGNGYFGM	f95-103	Legumin A2
	1001.465	REDPEERA	f113-120	Convicilin
		EDPEERAR	f114-121	Convicilin
	1001.480	TWNPNNKQ	f55-62	Legumin A2
	1001.483	NQLDQMPR	f173-180	Legumin A2
	1001.501	LLSGNQNQQ	f163-171	Vicilin
		VLSPNDRNS	f227-235	Convicilin
	1001.516	EYRAKPHT	f196-203	Convicilin
	1001.526	QEVDRILE	f423-430	Vicilin
		GTSSVINNLP	f472-481	Legumin A2
	1001.541	LEYKSKPH	f76-83	Vicilin
		VPQNFVVAQ	f493-501	Legumin MS
	1001.563	KNILEASLN	f296-304	Convicilin

	1001.567	VVDFVIPVN	f265-273	Convicilin
		SPSPQLIFI	f87-95	Legumin MS
	1001.574	SAKIRENIA	f397-405	Legumin MS
	1001.588	EIKEGSLLL	f292-300	Vicilin
1040.500	1040.410	NCNGNTVFDG	f420-429	Legumin A2
	1040.487	HFLSSFSLC	f4-12	Legumin MS
		RHQREGEE	f134-141	Convicilin
	1040.489	TTSYLVNQD	f250-258	Convicilin
	1040.505	IFENLQNY	f66-73	Vicilin
		LFENLQNY	f185-192	Convicilin
	1040.508	LASVCVTYAN	f20-29	Convicilin
	1040.519	HPELKCAGVS	f61-70	Legumin MS
	1040.527	QEHRNPFL	f149-156	Convicilin
	1040.552	FDQRSKIF	f60-67	Vicilin
		WLKLSAEHG	f379-387	Legumin A2
		QQRYSFLV	f204-211	Legumin MS
	1040.556	MATTVKSRF	f1-9	Convicilin
	1040.596	KPQQLRDR	f321-328	Convicilin
		PQQLRDRK	f322-329	Convicilin
	1040.599	GLSIISPPEK	f253-262	Legumin A2
1072.500	1072.404	WMYNDQDT	f151-158	Legumin A2
	1072.506	LFENENGHI	f46-54	Vicilin
			f165-173	Convicilin
	1072.513	VGQRNENQQ	f323-331	Vicilin
		INAENNQRN	f388-396	Vicilin

		RSPQDERGQ	f256-264	Legumin MS
	1072.517	NHEQEFLR	f187-194	Legumin A2
	1072.527	LEPDNRIES	f40-48	Legumin A2
	1072.531	LGGNPEIEFP	f184-193	Legumin MS
	1072.563	AQEVDRILE	f422-430	Vicilin
		VNEGKGNLEL	f430-439	Convicilin
		LVNEGKGNLE	f429-438	Convicilin
		PVAISASSNLN	f479-489	Convicilin
		AGTSSVINNLP	f471-481	Legumin A2
	1072.575	DNVISQIQR	f403-411	Vicilin
		REQIEELR	f345-352	Convicilin
		QDERGQIVK	f259-267	Legumin MS
	1072.590	HYNSRAIVI	f302-310	Vicilin
	1072.600	EERDAIIKV	f335-343	Convicilin
1089.400	1089.433	EPQESEQGEG	f114-123	Legumin A2
	1089.452	PEQNECQLE	f27-35	Legumin A2
	1089.458	SPELQEEEE	f276-284	Legumin MS
	1089.481	LQGRNEDEE	f235-243	Legumin A2
	1089.492	EQQEREDR	f445-452	Convicilin
1200.500	1200.576	AGNHEQEFLR	f185-194	Legumin A2
	1200.583	LFTTACLAHHS	f15-25	Legumin MS
	1200.587	HEPRQQRYS	f200-208	Legumin MS
1302.500	1302.556	EDEDEERQPR	f276-285 f296-305	Legumin A2
	1302.592	KQEQENEGNNI	f202-212	Legumin A2

1330.600	1330.577	IVFWMYNDQD	f148-157	Legumin A2
	1330.601	ISPELQEEEEQ	f275-285	Legumin MS
	1330.612	SDDNVISQIENP	f508-519	Convicilin
	1330.613	YQHQQGGKQEQ	f195-205	Legumin A2
	1330.635	DEGSETRVPGQR	f31-42	Convicilin
		VDRLQGRNEDE	f232-242	Legumin A2
	1330.648	DDNEELRVLDL	f139-149	Vicilin
	1330.660	GSAQEVDRILEN	f420-431	Vicilin
	1330.685	NEDEEKGAIVKV	f239-250	Legumin A2
	1330.693	QKGKSRRQGDNG	f325-336	Legumin A2
1434.500	1434.562	GQQQEEESEEQN	f216-227	Legumin MS
	1434.598	QRKEDDEEEEQ	f333-343	Vicilin
		QEQRKEDDEEE	f331-341	Vicilin
1523.000	1522.943	NSLTLPILRNLRL	f424-436	Legumin MS

6. CONCLUSION

Yellow field pea protein peptides derived from the four enzymes (alcalase, chymotrypsin, pepsin, and trypsin) had the ability to inhibit key enzymes involved in nutrient digestion. The inhibitory activity of the yellow field pea protein hydrolysates and peptide fractions against the enzymes were dose-dependent. Generally, based on the mean inhibitory activity and IC_{50} of the samples, it can be deduced that the fractionated and low molecular weight peptides exhibited more potency than the unfractionated hydrolysates in inhibiting α-amylase, α-glucosidase, trypsin, and chymotrypsin, with the exception of pancreatic lipase, where the unfractionated hydrolysates showed higher inhibitory activity against this enzyme.

With regards to enzymes involved in carbohydrate digestion, the low molecular weight peptide fractions were more potent α-amylase inhibitors than α-glucosidase, suggesting that the peptides may have interacted differently with the enzymes. The fractionated peptides of all sizes exhibited higher α-amylase inhibitory activity, whereas the fractionated peptides (<1 kDa and 3-5 kDa) exhibited higher α-glucosidase inhibitory activity.

Pertaining to pancreatic lipase inhibition, the unfractionated hydrolysates had greater ability to inhibit the enzyme compared to the peptide fractions. In the case of the enzymes involved in protein digestion, the peptides were more potent in inhibiting trypsin than chymotrypsin, indicating different interaction of the peptides with the enzymes. For trypsin inhibition, the fractionated peptides (<1 kDa and 5-10 kDa) exhibited a stronger ability to inhibit this enzyme, while for chymotrypsin activity, only the fractionated peptides of 5-10 kDa had significant inhibitory activity.

In this present study, out of all the four enzymes used for hydrolysis of yellow field pea isolate, chymotrypsin has been identified herein as the most potent enzyme suitable for the release of peptides from yellow field pea protein with α-amylase inhibitory activity. Pepsin has been identified as the most suitable enzyme for the production of peptides with chymotrypsin inhibitory activity, while trypsin has been identified as the enzyme suitable for the release of α-glucosidase, trypsin, and pancreatic lipase inhibitory peptides.

The mode of inhibition of the peptides against the enzymes α-amylase, chymotrypsin and trypsin was identified as competitive, while for α-glucosidase, it was observed to be non-competitive.

The peptides found in the hydrolysates ranged from a dipeptide to a polypeptide, with the highest polypeptide consisting of 13 amino acids.

Findings from this study suggest that pea-protein derived peptides have the potential to be developed into functional foods and/or nutraceuticals for management of caloric intake with respect to obesity and T2DM.

7. SIGNIFICANCE OF RESEARCH

This research contributes to scientific knowledge as well as the body of the literature about the ability of yellow field pea protein hydrolysates and its peptide fractions to function as inhibitors of key digestive enzymes. This research study is the first to show that yellow field pea protein hydrolysates and peptide fractions can inhibit key digestive enzymes. This study also provides information about the mode of inhibition of the peptides against the enzymes. In addition, this research work provides a foundation for further research studies to confirm the inhibitory activity of the peptides against digestive enzymes *in vivo* and perhaps produce a reduction in weight as there is no literature on yellow field pea derived peptides being able to produce a reduction in weight. Furthermore, this research work provides insight into the mode of interactions between yellow pea protein peptides and enzymes implicated in these metabolic disorders (obesity and T2DM).

Based on the results derived from this study, peptides derived from yellow field pea protein can be used as a dietary approach in caloric management. This would present a low cost, safe and effective approach with extensive implications for future directions in nutritional and healthcare research and practice improvement.

8. FUTURE RESEARCH

Based on the results of this study, yellow field pea protein unfractionated hydrolysates and peptides have the ability to inhibit digestive enzymes, as shown during the *in vitro* experiments. However, future research is needed on the further fractionation and purification of the inhibitory peptides using RP-HPLC coupled with MS/MS analysis to provide the amino acid sequences that are responsible for each MS ion signal.

In addition, further research will need to be conducted to determine the inhibitory activity of the hydrolysates and peptide fractions against digestive enzymes *in vivo* as well as the ability of the hydrolysates and peptides to produce weight loss. Following the successful short-term study with the peptides *in vitro*, it is worthwhile carrying out *in vivo* studies in animal models and in long-term, clinical trials in human subjects to validate the observed results. The first step will be to conduct the experiment on a suitable animal model, where the rodents will be treated with various concentrations of the peptides. If the findings are promising, a randomized clinical trial with human subjects (subjects who are overweight/obese, have type 2 diabetes mellitus) will be the next step.

If these are successful, the peptides can be developed into a nutraceutical and/or functional food. However, the viability of production will need to be assessed in terms of proper cost analysis to ensure profit while producing the desired effects in terms of caloric management and weight loss.

Future feeding studies using animal models and human volunteers are required to confirm the results obtained from this in vitro inhibition of enzyme activity.

The mode of inhibition of the peptides against pancreatic lipase will need to be determined in order to understand the interaction between the peptides and the enzyme.

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