Final report

2021G011R

Development of a nutritionally enhanced plant-based milk alternative beverage from Canadian oats and study of its glycemia-lowering effect

Supported by Results Driven Agriculture Research (RDAR)
Prairie Oat Growers Association (POGA)
Earth's Own Food Company Inc.

Reported by Dr. Lingyun Chen (Full Professor)

Department of Agricultural, Food and Nutritional Science, University of Alberta

October 2024

Non-technical summary

The rising rate of milk allergies and lactose intolerance in society is responsible for the growth of the plant-based milk alternatives market. This market has been further driven by consumers increasingly looking for plant-based healthy choices options including high protein and high fibre. The Canadian oat industry is well-positioned to capitalize on these trends, with breeding efforts leading to high-yield oat varieties that boast significantly increased protein (15-20%) and β-glucan (>5%) content.

In collaboration with the Prairie Oat Growers Association (POGA) and Earth's Own, our research compared protein and β-glucan recovery in oat milk products made from 14 oat varieties. Results showed a strong correlation between the protein content of oat milk and the original grain, indicating that high-protein oats yield higher-protein oat milk. Additionally, these varieties contain 4% or more β-glucan, making them highly suitable for developing nutritious oat milk beverages.

Additionally, the research introduced an ultrasound-based processing technique that enhanced β-glucan recovery by 39% while preserving its health benefits. This technology also improved protein solubility and foaming properties, unlocking new applications for oat milk in coffee beverages and desserts.

Moreover, the study found that oat protein hydrolysates (peptides) exhibited glycemia-lowering effects by inhibiting enzymes involved in glucose metabolism, suggesting potential for blood sugar management. Preliminary results indicate that chronic and acute consumption of these peptides may help regulate body weight and improve glucose tolerance. However, further studies are needed to confirm these findings in diet-induced obese models.

The findings highlight that the high-protein, high-β-glucan Canadian milling oats are advantageous for oat milk production at the global market, with promising opportunities for functional beverage development with glycemia-lowing effect by harnessing the combined benefits of oat β-glucan and oat protein peptides.

Project team

Dr. Lingyun Chen, Protein Chemistry and Technology, University of Alberta, served as the project leader and supervised three graduate students to develop nutritionally enhanced oat milk from Canadian milling oats and study its glycemia-lowering effect.

Dr. Caroline Richard, Human Nutrition, co-supervised graduate students to study the glycemia-lowering effect of the oat peptides in vivo using animal models.

Dr. Jean Buteau, Human Nutrition, co-supervised graduate students to study the glycemia-lowering effect of the oat peptides in vitro using cell models.

Abbreviations

AH: alcalase hydrolysate
AFH: alcalase-flavourzyme hydrolysate

DPP4: dipeptidyl peptidase IV
GLP-1: glucose like peptide-1
OPH: oat protein hydrolysates

Background

The rising rate of milk allergies and lactose intolerance in society is responsible for the growth of the plant-based milk alternatives market. Recently this market is driven by consumers increasingly looking for plant-based healthy choices options including high protein and high fibre. Oat milk is among the leading dairy alternatives in the global market because oats are a good source of both protein and dietary fibre. Oat β-glucan is known to reduce postprandial glucose response. In addition, oat is the only cereal containing globulin protein, avenalin, as its major (80%) protein components, thus is more nutritious than most cereals with greater essential amino acids lysine and threonine (Klose, 2012).

Canadian oat industry is well positioned to seize the current global trends for plant-based milks and functional drinks. The oat milk products currently available in the market contain 1-1.5% protein and < 0.4g β-glucan per serving (~250 mL). Breeding efforts have led to commercial high yield oats varieties in Canada that have significantly increased protein (15-20%) and β-glucan (>5%) compared to normal oats (~13% protein, 3% β-glucan) (Chen, 2018). Using these high quality oats provides a good opportunity to produce oat milk products with protein content more comparable to bovine milk (3.5-4%). Moreover an oat drink containing 0.75 g β-glucan per serving will provide a total of 3 g in 4 servings per day to justify a glycaemia reduction effect (Wursh, 1997, Tosh, 2013). However, these valuable oat varieties have not yet been exploited in making oat milks. Current research has been focused on oat milling quality for oatmeal production and β-glucan processing (Vasanthan, 2008; Izydorczyk, 2014; Gamel, 2015). Knowledge on processing of Canadian oats for plant protein milk production is lacking and the process optimization to improve protein and β-glucan recovery in the milk products has seldom been reported. Filling this knowledge gap is important to understand the opportunity of Canadian milling oats for new markets.

Our preliminary research revealed that oat protein enzymatic hydrolysates can effectively inhibit the dipeptidyl peptidase IV (DPP4). DPP4 inhibitors increase glucose like peptide (GLP)-1, a hormone involved in glucose metabolism, by stimulating insulin production, thus they are used as targets in anti-diabetic drug development (Gomez-Peralta, 2018). Consumers prefer food ingredients that can simulate mechanisms of action of pharmaceutical treatments. Thus, the ability to generate peptides with anti-diabetic activities in oat milk presents a new and secondary strategy to develop a diabetic-friendly beverage. However, the peptides responsible for the anti-diabetic effects need to be identified and concentrated in the oat drink. Research is also required to exploit the peptide efficacy for management of diabetes in vivo, which has been seldom studied currently. Filling these gaps is crucial for successful functional oat beverage development.

Objectives

This is collaborative research with the Prairie Oat Growers Association (POGA) and Earth's Own with the long-term goal developing new processing to improve protein and beta-glucan recovery in oat milk products, and from there to further develop functional drinks with additional health benefits.

The short-term objectives in the last three years include:

1. Screen the oat varieties and develop processing technique to increased protein and β-glucan recovery in oat milk
2. Develop anti-diabetic peptides from oat proteins
3. Study the feasibility of developing a functional oat beverage with glycemia–lowering effects in a pre-clinical study

Research design and methodology

1. Screen oat varieties and develop processing technique to increased protein and β-glucan recovery in oat milk

More than 10 commercial oat varieties were studied with varied protein and β-glucan content. The oat grains were dehulled and then prepare in oat milk products following the established industry protocol involving milling and subsequent liquification by starch hydrolysis. Afterward, the solid residues are separated from the oat beverage by centrifugation or descanting. The β-glucan contents in the oat milk beverage were determined using enzyme kits according to Approved Methods 32-23.01(AACC International 2010). Protein content (%N × 5.7) were determined by combustion nitrogen analysis using the Leco analyzer calibrated with EDTA according to Approved Method 46-30.01 (AACC International 2010).

In the second step, ultrasound, an environmentally friendly technology, was focused as a new processing technique to increase protein and β-glucan recovery in oat milk. Oat grains (mixed varieties) slurry after dehulling and wet milling was treated by an ultrasonic processor (Model JY98 – IIIDN, Ningbo Scientz Biotechnology Co., Ltd., China) equipped with a titanium probe (diameter 3/4 inch). The samples were treated at different power output levels (60, 240, and 960 W) for 1, 5, and 9 min (pulse duration: on-time, 5 sec; off-time, 5 sec). The temperature was controlled by using ice bath as not exceeding 45˚C. Then the treated oat slurries were hydrolyzed by α-amylase at 60˚C for 30 min, followed by inactivation in a 90℃ water bath for 10 min. After cooling, the oat slurries were observed for particle size and morphology analysis using optic and confocual microscopic techniques. Afterward, the slurries were centrifugated to collect the oat beverage(supernatant) for subsequent analysis of protein and β-glucan content and their structures. In addition, the foaming property of the oat beverage was studied to understand their feasibility for coffee-based beverages development. The oat beverage samples without ultrasound treatment were also prepared as control.

2. Develop anti-diabetic peptides from oat proteins

The protein was extracted from oat grains by wet method involving protein extraction in an alkaline solution at pH 9, followed by its precipitation at the protein isoelectric point of around 5. The protein content was determined to be 76%, using a combustion nitrogen analyzer (Leco Corporation, St Joseph, MI, USA), and a factor of 5.83 was used for protein conversion. Oat protein hydrolysis was conducted by adding alcalase (⩽5000 U/g) to the 1% (w/v) protein suspension in a ratio of 8 µL/100 mg protein powder. The reaction was carried out for 4 h at 37℃ and adjusted to pH 10 by adding NaOH 0.1 M. Continuous hydrolysis by alcalase and flavourzyme involved an initial alcalase hydrolysis as described above, followed by flavourzyme treatment (5 µl/100mg sample) at pH 7 for 2 h at 50℃. After the hydrolysis treatment, the enzyme was inactivated by heating at 85℃. The suspension was left to cool down at room temperature and then freeze dried to obtain the powder samples of hydrolysates. Both alcalase hydrolysate (AH) and alcalase-flavourzyme (AFH) filtered fractions were tested for enzymatic inhibition. The molecular weight (MW) distribution of the hydrolysates samples was evaluated using size exclusion chromatography (Agilent 1200 series HPLC equipped with a TSKgel G3000SWXL column).

The protein sample hydrolyzed by alcalase-flavourzyme was passed through an ultra/diafiltration system equipped with Centramate Cassettes filtration system (T-series Omega, Pall Life Sciences, Mississauga, ON, Canada) using membranes with MW cut off values of 5 and 1 kDa. The fractions with MW distribution of 1-5 kDa and 1kDa were collected, lyophilized, and stored at 4℃. In addition, the ultra-filtration fraction with high activity in antidiabetic assays (MW 1-5 kDa) was further fractionated based on its hydrophobicity using an Agilent 1200 series HPLC system with reversed-phase column (Zorbax SB-C18 column). Peaks were monitored at a UV wavelength of 280 nm and collected as four fractions. After collecting a suitable volume, samples were freeze dried and used to evaluate their antidiabetic properties including the α-Amylase assay, α-Glucosidase assay and DPP-IV assay. In addition, the fractions with strongest antidiabetic activities were studied for the peptide sequences by LC-MS/MS on a q-Tof premier mass spectrometer (Waters, Milford, MA) coupled with a nano Acquity UPLC system (Waters, Milford, MA).

3. Study the feasibility of developing a functional oat beverage with glycemia–lowering effects in a pre-clinical study

In vitro study using cell model: The effects of oat protein hydrolysates (OPH) on DPP-4 inhibition was evaluated using Caco-2 cell model. Caco-2 cells are human epithelial colorectal adenocarcinoma cells commonly used as an in vitro model of the intestinal barrier. These cells, when fully differentiated, mimic the properties of the human small intestine epithelium, making them useful for studying nutrient absorption, including glucose. Caco-2 cells from passage 15-30 were cultured in standard DMEM high glucose media. Once the cells reached 70-80% confluency, they were seeded in 24-well plate at a concentration of 10^6 cells/well and incubated for 21 days. Subsequently, treatments with OPH at concentrations of 1, 0.5, 0.25, 0.1 mg/ml, as well as

sitagliptin (0.1 mg/ml), were applied and incubated for 24 hours. Sitagliptin, a well-known DPP-4 inhibitor, was used as a positive control. After treatment, the media were aspirated, and the cells were washed three times with DPBS. Cells were then lysed with lysis buffer and the resulting supernatant was used to perform the DPP-4 assay according to the kit instruction (DPPIV-Glo™ Protease Assay).

In vivo pilot study using non-obese mice model: Male C57BL/6 mice, a widely used strain for metabolic disease study, were chosen for this experiment. A total of 36 mice, all 12 weeks old, were housed under controlled environmental conditions, including a temperature of 22 ± 2°C, 55% relative humidity, and a 12-hour light/dark cycle. All mice had ad libitum access to food and water throughout the study.

Mice were divided into five different diet groups as follows:

1- Control group: Mice were fed a diet consisting of 60% calories from fat, and casein as the main source of protein (n=6).
2- Oat protein group (OP): Mice received a high fat diet (HFD) supplemented with 25% oat protein (n=6).
3- OPH group: Mice fed a HFD supplemented with 25% OPH (n=6).
4- β-glucan group (BG): Mice were given a HFD supplemented with 5% β-glucan (n=6).
5- OPH+ β-glucan group (OPH+BG): Mice were fed a HFD supplemented with 25% OPH and 5% β-glucan (n=6).

All diets were formulated to ensure isocaloric intake across groups, except for the variation in protein and β-glucan supplementation. The study duration was 5 weeks, during which the mice were closely monitored for food intake and weight gain. In addition, the following measurements were conducted including:

a) Fasting Blood Glucose (FBG) level: Blood samples were collected from the tail vein after about 16 hours of fasting, and glucose levels were measured using a glucometer in weeks 2, 3, 4, and 5 after intervention.

b) Oral Glucose Tolerance Test (OGTT): At the third week of the experiment, an OGTT test was performed to assess how well the mice metabolized glucose. After overnight fasting, FBG was measured at timepoint 0, then each mouse was administered a glucose solution (2 g/kg body weight), and blood glucose levels were measured at 15, 30, 60, 90, and 120 minutes afterward.

c) Insulin Tolerance Test (ITT): ITT is a procedure used to assess insulin sensitivity, which is impaired in obese and diabetic mice. In this test, insulin is injected to lower blood glucose levels, and the rate at which the body responds to this insulin is measured. After 4 hours of fasting, a baseline glucose level was measured. After a dose of insulin (0.5 units/kg body weight) injected intraperitoneally, the glucose levels were assessed at 15, 30, 60, 90, and 120 minutes afterward.

d) Meal Tolerance Test (MTT)

MTT is a method to evaluate the acute effects of OPH on glucose level in HFD-fed mice. Specifically, this test was conducted to determine whether OPH can quickly influence glucose metabolism through incretin hormones like GLP-1, which play a role in insulin release and overall glucose regulation. In this experiment, the mice were divided into three distinct treatment group (n=2) to evaluate the different dietary protein sources: 1) Casein group: Mice in this group were orally administered a dose of casein protein at 2 g/kg body weight; 2) Oat protein group: Mice in this group received oat protein solution at the same dose (2 g/kg body weight); 3) OPH group:

The third group was given OPH at 2 g/kg body weight. After overnight fasting, glucose levels were measured before and 15 minutes after administration a dose of protein.

Statistical analysis: All quantitative analysis were performed at least in triplicate. Origin version 2022b (OriginLab Corporation, Northampton, MA, USA) software was used for statistical analysis and results presented as the mean ± standard deviation. Statistical analysis was conducted by one-way analysis of variance (ANOVA) at a confidence interval of 95% utilizing Tukey's test at $p>0.05$. Additionally, a paired t-test was utilized to compare paired observation for in in vivo study with significant set at p values< 0.05. All statistical analysis were performed using GraphPad prism and Origin.

.

Results, discussion and conclusions

Objective 1- Screen oat variety and develop processing technique to increased protein and β-glucan recovery in oat milk

1.1 The impact of variety on protein and β-glucan content in oat milk products

With the help of POGA, 14 commercial oat varieties were obtained from seed companies or breeders including AAC Douglas, AC Morgan, CDC Endure, CS Camden, Kyron, Hot 602, Ore 3541M, Ore 3542M, Ore level 48, ORe level 50, Ore 6251M, OT 2129, OT 3112, Summit.
The oat grains were milled in flours and tested for their original protein and β-glucan content. As shown in Figure 1-1 and 1-2, the original protein content in the oat grains varied from10.9% to 17.5% and the original β-glucan content varied from 3.2% to 6.1%.

Some varieties have large acreage in western Canada with high yield, but relatively lower protein (11.3%) and β-glucan content (3.2%) such as AC Morgan, and some are relatively new varieties with either higher protein content such as HOT 602 (17.5%) or high β-glucan content such as OT 3112 (6.1%).

Oat milk samples were prepared from different oat grain varieties and the protein and β-glucan contents in the resulting oat milk were measured. As shown in Figures 1-2, the protein content ranged from 10.3% to 16.3%, while β-glucan content ranged from 1.0% to 5.0%. Notably, a strong positive correlation (Pearson correlation coefficient of 0.947) was observed between the protein content in the oat milk and the original protein content in the oat grains. This indicates that oat varieties with higher protein levels may yield oat milk with higher protein content. In contrast, no clear correlation was found for β-glucan content. Factors such as the hardness of the oat kernel, which affects the breakdown of cell walls and water penetration into the inner layers where β-glucan is located, may play a role in influencing β-glucan recovery in the oat milk products.

Based on above results, oat varieties of high protein content are recommended for oat milk production such as HOT 602 (17.5% protein in oat grain), Kyron (15.8%), ORe Level 48 (15.7%) and CS Camden (14.8%). In addition, these varieties contain ~4% or higher β-glucan, thus have

high potential to lead to nutritionally enhanced oat milk products high in both protein and dietary fiber. Nevertheless, it should be mentioned that the above values are based on a single year's harvest, and the protein content in the oat grains may vary depending on the harvest years, growing conditions and other factors such as fertilizer.

1.2. The effect of ultrasound on oat grain tissue

Ultrasound, an environmentally friendly technology, has garnered increased attention in food processing for its ability to enhance extraction yields and modify physicochemical properties of plant-based materials. The cavitation effect of ultrasound ruptures plant cell walls, enhancing the extractability of intercellular materials through high shear rates and the collapse of microbubbles in a liquid medium.

The oat beverage residues were observed by the confocal microscopy to study the impact of ultrasound on the oat grain materials, as shown in Fig. 1-3. The protein bodies were stained with Acid Fuchsin (red), and the cell wall was stained with Calcofluor White (blue). Without the ultrasound treatment, large pieces of intact cell wall structure remained with protein bodies embedded in the cells. When 60 W was applied, the cells adjacent to the disintegrated edge became less packed, but the center of the cells was remained intact and fully packed with protein bodies. When the higher ultrasound power level was applied, the destruction of cell walls not only limited to the edge of the residue fragment, with the observable damage towards its centre. This phenomenon was attributed to the greater exposure of cavitation effect on these cell walls. Prolonged treatment at 960 W for 9 min further intensified this impact, resulting in more damage in the centre of cell walls as indicated by the arrows in Fig. 1-4. The generated hydrodynamic shock wave can damage the cell structure including breakages and loosening of the cell structure. Although the cell walls were damaged, the overall shape of the cell walls was retained. Whereas several studies demonstrated completely rupture of cell walls when fruit juices were treated by a high-power level of ultrasound with a longer duration (Rojas, 2016; Campoli, 2018). Due to the difference in rigidity of the plant cell walls, ultrasound had less destructive effects on oat cell walls than those of fruits. The mechanism of intercellular materials extraction through ultrasound involves cell disruption, could result in increasing the contact area of intracellular materials and extraction solvent (water) to promote mass transfer. It was observed that some of the cells still contained fully packed protein bodies, even after treatment with 960 W for 9 min. Similar result was observed from the study of Preece et al. (2017) in which protein bodies remained within the cells when the soy slurry was treated with 1700 W for 2.3 min.

1.3. The effect of ultrasound on β-glucan content and structure in oat beverage

The β-glucan contents of oat beverages by ultrasound treatments are shown in Fig. 1-4. The oat beverages treated with ultrasound of 60 and 240 W showed gradually increased β-glucan content. At 240 W for 9 min the highest content of β-glucan of 7.45 mg/mL was observed in the oat beverage which increased by almost 2 folds compared to the control (4.58 mg/mL). The explosions of bubbles generate energy to loosen the outer layer of oat cell walls, allowing more

water to penetrate to the cell wall inner layer where β-glucan is located. However, when the ultrasound power level was further raised to 960 W and time prolonged to 9 min, the β-glucan content was reduced to 5.49 mg/mL, even no significant difference compared to the control sample ($p>0.05$). This reduction could be attributed to more insoluble materials such as cellulose and arabinoxylan were also released and suspend in the solvent (water), which possibly lower the permeability of water to extract β-glucan (Prakash Maran, 2013). The prolonged exposure time (9 min) may cause excessive release of these insoluble materials, leading to the further reduction of the β-glucan content in oat beverage.

Meanwhile, only minor change was observed for the β-glucan molecular weight (M_w). The samples showed β-glucan M_w range of 180.51 to 204.23 kDa. Even with the highest ultrasound power level with prolonged treatment time (960 W for 9 min), only approximately 4.2% decrease of β-glucan M_w when ultrasound timing was extended from 0 to 9 min at 960 W. Since the large amount of microbubble formation at a higher ultrasound power level which could act as a barrier of energy transmission within the system. These bubbles may not effectively to produce the required energy to further decrease the M_w. Comparing to ultrasound, multiple enzymatic (e.g. the combination of α-amylase, protease and xylanase) and chemical (alkaline and acidic solutions) extraction methods can lead to extensively fragmentation of β-glucan M_w into shorter chains, which may interfere its blood-glucose and blood-cholesterol lowering abilities (Bozbulut, 2019; Goudar, 2020). Significantly increase in extraction of β-glucan content by the ultrasound treatment (240 W for 9 min) without extensively reducing its M_w makes this technology favorable for improving the nutritional quality of oat beverage.

1.4 The effect of ultrasound on protein content and structure in oat beverage

The effect of ultrasound on protein content in the oat beverages was presented in Fig. 1-5 (A,B,C) Comparing with the control, the ultrasound treated the oat beverages showed either no significant difference or a reduction in the protein content. One explanation could be due to the rigid cell wall protected the intercellular proteins from cavitation effects, which limiting the extraction of cellular proteins. Another explanation was ultrasound not only released the protein, other components including dietary fibers such as β-glucan and oil were also being released in the oat beverages. These released components might interact with the oat protein which inhibited the dissolution of protein during extraction, leading to its precipitation by centrifugation (Jaramillo, 2011; Byanju, 2020). Similar findings were found when using ultrasound to extract protein from chickpea as the authors suggested high contents of carbohydrates and lipids lowered the protein yield in the products (Byanju et al., 2020). Increase water ratio might increase protein content in the oat beverages as water allowed more cavitation effects. However, from the industrial perspective, increase in water usage is not desirable because the oat beverage will be diluted, which also leads to diminish of its nutritive quality and desirable characteristics such as mouthfeel.

FT-IR was used to understand the secondary structure of protein. The absorption changes in amide region (amide I) were mainly studied and the results are shown in Fig. 1-5 (F,G,H). The amid I band with and without ultrasound treatment showed 7 bands, including 1691 cm-1 (β-

sheets/ turns), 1670 cm1 (β-turns), 1656 cm-1 (α-helix), 1641 cm-1 (random coil), 1629 cm-1 (β-sheet), 1618 cm-1 (intermolecular β-sheet), and 1609 cm-1 (vibration of amino acid residues. When oat grain underwent ultrasound treatment, no changes in amid I region were observed This result suggests that ultrasound leave the secondary structure of oat protein intact.

Fluorescence can be applied to characterize the tertiary structure changes of protein, mainly due to the presence of chromophores (tryptophan, tyrosine, and phenylalanine residues). As presented in Fig. 1-5 (I,J,K), the λmax of control was 334 nm and ultrasound treated oat beverages were between 334 and 340 nm. When the ultrasound duration was increased to 9 min at 60 W, the λmax was shifted to 336 nm. Further peak shift was observed as the oat beverage was treated with 240 W for 9 min. The shift of λmax to the longer wavelength wave (red shift) indicates that tryptophan side chains in protein were exposed to the polar environment. This was aligned with the results of surface hydrophobicity (H0) measurement. H0 indicates the number of hydrophobic groups exposed to the protein molecular surface. Treatment by 240 W for 9 min resulted the significant increased in H0 when compared to the samples treated by 1 min and the control. Both results indicates that ultrasound partially unfolded oat protein, led to exposure of buried hydrophobic groups to the protein surface. When the power level increased to 960 W, a reduction in H0 was observed when the treatment duration was prolonged to 9 min. This could be related to the aggregation of partially unfolded proteins through hydrophobic and other interactions, and thus the exposed hydrophobic groups were buried inside the protein aggregates (Mir, 2019; Liu et al., 2022). Consequently, a blue shift occurred as the λmax shifted back from 338 to 336 nm when the ultrasound treatment was prolonged from 1 to 9 min.

1.5. The effect of ultrasound on protein solubility and foaming in oat beverage

Protein solubility is a critical factor in plant-based beverages as having a good protein solubility will improve physiochemical properties, including foaming properties of plant-based beverages. In this study, the oat beverage samples have a range of pH from 6.26 to 6.40, and oat protein is known to have limited solubility in such pH range (Yu et al., 2023). As displayed in Fig. 1-5 (L,M,N), the protein solubility showed no statistical change compared with the control at low power ultrasound treatment. As the power level was increased to 240 W and treated for 9 min, the protein solubility was significantly enhanced from 24.7 to 52.8%. This could be attributed to partial unfolding structure of oat protein, increasing the interaction between the protein and water molecules. When the ultrasound power level and treatment time increased, more hydrophobic groups were exposed that could caused protein to reaggregate through non-covalent interactions and thus, reduce its solubility (Mir et al., 2019; Li, 2021). Despite an increase in solubility at 240 W, the protein recovery did not improve. This contracts with the expectations, as higher water solubility usually correlates with a better recovery (Karabulut et al., 2023; Li et al., 2023). Further studies are needed to understand this phenomenon.

The values for foaming capacity and stability are presented in Fig. 1-6. Notably, the foaming capacity of oat beverage showed an improvement after ultrasound treatments. The foaming capacity can be impacted by the viscosity of oat beverage. For example, the oat beverage treated

at 960 W showed a significant decrease in viscosity, making proteins easier to absorb to the air-water interfaces (Sheng et al., 2018). Furthermore, the foaming stability was also significantly improved when ultrasound was applied than that of the control, typically at 240 and 960 W ($p<0.05$). The increase of foaming stability could be due to the enhanced β-glucan content, providing a certain level of viscosity to lower the mobility of air bubbles (Eghbaljoo, 2022). More importantly, ultrasound induced the partial unfolding of oat proteins, increasing the number of hydrophobic groups exposure to surface, which enhanced their ability to absorb at the air/water interface and formation of a cohesive layer around the air bubbles to prevent coalescence and thereby increase the foaming ability (Mir, 2019). The improved foaming capacity and stability of ultrasound treated oat beverage will be desirable when using as an ingredient in such as coffee-based beverages and desserts.

Summary

- A strong positive correlation was observed between the protein content in the oat milk and the original protein level in the oat grains, suggesting that oat varieties with higher protein levels may yield oat milk with higher protein content such as HOT 602 (17.5% protein in oat grain), Kyron (15.8%), ORe Level 48 (15.7%) and CS Camden (14.8%).

- However, no clear correlation was found between the β-glucan content in the oat milk and the original β-glucan level in the oat grains.

- Ultrasound technique led to 39% enhancement in β-glucan recovery in oat milk when treated at the power level at 240 W for 9 min, while exhibiting minimal impact on the β-glucan molecular weight that could preserved its inherent health benefits.

- The protein recovery in oat beverage was not improved by ultrasound treatment, instead, ultrasound treatment led to increased protein solubility and foaming properties, which would be advantageous for developing coffee-based beverages and desserts.

- Therefore, a new processing technique based on ultrasound has been developed to enhance the β-glucan recovery and the oat protein functional properties to improve the overall quality of oat beverage.

Study innovations and implications for the advancement of agricultural science

The research in this objective has led to a new processing technique based on ultrasound to enhance the β-glucan recovery and the oat protein functional properties to improve the overall quality of oat beverage. Interestingly, ultrasound exhibited minimal impact on the β-glucan molecular weight that could preserved its inherent health benefits. The enhanced foaming properties would be advantageous for developing coffee-based beverages and desserts from

milling oats. In addition, this research has provided scientific insight into the oat protein and β-glucan extraction and modification by ultrasound as a pre-treatment technology during oat beverage preparation. Increasing nutrients recoveries through ultrasound will provide a more sustainable method to help oat beverage industry in improving the product nutritive quality and enhancing competitiveness in the global market. To further enhance the extractability of protein in oat beverage, it will be worthy of investigation to combine ultrasound with other technologies such as cell wall degrading enzymes for a better disruption of the rigid cell walls, thereby further improving the nutritional quality of oat beverage.

Objective 2 - Develop anti-diabetic peptides from oat proteins

2.1. Enzymatic hydrolysis of oat protein

The SE-HPLC chromatograms in Figure 2-1 show oat protein's molecular weight (MW) distribution and hydrolysates by alcalase and alcalase-flavourzyme treatment. The major peaks in oat protein were in the range of 30 to 7.2 kDa, which progressively shifted to a more defined 6.1 kDa peak when hydrolyzed with alcalase. However, the obtention of smaller peptides was not efficient with the use of alcalase alone. Therefore, further hydrolysis was conducted with flavourzyme because it has been shown to produce smaller peptides due to its endo and exopeptidase action (Walters 2020). The peptides MW was significantly reduced after flavourzyme hydrolysis with major peaks in the range of 5.7 kDa and 1.5 kDa because the pre-digestive effect of alcalase over internal peptide bonds of the protein structure favored flavourzyme cleavage of amino acids at the chain terminus.

2.2 Antidiabetic activities of oat hydrolysates.

α-amylase inhibitory essay: Figure 2-2A shows the α-amylase inhibitory effect of oat protein hydrolysates by alcalase treatment (AH) at different doses. With increasing hydrolysate concentration from 0.33 to 1.0 mg/mL, the inhibitory activity increased from 18.1 up to 49.5 %. Further increasing AH concentration reduced the α-amylase inhibitory effect. A possible explanation could be attributed to the peptides' aggregation when increasing the concentrations instead of binding to the enzyme for inhibition. Inhibitory effect of AH from oat protein was higher than the inhibition activity reported for seaweed alcalase hydrolysates (~30%) at 1.86 mg/mL (Admassu, 2018)

Figure 3-2B shows the α-amylase inhibitory effect of oat peptide fractions from the alcalase-flavourzyme hydrolysate (AFH) with the M_W of 1-5 kDa and 1 kDa. Similar inhibitions of up to 42.6 ± 0.5 % and 56.0 ± 3.7 % were achieved for both fractions, respectively, at a concentration almost six times lower than the one required from AH. The 1-5 kDa fraction showed ~33% α-amylase inhibition even at a low concentration of 30 µg/ml, but further increasing the fraction concentration from 100 to170 µg/ml did not significantly improve the α-amylase inhibition. For the ≤1 kDa fraction, the α-amylase inhibition effect increased from ~7% to 44%, and 56% when the concentration increased from 30 to 100 and 170 µg/ml. Further increasing peptide concentration to 330 µg/ml reduced the inhibitory activity for both 1-5 kDa and ≤1 kDa fractions. The dose

dependency effect was not clear for α-amylase inhibitory effect in this study, which is worthy of investigation in the future. Nonetheless, the trend indicates that the obtention of smaller peptides by combined alcalase and flavourzyme hydrolysis, followed by the hydrolysate filtration to recover the low MW fraction, effectively concentrated the peptides with α-amylase inhibitory capacity.

It is noticed that the oat peptide fraction (AFH) exhibited a similar or higher α-amylase inhibitory effect when compared to pea protein hydrolysate fractions. For example, AFH of 1-5 kDa showed an α-amylase inhibitory effect of 42.6 ± 1.1% at 100 µg/mL. To reach a similar level of α-amylase inhibitory, about 225 µg/mL was required for pea protein hydrolysate fraction of 1-3 kDa (Awosika & Aluko, 2019). Although a similar molecular weight cut off was used in chia protein hydrolysate fraction with M_w of 1-3 kDa showed the inhibitory effect of 18% at a concentration of 10 mg/mL The above comparison allows us to confirm that fractions from oat protein hydrolysates can potentially generate α-amylase inhibitory peptides.

The positive control, acarbose (82.9± 1.65 to 98.7 ± 1.0 %), displayed a significantly higher α-amylase inhibitory effect even at a low concentration of 30 µg/mL *($p < 0.05$)*, which is expected for a well known synthetized antidiabetic drug. The obtained inhibitory activity is still not comparable to acarbose; however, oat peptides present potential inhibitory activity that could be the base to generating a more natural source of antidiabetic ingredient to delay α-amylase digestion of starch because peptides are natural with lower side effects. In addition, the use of bioactive peptides from food proteins may provide other biological functions as antihypertensives, antioxidants, and bactericides (Kannan, 2011), among others.

α-Glucosidase inhibitory assay: In this study, the oat AH showed a significant difference in the inhibition percentage over time (Figure 2-3). The enzymatic assay showed α-glucosidase inhibitory effects of up to 38% in the first minute; however, the inhibitory effect significantly dropped by half after 5 minutes and then decreased to less than 10% after 10 minutes. The results obtained in this assay suggests that oat peptides presented a low affinity to the enzyme as the inhibitory effect was not observed for a long time. In this study, no concentration dependent effect was observed for oat AH. This could be due to the presence of a diversity of peptides with a wide range of molecular weights that could interfere with the inhibition of α-glucosidase. Higher concentrations of peptides may need to be tested in the future because other protein sources like hemp seeds alcalase hydrolysates showed inhibitory effects of around 58% and 25% at concentrations of 100 mg/mL and 100 µg/mL, respectively (Ren et al., 2016). It was also noticed that oat AFH fractions of ≤1 kDa and 1-5 kDa showed no obvious inhibitory effect (data not shown). These findings lead to the assumption that AFH in our research might be less efficient to generate peptides with a strong α-glucosidase inhibition effect compared to trypsin hydrolysates.

***DPP-IV inhibitory assay*:** Oat AH tended to a dose dependent DPP-IV inhibitory effect (Figure 3-3A) starting at 4.9 ± 1.5% and increasing to 48.7 ± 13% when increasing concentration from 100 to 500 µg/mL. However, the high inter sample variability prevented from reaching statistical differences. Nevertheless, a dose dependent inhibition was observed for the 1-5 kDa AFH fraction (Figure 3-3B), which showed inhibition of 56.2 ± 4.3% when the peptide concentration increased

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

In previous work by Wang et al. (2015), hydrolysates of oat globulin showed good DPP-IV inhibition (IC_{50} 2.04 mg/mL) after 14 h of trypsin treatment. In our study, the continuous hydrolysis by alcalase and flavourzyme was more efficient to release bioactive peptides with strong DPP-IV inhibition capacity (IC_{50} of 0.413 ± 15 mg/mL) in only 6 h. It is likely related to the fact that alcalase belongs to the endoprotease classification and flavourzyme exhibits both endo and exoprotease activity with non specific cleavage; thus, such combination allowed extensive hydrolysis of oat protein for more rapid generation of DPP-IV inhibitory peptides.

In comparison, oat protein hydrolysates presented a similar activity at a lower concentration of 500 µg/mL. Velarde-Salcedo et al. (2013) showed that hydrolysates from amaranth, black bean, soybean, and wheat by enzymes (pepsin, trypsin, and pancreatin) in simulated gastrointestinal digestion had a DPP-IV inhibitory effect of 20 to 60% at the concentration of 1.4 mg/mL. Moreover, AFH fractions from oat protein were comparable to hydrolysates from lactoferrin and bovine serum albumin that displayed IC_{50} values of 0.379 and 0.513 mg/mL, respectively (Lacroix, 2017). As expected, DPP-IV positive control inhibitor, sitagliptin, had the highest inhibitory effect (86.8 % at a much smaller concentration of 50 µg/mL). Although oat hydrolysates did not reach as high inhibitory effects as the positive control, findings in this study confirm that DPP-IV inhibitory peptides from oat protein are comparable to other peptides from various plant and animal protein sources. It would also be necessary to study the inhibitory effect at higher concentrations in the future to understand the peptide's inhibitory dose dependent effect.

2.3 RP-HPLC peptide fractionation

Since the AFH fraction of 1-5 kDa showed a weak inhibitory effect in α-glucosidase and medium inhibitory activities in α-amylase and DPP-IV assays, it was chosen for further fractionation using RP-HPLC. In this study, oat AFH was separated into four fractions (F1, F2, F3, and F4), as shown in Figure 2-4, with F1 being the most hydrophilic and F4 the most hydrophobic fraction. All four fractions were then collected and tested for their capacity to inhibit α-amylase, α-glucosidase, and DPP-IV.

Specific fractions exhibited better inhibitory effects. For instance, a stronger α-amylase inhibition effect was observed for F2 and F3, as shown in Figure 3-5A. Specifically, the α-amylase inhibitory effect was 53.8 ± 5.6% for F2 even at a low concentration of 30 µg/mL when compared to 33% for 1-5 kDa fraction at the same concentration. It was noticed that the inhibitory effect decreased with increasing concentration of the F2 to 170 µg/mL. For F3, concentrations of 30 and 100 µg/mL generated the strongest α-amylase inhibitory effects of 46.6 ± 6.3 and 57.3 ± 9.5%, respectively. The α-amylase inhibitory effect observed for F2 and F3 (F1 and F4 effect not shown as they

showed none or very weak inhibitory effects) suggests that peptides with medium polarity are necessary for interaction with the active site of α-amylase.

For the α-glucosidase assay, the inhibition values at 10 min were used to compare the effect of four fractions, as 10 min was the longest time that oat peptides generated an inhibitory activity. The results in Figure 3-5B showed the α-glucosidase inhibitory effect for F1 and F2 since F3, and F4 show little or no inhibitory effect. The inhibitory effect of F1 at a low concentration of 25 μg/mL was 19.7 %, which decreased with increasing concentration to 75 and 125 μg/mL. The inhibitory effect of F2 was 33.4 ± 1.8% at the highest concentration of 125 μg/mL, suggesting that the oat peptide fractions with medium polarity and the possible presence of hydrophilic amino acids might better inhibit α-glucosidase. However, studies report that the hydrophobicity of peptides and α-glucosidase inhibitory effects were poorly correlated (Ibrahim, Bester, Neitz, & Gaspar, 2018). Similar α-glucosidase inhibitory effects that ranged from 27 to 33% at 1000 μg/mL were found for peptide fractions generated from germinated soy protein hydrolysate (González-Montoya, 2018). As a positive control, acarbose showed an α-glucosidase inhibitory effect of 37.1 % at a concentration of 125 μg/mL, which is in accordance with findings reported in the literature (Lordan, 2013; Moon., 2011).

F1, F2, F3, and F4 all exhibited DPP-IV inhibitory effects. Especially, F3 showed an inhibitory effect of 43.3 % at a relatively low concentration of 100 ug/mL (Figure 2-5C). Further increasing concentration to 500 μg/mL led to an increased inhibitory effect of 78.0 %. The higher inhibitory effect observed for F3 and F4 suggests that hydrophobic amino acids strongly contribute to peptides' DPP-IV inhibition. The existence of some hydrophilic amino acids might further improve the peptide bioactivity as F3 shows the highest inhibitory effect. Nongonierma, 2019) also reported that the hydrophobic or aromatic amino acids in peptide sequences contributed to DPP-IV inhibition. Moreover, structural analysis of known inhibitors of DPP-IV suggests their capacity to bind to the active site of the enzyme by hydrogen bonding and hydrophobic interactions (Chakraborty, 2014). Thus, it is believed that the exposure of hydrophobic and aromatic amino acids, obtained by the hydrolysate of low M_W (1-5 kDa), contributed to the strong capacity of oat peptides to inhibit DPP-IV and the fractionation by RP-HPLC to further concentrate the peptides with DPP-IV inhibitory effects. It should be mentioned that the highest DPP-IV values reached ~50% for oat protein hydrolysate fractions in this study. It would be necessary to study the inhibitory effect at higher concentrations in the future to understand the peptide's inhibition dose dependent effect.

2.4 Peptide sequencing of the effective fractions

De novo sequencing of our sample identified new sequences (Table 2-1) varying from 4 to 7 amino acid peptides with similar amino acid content and position characteristics for α-amylase inhibition. For example, Phe-Pro-Leu-Leu-Gln (FPLLQ), Phe-Pro-Leu-Leu-Phe (FPLLF), and Phe-Pro-Leu-Leu-Leu (FPLLL) all have Phe at the N-terminus and the presence of Leu and Phe at the C-terminus. Moreover, two peptide sequences of 8 amino acids each were identified from

F3: Gly-Asp-Val-Val-Ala-Leu-Pro-Ala (GDVVALPA) and Asp-Val-Val-Ala-Leu-Pro-Ala-Gly (DVVALPAG). Both peptides are constituted by hydrophobic amino acids and one negatively charged amino acid (Asp).

For the α-glucosidase assay, molecular docking studies suggest that an albumin peptide Lys-Leu-Pro-Gly-Phe (KLPGF) is comparable to acarbose as a positive control for α-glucosidase inhibition with similar IC_{50} values of 33 and 39 mg/mL, respectively (Yu, 2012). A similar pattern was observed in the effective sequences identified in this study. For example, Leu-Pro-Pro-Gln-Leu (LPPQL), Phe-Pro-Leu-Leu-Gln (FPLLQ), and Leu-Pro-Glu-Leu-Gln (LPELQ) (Table 3-1) both contain Leu-Pro or Pro-Leu as found in other peptides registered as α-glucosidase inhibitors (Minkiewicz, 2019).

Diprotin A and B are strong DPP-IV inhibitors with IC_{50} values of 1.1 and 5.5 μg/mL with amino acid sequences: Ile-Pro-Ile and Val-Pro-Leu, respectively, as reported by Umezawa et al. (1984). Interestingly, de novo peptide sequencing of F3 disclosed the presence of sequences such as Leu-Pro-Val-Asp-Val (LPVDV), Leu-Pro-Leu-Pro-Gln (LPLPQ), and Tyr-Pro-Thr-Asn-Thr-Tyr (YPTNTY) (Table 3-1), which resemble the known inhibitors in that they both contain Val, Pro, and Leu in their N-terminal structures, and Pro in the chain's second position. In addition, dipeptides Leu-Pro and Ile-Pro have been demonstrated to be some of the main DPP-IV inhibitors present in rice bran, and Tyr-Pro was found as an inhibitor in milk protein (Hatanaka et al., 2012; Nongonierma, 2014). Therefore, we can speculate that the presence of these dipeptides in oat peptide sequences such as Gly-Asp-Val-Val-Ala-Leu-Pro-Ala (GDVVALPA) and Asp-Val-Val-Ala-Leu-Pro-Ala-Gly (DVVALPAG) might have significantly contributed to the AFH antidiabetic activity.

Due to its abundance in oat protein, Pro content is high in the identified sequences in our study. It is possible that those peptides with antidiabetic effects could better resist gut enzymatic digestion; thus, maintaining their stability and bioavailability. Yet, the release of the smaller peptides from the identified sequences in this study after gut digestion needs to be investigated, as well as their antidiabetic activities. Moreover, the peptides with antidiabetic activities identified have a relatively small M_W, with most of them having a 5 amino acid length. Thus the absorption of those peptides is possible through specific peptide transporters, paracellular transport, or transcytosis route (Oseguera-Toledo, 2014). Other studies using molecular docking, cell culture and *in vivo* models are required to understand the underlying mechanisms of oat peptides digestion, absorption, and interactions with DPP-IV.

Summary

- Peptides from oat protein hydrolysis demonstrated good capacity to inhibit enzymes involved in glucose metabolism like DPP-IV, α-amylase, and α-glucosidase.

- Ultrafiltration and RP-HPLC fraction techniques were effective to concentrate DPP-IV and α-amylase inhibitory peptides from oat protein hydrolysates.

- LC-MS/MS analysis disclosed the presence of two amino acid sequences GDVVALPA and DVVALPAG, as well as 25 new sequences rich in hydrophobic and aromatic amino acids and proline from oat protein with potential glycemia-lowering effect.

Study innovations and implications for the advancement of agricultural science

This research suggests that proline and hydrophobic amino acids play a crucial inhibitory role and may favor hydrophobic interactions and hydrogen bonding at the active site of these enzymes. Although the information in this research might seem basic, it is the first necessary stage to develop peptides with antidiabetic activities from oat as a relatively new source of protein of plant origin. Our data indicate that it is possible to generate antidiabetic peptides from oat protein that targets three of the most studied enzymes for glucose regulation, which casts a new light for the study of oat peptides and their future in pharma and nutraceutical applications for T2DM management. This research also justifies studying the antidiabetic effects of peptides through cell and animal models in the next step. This knowledge may allow the industry to implement bioactive peptides in diabetic friendly foods and general products in addition to oat β-glucans and phenolic compounds and add value to oats as a globally beneficial crop.

Objective 3- Study the feasibility of developing a functional oat beverage with glycemia–lowering effects in a pre-clinical study

Figure 3-1 illustrates the effect of OPH on DPP-4 activity in Caco-2 cells. All concentrations, except the lowest, significantly inhibited the enzyme compared to the control group, with a clear dose-response relationship. Moreover, OPH at concentrations of 1, 0.5, and 0.25 mg/ml demonstrated relative DPP-4 activity of 20%, 77%, and 83%, respectively, indicating higher OPH concentration led to a higher level of DPP 4 inhibition.

The in vivo study results are shown in Figure 3-2. At the start of the experiment, body weight was comparable across all diet groups; however, the OPH group exhibited the lowest body weight gain percentage. After three weeks of intervention, FBG levels showed a marked increase in the HFD group, whereases no significant changes were observed in the intervention groups. Additionally, OGTT and ITT results indicated no significant differences among the groups, while MTT results demonstrated highest glucose level in Casein and lowest in OPH group at baseline and 15 minutes.

Despite the overall increase in body weight across all groups, the OPH group showed the lowest body weight gain, though the difference was not statistically significant compared to other groups. This suggests that OPH may have a positive role in regulating body weight. However, a larger sample size might be necessary to more accurately capture and represent this potential effect. In another study, sixteen weeks of a high-fat/high-sucrose (HF/HS) diet supplemented with oat protein had no significant effect on body weight in Wistar rats(7). Thus, OPH may contain unique bioactive peptides that the body’s digestive enzymes are unable to produce naturally. To

investigate this further, we plan to evaluate the individual effects of oat protein and peptides on body weight regulation in an obese mouse model.

The evaluation of the acute effects of OPH on glucose regulation through the MTT test revealed that lower blood glucose levels compared to both casein and control group. According to the in vitro results, it is hypothesized that in mice treated with OPH, the inhibition of DPP-4 activity led to an increased level of GLP-1, which stimulated insulin secretion and lowered blood glucose level. While our finding suggests a potentially beneficial role for OPH in glucose regulation, it is important to note that the limited sample size in this pilot experiment (n=2) may not provide a reliable representation of the effects. Further studies with larger sample sizes are necessary to confirm the acute impact of oat peptides.

Summary

- Oat protein hydrolysate appears to have positive effects on glucose regulation, such as inhibiting DPP-4 and reducing glucose absorption in intestine.
- Further studies with larger sample sizes are necessary to confirm the acute impact of oat peptides.

Study innovations and implications for the advancement of agricultural science

This is one of the first studies that test the glycemia-lowering effects of oat peptides in vivo using animal model. Our preliminary in vivo study suggests that chronic and acute consumption of OPH may regulate body weight and improve glucose tolerance, respectively. We believe that using a diet-induced obese (DIO) mouse model with adequately sized sample, will offer more reliable insights into the potential of oat peptides role in diabetes management.

Literature cited

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

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

Byanju, B., Rahman, M. M., Hojilla-Evangelista, M. P., & Lamsal, B. P. (2020). Effect of high-power sonication pretreatment on extraction and some physicochemical properties of proteins from chickpea, kidney bean, and soybean. International Journal of Biological Macromolecules, 145, 712-721. https://doi.org/10.1016/j.ijbiomac.2019.12.118.

Campoli, S. S., Rojas, M. L., do Amaral, J. E. P. G., Canniatti-Brazaca, S. G., & Augusto, P. E. D. (2018). Ultrasound processing of guava juice: Effect on structure, physical properties and lycopene in vitro accessibility. Food Chemistry, 268, 594-601. https://doi.org/10.1016/j.foodchem.2018.06.127.

Chakraborty, C., Hsu, M. J., & Agoramoorthy, G. (2014). Understanding the Molecular Dynamics of Type-2 Diabetes Drug Target DPP-4 and its Interaction with Sitagliptin and Inhibitor Diprotin-A. Cell Biochemistry and Biophysics, 70(2), 907–922. https://doi.org/10.1007/s12013-014-9998-0
Chen, L. (2018) Final report of project 2015C010R “Optimizing protein quality of Alberta oats and food application”, sponsored by Alberta Crop Industry Development Fund.

Eghbaljoo, H., Sani, I. K., Sani, M. A., Rahati, S., Mansouri, E., Molaee-Aghaee, E., Fatourehchi, N., Kadi, A., Arab, A., Sarabandi, K., Samborska, K., & Jafari, S. M. (2022). Advances in plant gum polysaccharides; Sources, techno-functional properties, and applications in the food industry - A review. International Journal of Biological Macromolecules, 222, 2327-2340. https://doi.org/10.1016/j.ijbiomac.2022.10.020.

Gamel, T.H., Abdel-Aal, E.S., Tosh, S.M. (2015) Effect of yeast-fermented and sour-dough making processes on physicochemical characteristics of β-glucan in whole wheat/oat bread, LWT-Food Science and Technology, 60, 78-85.

Gomez-Peralta, F., Abreu, G., Gomez-Rodriguez, S., Barranco, R.J., Umpierrez, G.E. (2018) Safety and efficacy of DPP4 inhibitor and basal insulin in Type 2 Diabetes: an updated review and challenging clinical scenarios, Diabetes Therapy, 9, 1775-1789.

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

Goudar, G., Sharma, P., Janghu, S., & Longvah, T. (2020). Effect of processing on barley β-glucan content, its molecular weight and extractability. International Journal of Biological Macromolecules, 162, 1204-1216. https://doi.org/10.1016/j.ijbiomac.2020.06.208.

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

Izydorczyk, M.S., McMillan, T., Bazin, S., Kletke, J., Dushnicky, L., Dexter, J., Chupurna, A., Rossnagel, B. (2014) Milling of Canadian oats and barley for functional food ingredients: Oat bran and barley fibre-rich fractions, Canadian Journal of Plant Science, 94, 573-586.

Jaramillo, D. P., Roberts, R. F., & Coupland, J. N. (2011). Effect of pH on the properties of soy protein–pectin complexes. Food Research International, 44(4), 911-916. https://doi.org/10.1016/j.foodres.2011.01.057.

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

Klose, C., Arendt, E.K. (2012) Proteins in oats; their synthesis and changes during germination: a review, Critical Review in Food Science and Nutrition, 52, 627-639.

Lacroix, I. M. E., Chen, X.-M., Kitts, D. D., & Li-Chan, E. C. Y. (2017). Investigation into the bioavailability of milk protein-derived peptides with dipeptidyl-peptidase IV inhibitory activity using Caco-2 cell monolayers. 8, 701. https://doi.org/10.1039/c6fo01411a

Li, R., & Xiong, Y. L. (2021). Ultrasound-induced structural modification and thermal properties of oat protein. LWT, 149, 111861. https://doi.org/10.1016/j.lwt.2021.111861.

Liu, X., Wang, M., Xue, F., & Adhikari, B. (2022). Application of ultrasound treatment to improve the technofunctional properties of hemp protein isolate. Future Foods, 6, 100176. https://doi.org/10.1016/j.fufo.2022.100176.

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

Minkiewicz, P., Iwaniak, A., & Darewicz, M. (2019). BIOPEP-UWM Database of Bioactive Peptides: Current Opportunities. International Journal of Molecular Sciences 2019, Vol. 20, Page 5978, 20(23), 5978. https://doi.org/10.3390/IJMS20235978

Mir, N. A., Riar, C. S., & Singh, S. (2019). Physicochemical, molecular and thermal properties of high-intensity ultrasound (HIUS) treated protein isolates from album (Chenopodium album) seed. Food Hydrocolloids, 96, 433-441. 10.1016/j.foodhyd.2019.05.052.

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

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

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

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

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

Prakash Maran, J., Manikandan, S., Thirugnanasambandham, K., Vigna Nivetha, C., & Dinesh, R. (2013). Box–Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. Carbohydrate Polymers, 92(1), 604-611. https://doi.org/10.1016/j.carbpol.2012.09.020.

Preece, K. E., Hooshyar, N., Krijgsman, A., Fryer, P. J., & Zuidam, N. J. (2017). Intensified soy protein extraction by ultrasound. Chemical Engineering and Processing: Process Intensification, 113, 94-101. https://doi.org/10.1016/j.cep.2016.09.003.

Rojas, M. L., Leite, T. S., Cristianini, M., Alvim, I. D., & Augusto, P. E. D. (2016). Peach juice processed by the ultrasound technology: Changes in its microstructure improve its physical properties and stability. Food Research International, 82, 22-33. https://doi.org/10.1016/j.foodres.2016.01.011.

Tosh, S.M. (2013) Review of human studies investigating the post-prandial blood-glucose lowering ability of oat and barley food products, European Journal of Clinical Nutrition, 67, 310-317.

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

Vasanthan, T., Temelli, F. (2008) Grain fractionation technologies for cereal beta-glucan concentration, Food Research International, 41, 876-881.

Velarde-Salcedo, A. J., Barrera-Pacheco, A., Lara-González, S., Montero-Morán, G. M., Díaz-Gois, A., González De Mejia, E., & Barba De La Rosa, A. P. (2013). In vitro inhibition of dipeptidyl

peptidase IV by peptides derived from the hydrolysis of amaranth (Amaranthus hypochondriacus L.) proteins. Food Chemistry, 136(2), 758–764. https://doi.org/10.1016/j.foodchem.2012.08.032

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

Wursch, P., Pi-Sunyer, F.X. (1997) The role of viscous soluble fiber in the metabolic control of diabetes, Diabetes Care, 20, 1774- 1780.

Yu, Y., Li, X., Zhang, J., Li, X., Wang, J., & Sun, B. (2023). Oat milk analogue versus traditional milk: Comprehensive evaluation of scientific evidence for processing techniques and health effects. Food Chemistry: X, 19, 100859. https://doi.org/10.1016/j.fochx.2023.100859.

Yu, Z., Yin, Y., Zhao, W., Liu, J., & Chen, F. (2012). Antidiabetic activity peptides from albumin against α-glucosidase and α-amylase. Food Chemistry, 135(3), 2078–2085. https://doi.org/10.1016/j.foodchem.2012.06.088

Benefits to the industry

The oats industry has made significant investments in research for varietal development and optimal crop practices to ensure the production and supply of high-quality oats. Milling oats typically contain at least 4.5% β-glucan and 10.0% total dietary fiber, and certain varieties boast high protein content (15-20%). While past research has primarily focused on enhancing oat milling quality for oatmeal production and β-glucan extraction, there is a substantial knowledge gap regarding the processing of Canadian oats for plant-based milk production.

This research analyzed and compared the protein and β-glucan recovery in oat milk products made from various oat varieties. Results revealed a strong positive correlation between the protein content in oat milk and the original protein content in the oat grains, suggesting that varieties with higher protein content, such as HOT 602, Kyron, Ore Level 48, and CS Camden, can yield oat milk with higher protein levels. Additionally, these varieties contain approximately 4% or more β-glucan, making them highly suitable for developing nutritious oat milk beverages.

The findings highlight the potential of high-protein, high-β-glucan Canadian milling oats for oat milk production. Recommendations for oat growers and processors will enable the oat industry to develop nutritionally enhanced oat milk products, benefiting consumers and promoting the use of

Canadian oats in the international plant-based beverage market. This knowledge dissemination will further elevate the demand for Canadian oats as a high-value crop for human consumption globally.

This research also introduces an innovative ultrasound-based processing technique to oat milk manufacturing industry that enhances β-glucan recovery in oat milk by 39%, while preserving its molecular weight, ensuring that the inherent health benefits of β-glucan are retained. Furthermore, this technology significantly improves protein solubility and foaming properties in oat milk products, opening new market opportunities, such as using oat milk as a foaming ingredient in coffee-based beverages and desserts.

Additionally, the study demonstrated that oat protein hydrolysates (peptides) exhibit promising glycemia-lowering effects by inhibiting key enzymes involved in glucose metabolism, such as DPP-IV and α-amylase. Preliminary in vivo results also suggest that both chronic and acute consumption of oat protein hydrolysates may help regulate body weight and improve glucose tolerance. In the long run, these findings could guide the oat milk manufacturing industry in formulating new functional beverages aimed at blood sugar management, benefiting prediabetic and diabetic populations by harnessing the combined benefits of oat β-glucan and oat protein peptides.

2021G011R

Project title: Development of a nutritionally enhanced plant-based milk alternative beverage from Canadian oats and study of its glycemia-lowering effect

Final report: October 2024

Technology Transfer & Commercialization Activities

# of Peer reviewed scientific publications: 4

Kwok, E., Yang, J., Taheri, P., Chen, L (2024) Impact of ultrasound on nutrition recovery and physicochemical properties of oat beverage, LWT, 116303.

Alsado, C., Palaruan, J., Chen, L., Wismer, W. (2024) Consumer evaluation of health and environmental label concepts on sensory acceptance and wellness perception of fortified oat beverage, Food and Humanity 3, 100362.

Alsado, C., Lopez-Aldana, L., Chen, L., Wismer, W. (2023) Consumer perception and sensory drivers of liking of fortified oat milks, Foods, 12, 4097.

Fuentes, L.R., Richard, C., Chen, L. (2021) Sequential alcalase and flavourzyme treatment for preparation of α-amylase, α-glucosidase, and dipeptidyl peptidase (DPP)-IV inhibitory peptides from oat protein, Journal of Functional Foods, 87, 104829.

# of scientific presentations, posters, and abstracts: 3

Kwok, E., Yang, J., Taheri, P., Chen, L. (2024) Impact of Ultrasound on Nutritional Quality and Functional Properties of Oat Milk, Canadian Protein and Lipids Conference. September 25-28, Ottawa, Canada (Oral presentation).

Nastaran, M., Chen, L., Richard, C. (2023) Antidiabetic effects of oat protein hydrolysates, Sustainable Protein Research Symposium, June 20-21, Winnipeg, MB, Canada. (Poster)

Kwok, E., Yang, J., Chen, L. (2023) The effect of ultrasound treatment on oat milk development, Sustainable Protein Research Symposium, June 20-21, Winnipeg, MB, Canada. (Poster)

# of industry communications: 4

Chen, L. (2021) The Oat & Oat-Based Research Plan: From Crop to Ingredients, Research Conference Plant-based Foods & Protein American 2021, October 19-10 (Virtual).

Yang, J., Chen, L. (2021) The Oat research and development, Research Conference Plant-based Foods & Protein American 2021, October 19-10 (Virtual).

Chen, L. (2021) Oat gelling properties and applications, 1st Food Oat Conference, June 8-9 (Virtual)

Yang, J., Chen, L. (2021) The oat research in Canada, Research Conference Plant-Based Foods & Proteins Europe 2021, May 20-21 (Virtual).

Supplementary documents

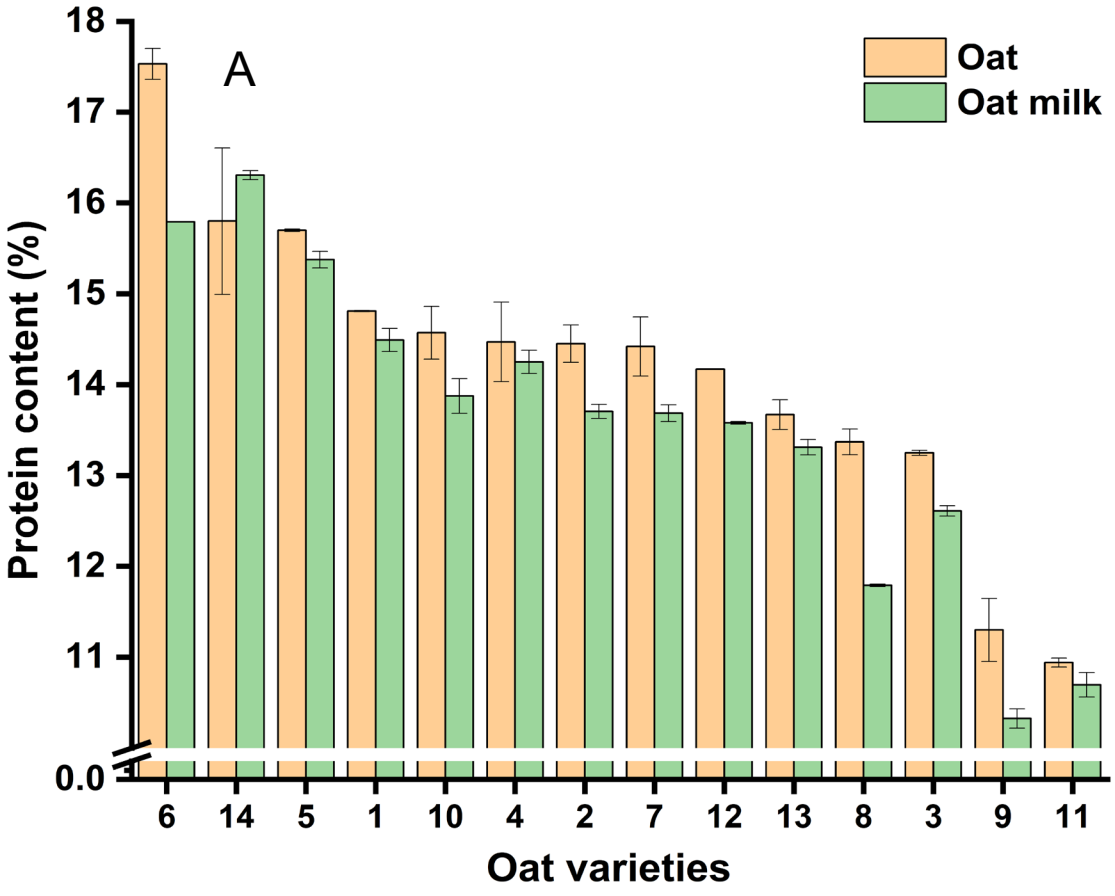
2021G011R "Development of a nutritionally enhanced plant-based milk alternative beverage from Canadian oats and study of its glycemia-lowering effect"

Figures and Tables

Objective-1:

Table 1.1 Oat varieties tested in this study

AAC Douglas	AC Morgan	CDC Endure	CS Camden	Kyron	HOT 602	ORe 3541M
ORe 3542M	ORe level 48	ORe level 50	ORe 6251M	OT 2129	OT 3112	Summit



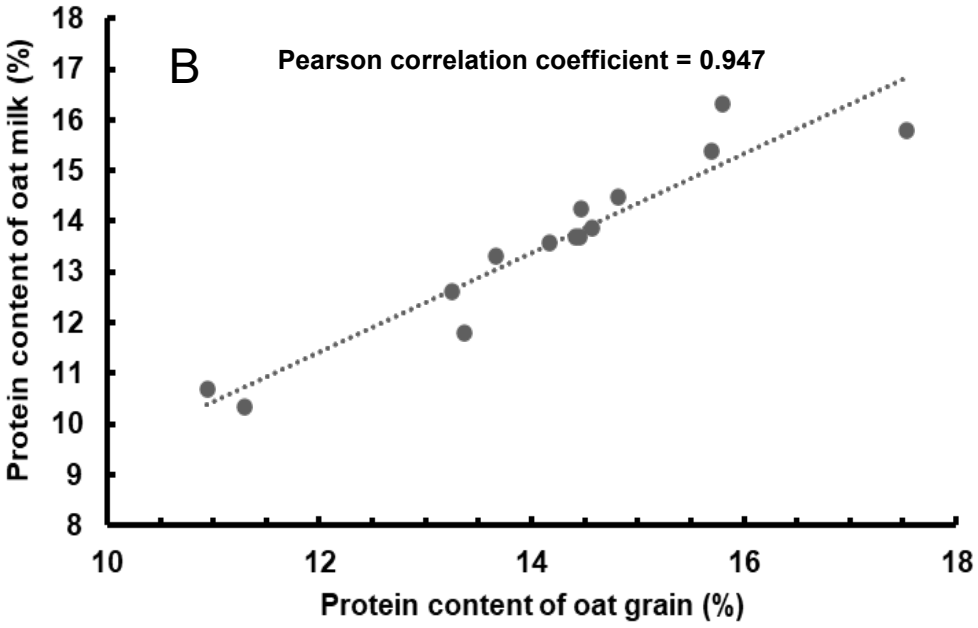


Figure 1-1. Protein content in oat grain and oat milk from different varieties (A); and the correction of the protein content in the oat grain and the protein content in the oat milk products

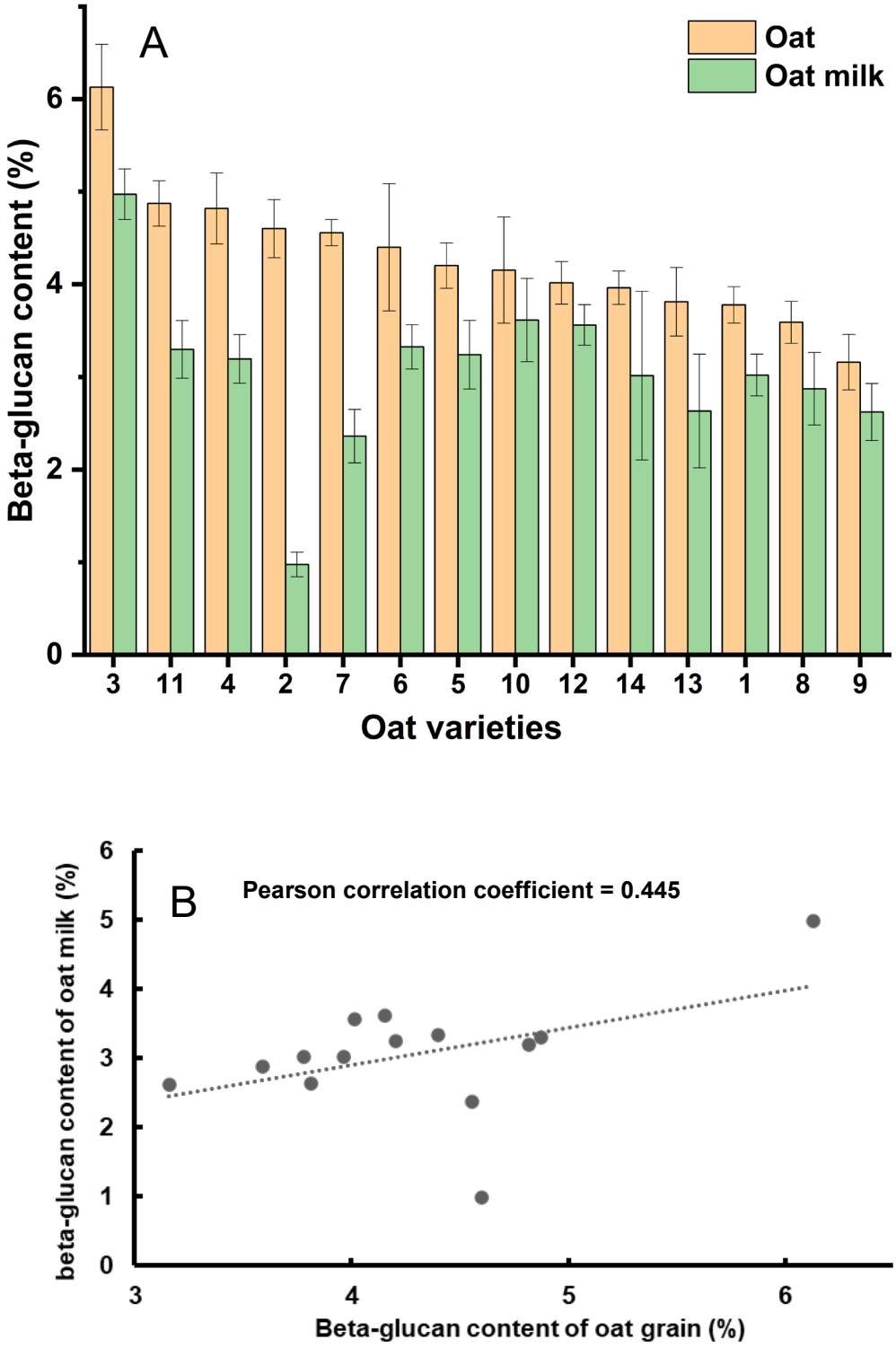


Figure 1-2. Beta-glucan content in oat grain and oat milk from different varieties (A); and the correction of the β-glucan content in the oat grain and the protein content in the oat milk products

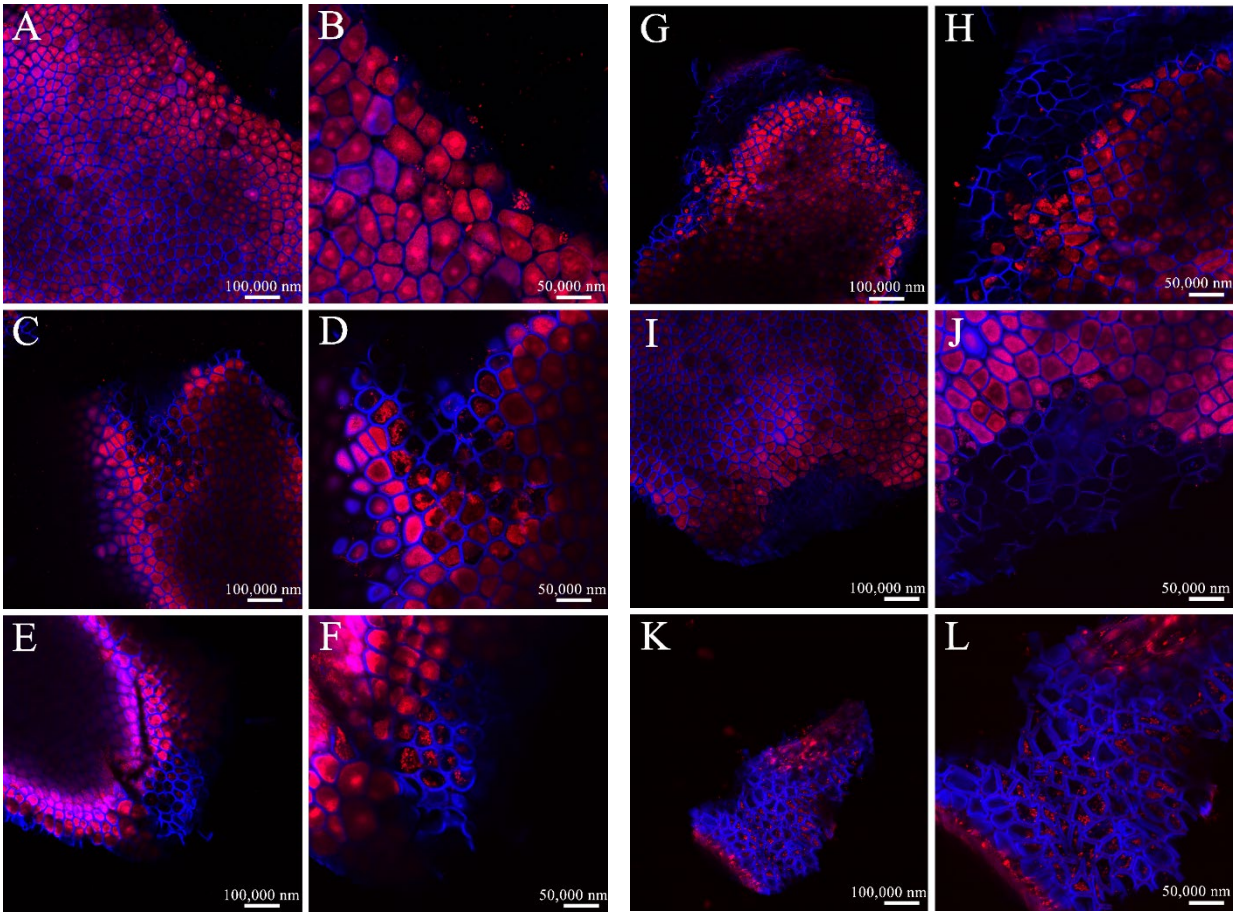


Figure 1-3. Confocal micrographs of oat milk residues undergo different ultrasound conditions. (A,B) Control; (C,D) 60 W for 9 min; (E,F) 240W for 1 min; (G,H) 240 W for 5 min; (I,J) 960W for 1 min; (K,L) 960 W for 9 min.

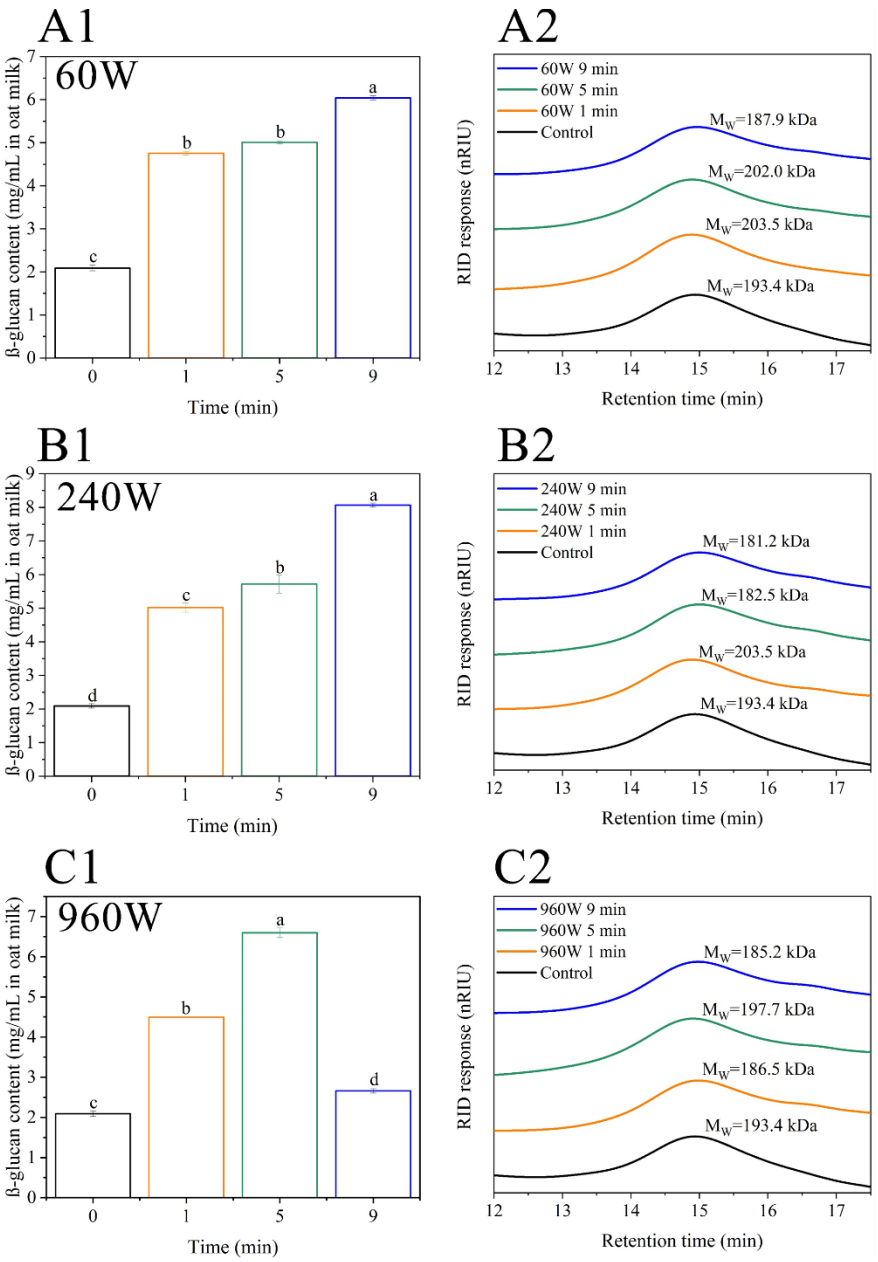


Figure 1-4. Effect of ultrasound treatment (60, 240, 960 W for 1, 5, and 9 min) on β-glucan content (A1 – C1) and M_w (A2 – C2) in the oat milks . Control indicated no ultrasound treatment. Different letters above column indicate significant differences (p <0.05).

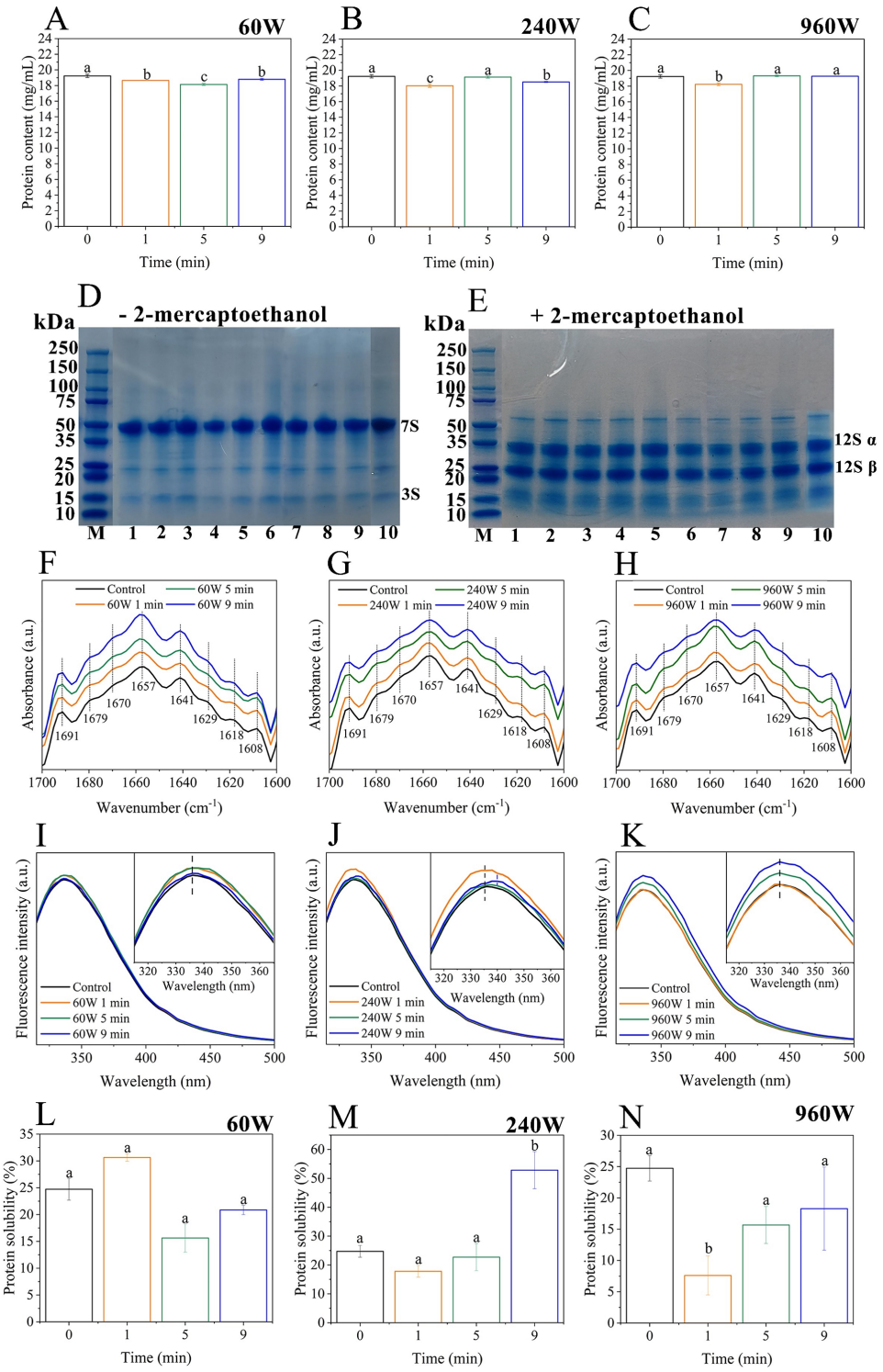


Figure 1-5. Effect of ultrasound treatment (60, 240, 960 W for 1, 5, and 9 min) on the (A–C) protein content in the oat milk, as well as (D–E) the SDS-Page profiles (Marker, M; Control, lane 1; 60W – 1 min, lane 2 ; 60 W – 5 min, lane 3;60 W – 9 min, lane 4; 240 W – 1 min, lane 5; 240 W – 5 min, lane 6; 240 W – 9 min, lane 7;960 W – 1 min, lane 8; 960 W – 5 min, lane 9; 960 W – 9 min, lane 10) , (F–H) secondary structure, (I–K) tertiary structure, and (L–M) protein solubility. Different lower-case letters denote significant differences (p<0.05).

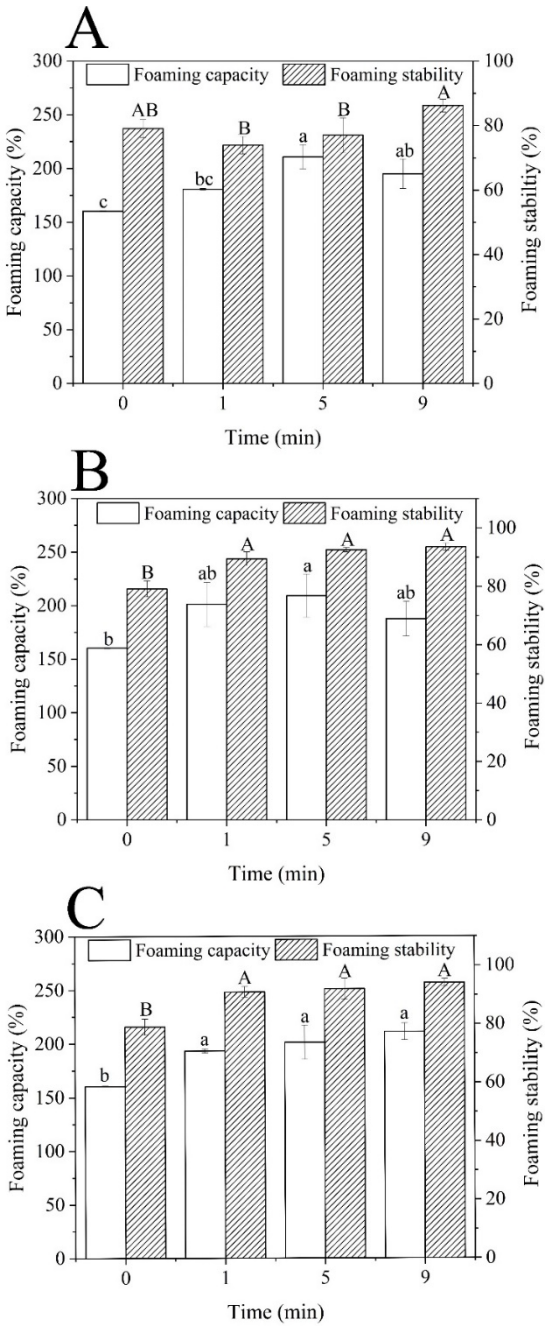


Figure 1-6. The impact of ultrasound on the foaming properties of oat milk at different ultrasound conditions A) 60 W, B) 240 W and C) 960 W for 1, 5, and 9 min. Control represented no ultrasound treatment. Different letters above column indicate significant differences in the same characteristic (p <0.05).

Objective-2

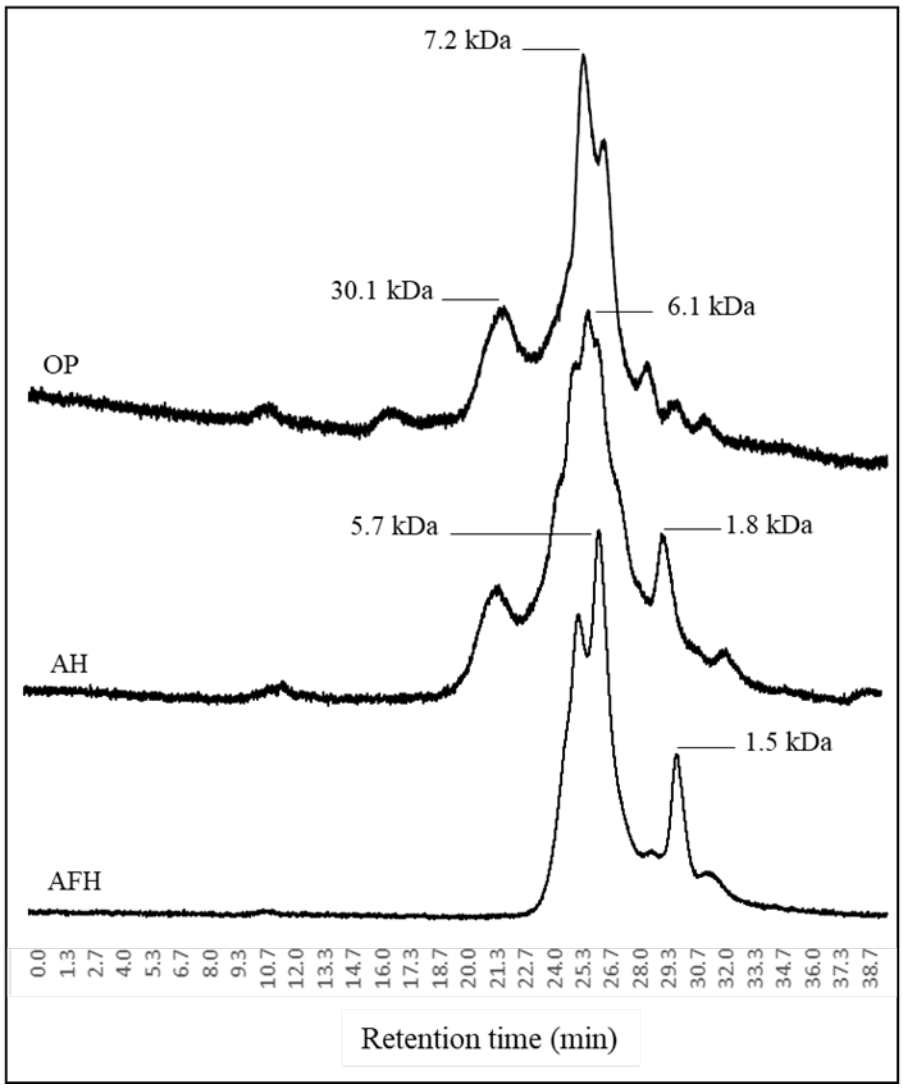


Figure 2-1. Size exclusion chromatogram (UV wavelength 280 nm) of oat protein and oat protein alcalase (AH) and alcalase-flavourzyme (AFH) hydrolysates. MW of oat protein and hydrolysates were obtained using a protein standard mix containing Ribonuclease A, Thyroglobulin, γ-Globulin, Albumin and p-aminobenzoic acid markers to calculate their log MW and respective elution times ($R^2 = 0.98$).

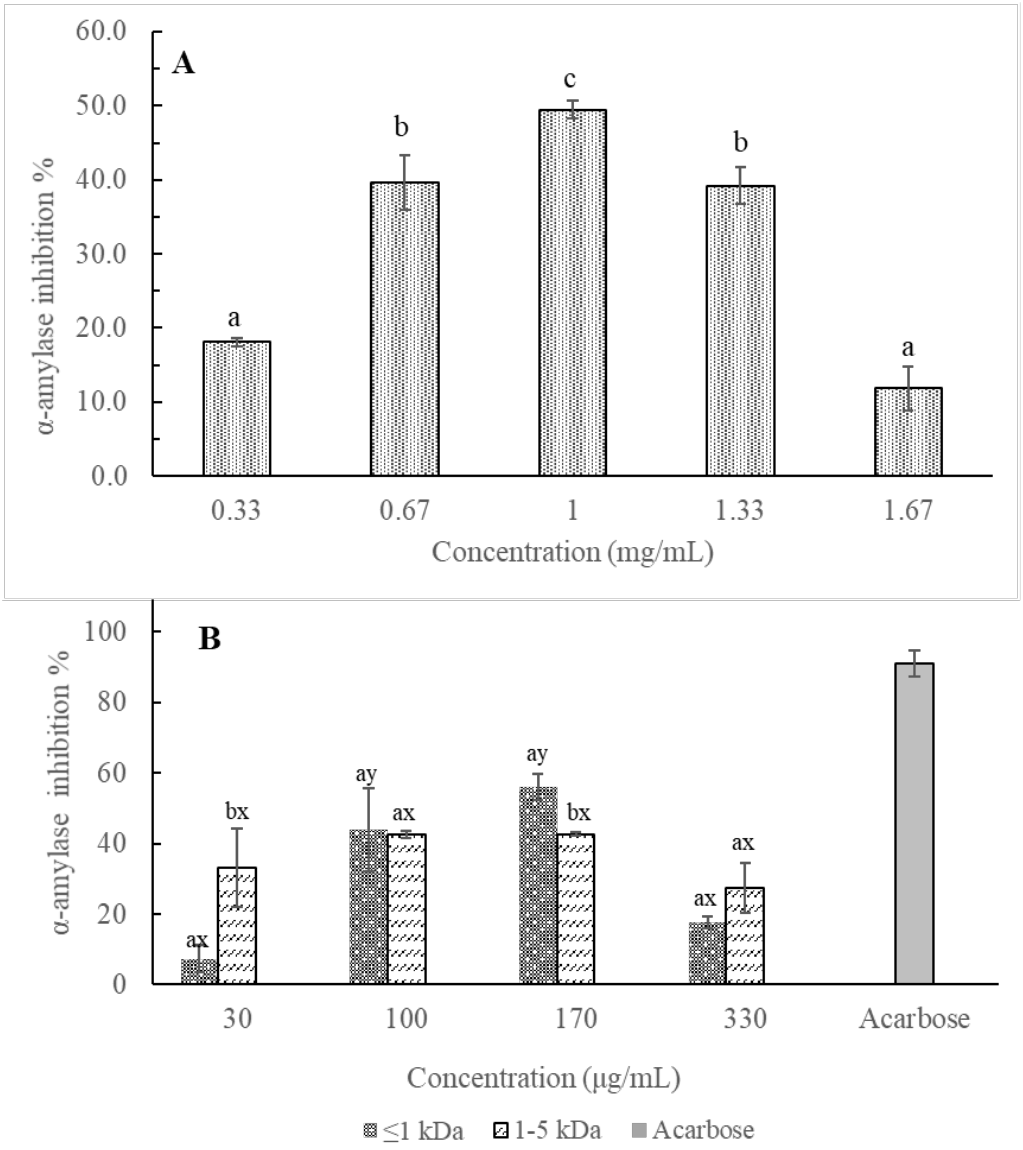


Figure 2-2. (A) Inhibitory effect of α-amylase by alcalase hydrolysate (AH) at different concentrations. Different letters on top of the bars represent a significant difference between doses ($p < 0.05$). (B) Inhibitory effect of α-amylase by peptide fractions from alcalase-flavourzyme hydrolysate (AFH) at different concentrations compared to the control, acarbose at 30 µg/mL. Different letters (a-c) on top of the bars represent a significant difference between groups at the same concentrations ($p < 0.05$). Different letters (x-z) on top of the bars represent significant difference between the same fractions at various concentrations ($p < 0.05$).

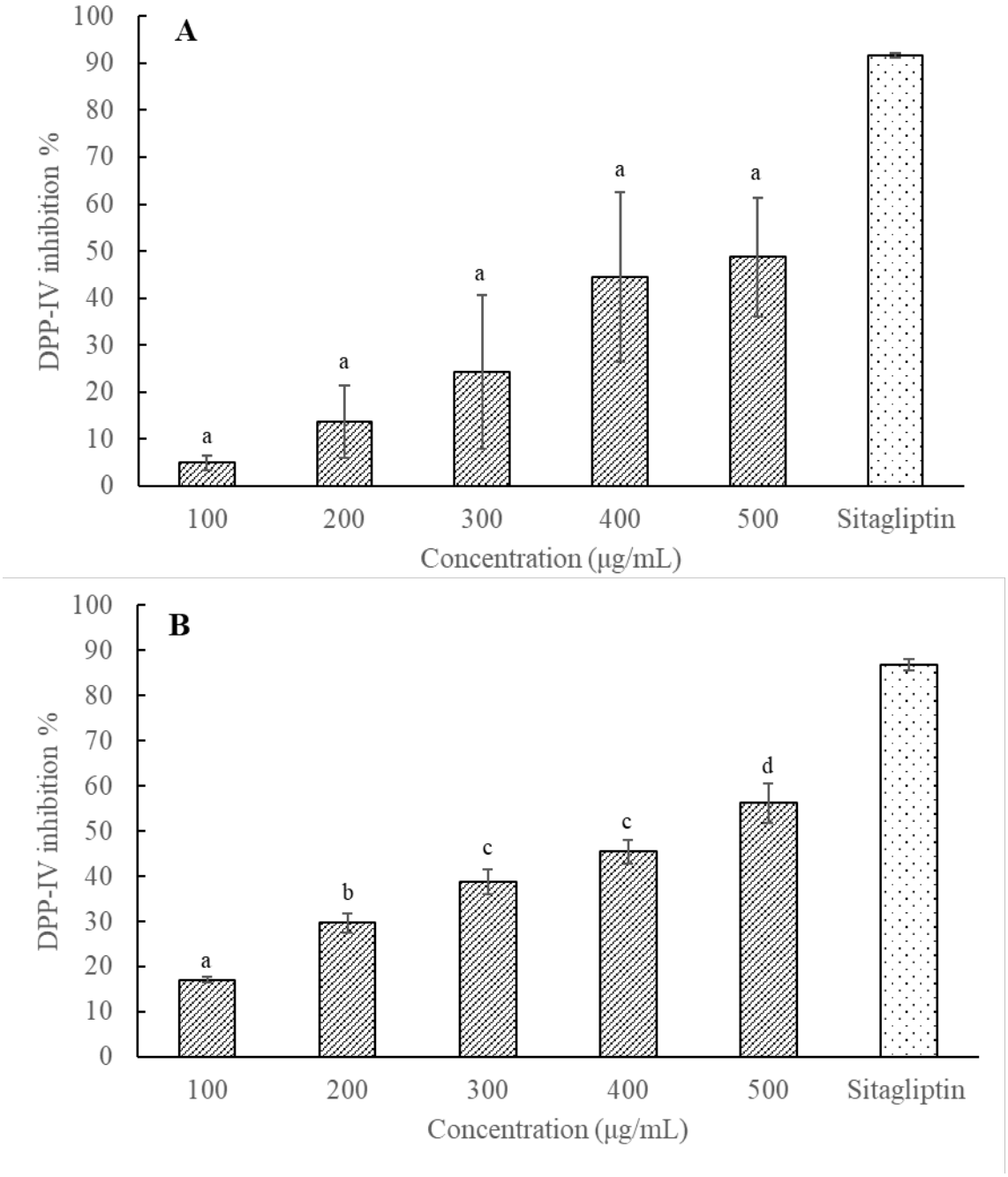


Figure 2-3. Oat alcalase hydrolysate (AH) (A) and alcalase-flavourzyme hydrolysate (AFH) 1-5 kDa fraction (B) concentration dependent inhibitory effect of DPP-IV. Different letters on top of the bars represent significant difference between concentrations ($p < 0.05$).

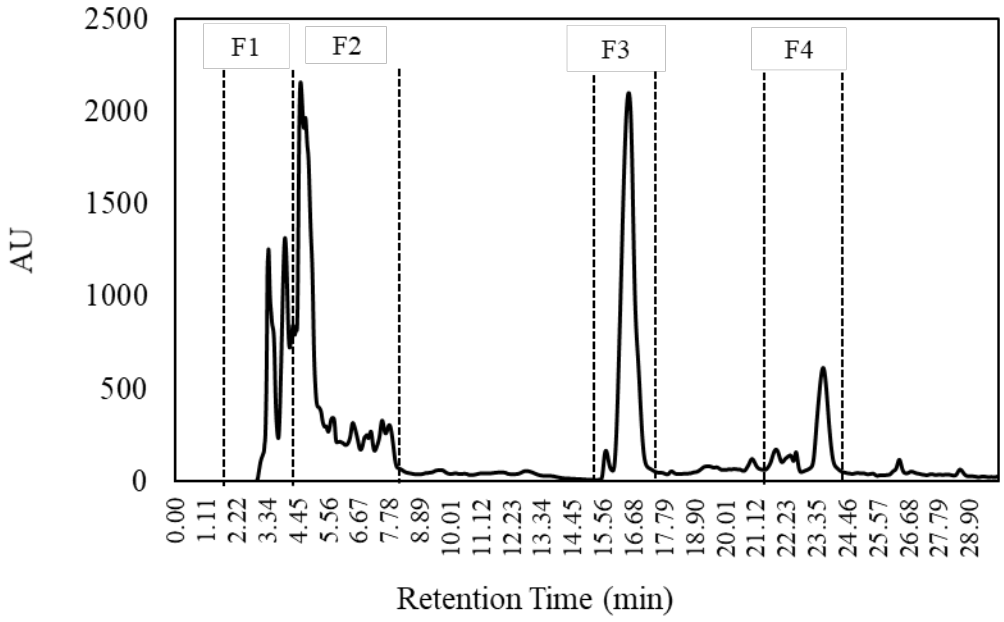


Figure 2-4. Reverse phase chromatogram of four fractions obtained from AFH sample of 1-5 kDa.

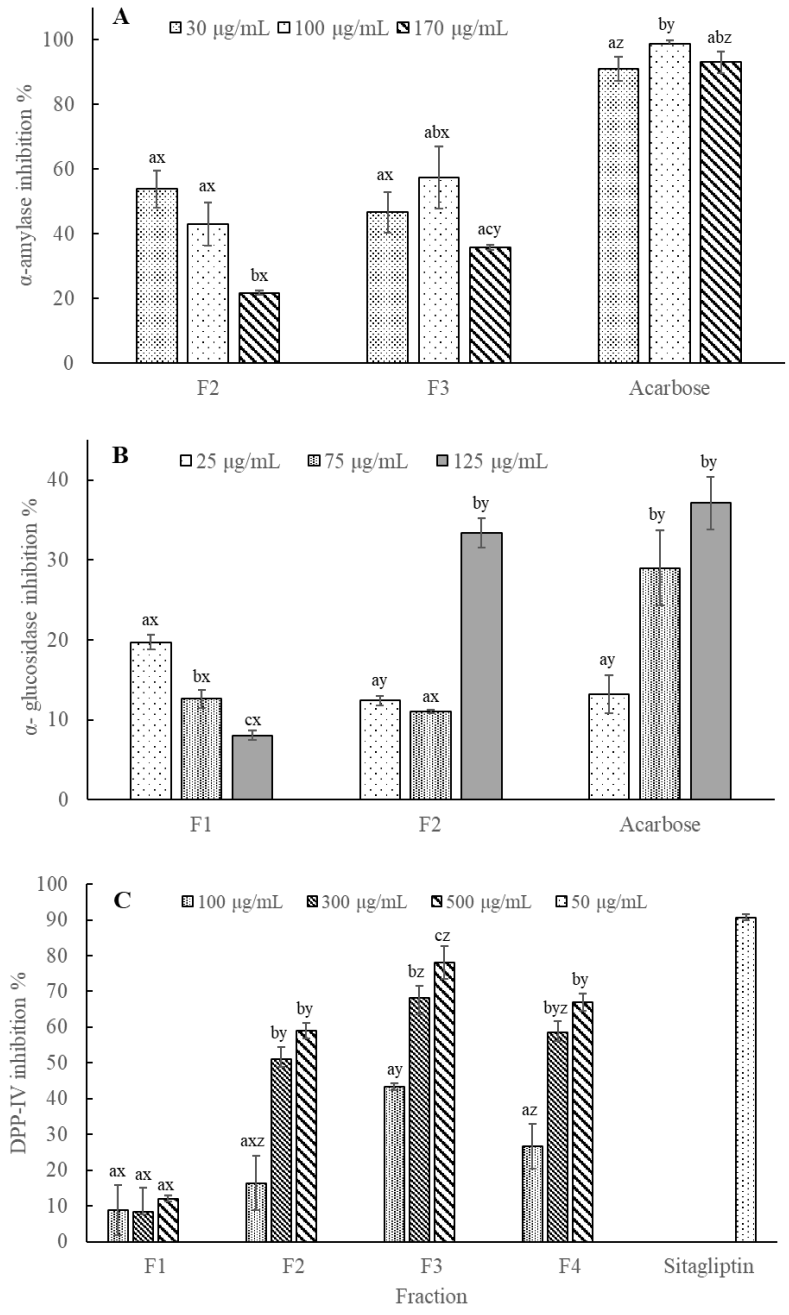


Figure 2-5. α-Amylase inhibition by fraction 2 (F2), fraction 3 (F3), and the control, acarbose (A); α-glucosidase inhibition by fraction 1 (F1), fraction 2 (F2), and acarbose after 10 min incubation (B); DPP-IV inhibitory effect by fraction 1 (F1), fraction 2 (F2), fraction 3 (F3), fraction 4 (F4), and the control, sitagliptin. Different letters (a-c) on top of the bars represent significant difference between concentrations of the same fraction ($p < 0.05$). Different letters (x-z) on top of the bars represent a significant difference between fractions at the same concentrations ($p < 0.05$)

Table 2-1. Identification of amino acid sequences from the most potent oat peptide fractions by LC-MS/MS analysis with average local confidence > 90%.

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

Objective-3

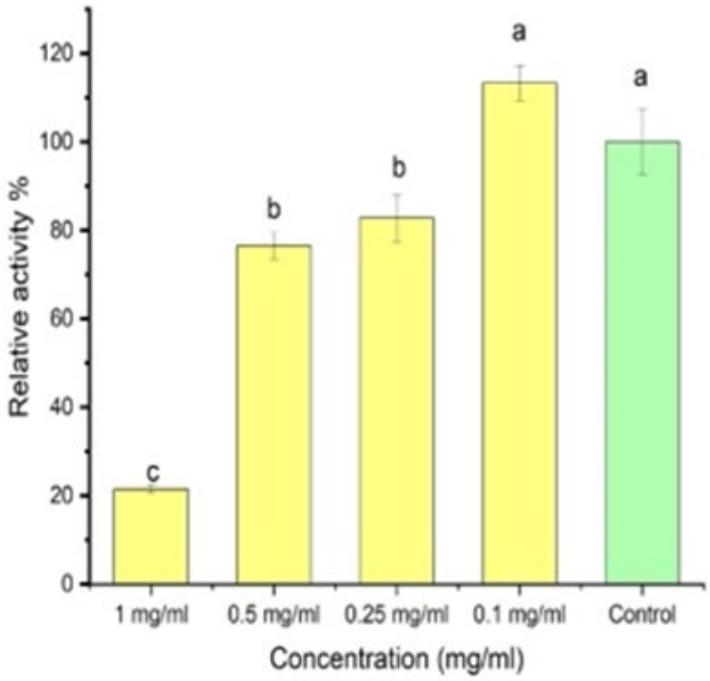
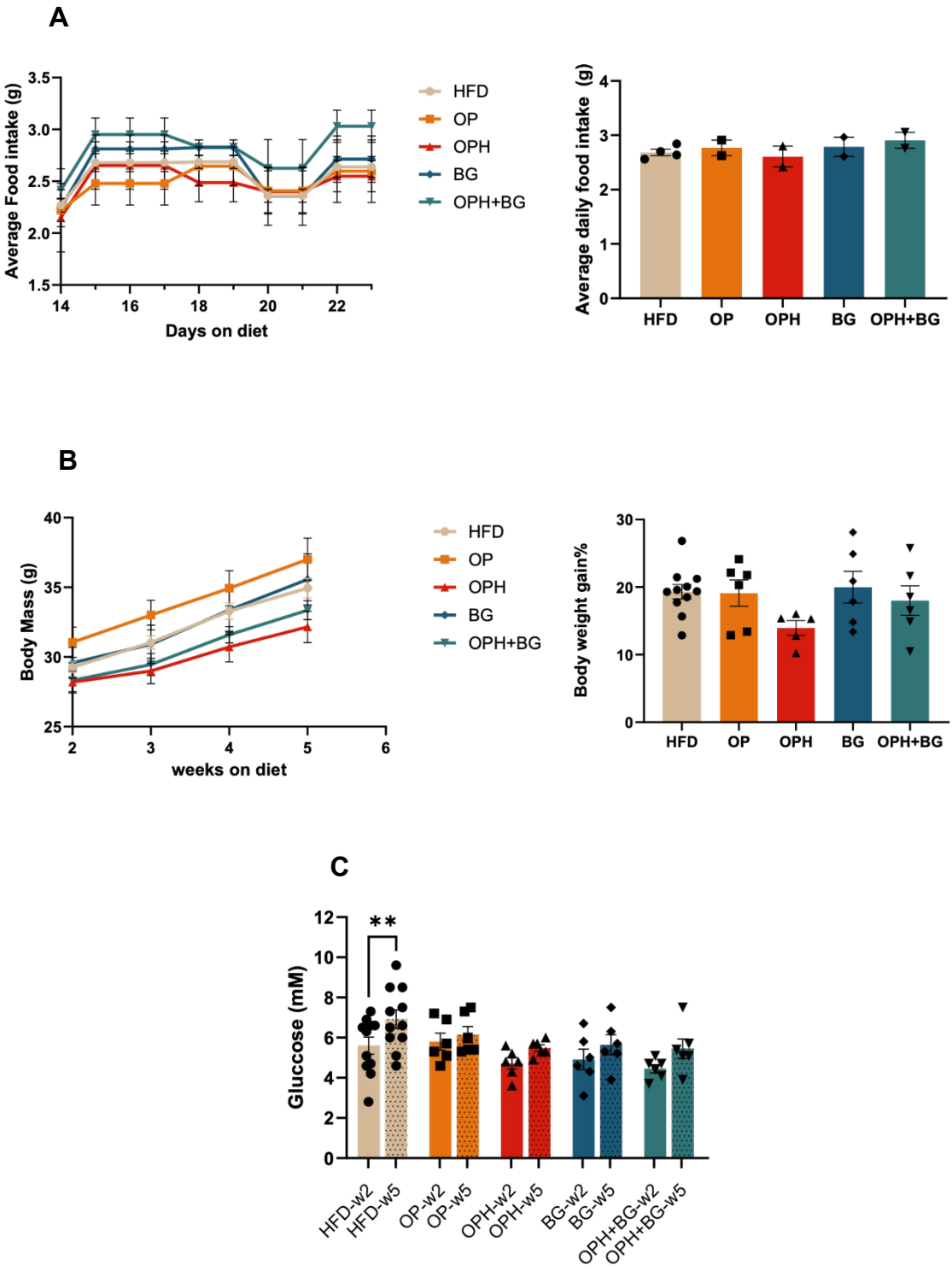


Figure 3-1 Effects of OPH on DPP-4 inhibition (data present the relative activity of DPP4). Different letters (a-c) above the bars indicate a statistically significant difference between groups ($p<0.05$).


A
Average Food intake (g)
Days on diet
HFD
OP
OPH
BG
OPH+BG
Average daily food intake (g)
B
Body Mass (g)
weeks on diet
Body weight gain%
C
Gluccose (mM)
**
HFD-w2
HFD-w5
OP-w2
OP-w5
OPH-w2
OPH-w5
BG-w2
BG-w5
OPH+BG-w2
OPH+BG-w5

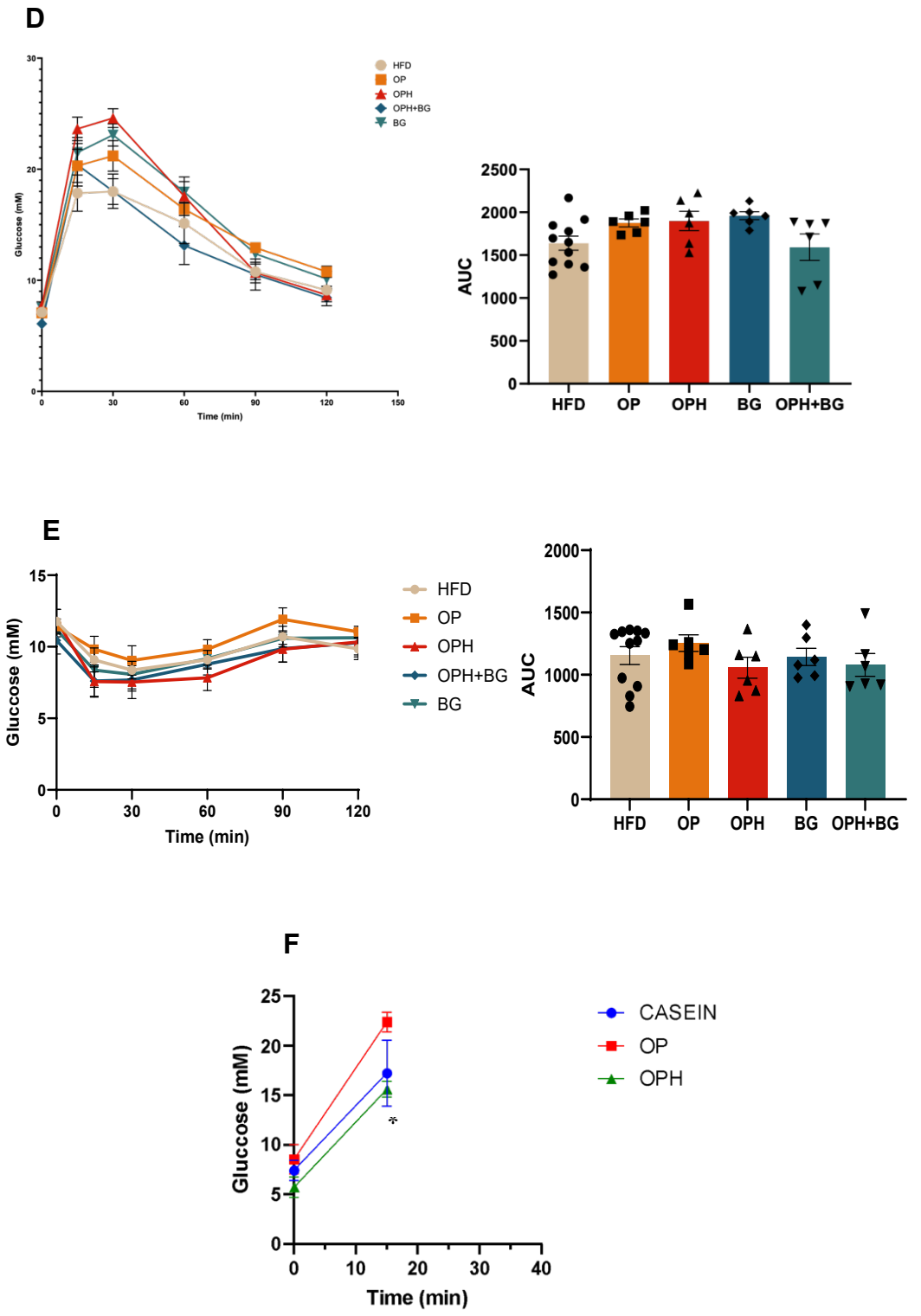


Figure 3-2 Effects of different experimental diet on A) food intake, B) Body weight and body weight gain percentage, C) FBG levels at week2 and week5, D) OGTT test and area under the curve (AUC), E) ITT test and area under the curve (AUC), and F) MTT test. The asterisk (*) above the bars indicates a statistically significant difference between the groups ($p<0.05$).