MODULATING GLYCEMIC RESPONSE THROUGH THE INHIBITION FUNCTION OF POTATO PEELS ON STARCH DIGESTIVE ENZYMES

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ABSTRACT

Potato peel waste is a significant financial burden to the potato processing industry. Currently, this waste is primary used as compost. However, potato peels contain several functional molecules that have the potential to modulate the post-prandial glycemic response by inhibiting starch degrading enzymes. The objectives of this study were to 1) investigate industrial potato peel waste for inhibition of α-amylase and mucosal α-glucosidase, 2) quantify and characterize the inhibitors, and 3) determine the impacts of potato variety and peeling methods on inhibition. Potato peels were extracted with water and analyzed for in vitro inhibition of α-amylase or mucosal α-glucosidase. All extracts inhibited both enzymes in a dose-dependent manner by a mix inhibition mechanism. A rat feeding study verified the impact of potato peel extract consumption on the glycemic response. Starch digestion, following consumption of potato peel extract, delayed the serum glucose spike from 30 to 60 mins. Five potato varieties, common to the United States Pacific Northwest, and grown during two consecutive years were analyzed for concentrations of pectin, pectic acids, minerals, and calystegine by HPLC, ICP-AES and uranic acid assay. Potato peel waste from two commercial processors were analyzed for modulating of the glycemic response. Inhibition correlated with inhibitor concentration *(r* = 0.74*).* Phenolic acids, pectin, minerals and calystegine were major inhibitors in the potato peel extract. Four phenolic acids were identified in the potato peel extract and associated with both α-amylase (*r* = 0.62) and mucosal α-glucosidase (*r* = 0.50) inhibition. Pectin also inhibited α-amylase at a dose of 5.6 mg/mL. However, the concentration of pectin in the potato peel extract was between 160.7 µg/mL and 916.3 µg/mL and not sufficient to inhibit α-amylase activity. The total copper, zinc, and nickel ranged from 1.8 µg/mL to 20.2 µg/mL potato extract, levels known to influence in the hydrolytic activity of both α-amylase and mucosal α-glucosidase. Four isoforms of calystegine were identified in the potato peel extracts with concentrations from 4.2 µg/mL to 20.2 µg/mL. Among the four isoforms, A_3 and B_2 are known to strongly inhibit mucosal α-glucosidase. The inhibition of the glycemic response by peel extracts from hand, steam, and abrasion peeling methods were investigated. The steam peeling preserved the highest inhibition power among the three methods (*p <.000*). Variations among the varieties were observed, and Ranger Russet had the highest inhibition power among the five varieties. The growing environment had a significant impact on the inhibition power, which the

potatoes grown in 2016 and 2017 with different growing temperatures had significant differences in their inhibition power *(p =.003*). These findings support the hypothesis that potato peel wastes generated by potato processors in the United States Pacific Northwest have potential as a natural food ingredient to modulate the post-priandial glycemic response.

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DEDICATION

To my parents and grandparents

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CHAPTER 1

MODULATING GLYCEMIC RESPONSE THROUGH THE INHIBITION FUNCTION OF POTATO PEELS ON STARCH DIGESTIVE ENZYMES

1.1 ABSTRACT

Type II diabetes and obesity are critical health concerns and are known to be associated with the high amount of rapid generation of dietary glucose, which is the product of starch digestion. Potato peels, as industrial waste, contain some molecules (i.e., calystegine) with the ability to interfere with the digestion of starch degrading enzymes in humans, therefore, can delay starch digestion and dietary glucose generation. The objective of the study was to examine the inhibition of potato peels on starch digestion enzymes and understand the inhibition mechanism in order to develop a food approach strategy for modulating the postprandial glycemic response. Potato peels were collected from the potato processors in Idaho and were dehydrated and ground. Our preliminary study showed that the water-soluble components of potato peels have significant influence in starch digestion enzymes, therefore, was used as the materials for the inhibition study. For examining the influence of potato peel extracts in two starch degrading enzymes, α-amylase and mucosal α-glucosidase, soluble potato starch and maltose were used as the substrate of two enzymes in an in vitro system. Reaction velocity plots and Lineweaver-Burk plots were constructed to study the mechanism of inhibition. The primary inhibitors existing in potato peel extracts were identified, which included phenolic acids, minerals, pectin, and calystegine. We quantified the primary inhibitors and applied multiple linear regression analysis to identify the significance of inhibition. The in vitro studies demonstrated the significant inhibition power of potato peel extract on both α-amylase and mucosal α-glucosidase with a non-competitive and a mixed inhibition mechanism, respectively. For the non-competitive inhibitor on α-amylase, phenolic acids were the primary inhibitor; for the mixed inhibition on mucosal α-glucosidase, phenolic acids were the primary non-competitive inhibitor while calystegine was the primary competitive inhibitor. Pectin, copper, and nickel did not show significant correlation with inhibition in our study because the quantity existing in potato peels is not high enough to

interfere with starch digestion. The in vivo study performed with healthy rats showed that the potato peel extracts delayed a postprandial glucose spike from 30 min to 60 min. Our findings suggest potato peel waste has the potential to be used as a functional ingredient or supplement to modulate postprandial glycemic response.

1.2 INTRODUCTION

Type II diabetes, which affects 442 million people in the world and 25.9 million people in the United States in 2011 has become a major worldwide health concern (Smyth & Heron, 2006). Obesity is one of the key independent factors related to Type II diabetes, and over 90% of the population with Type II diabetes are obese (Smyth & Heron, 2006). A cause of obesity is the imbalance between energy intake and expenditure; thus, the control of diets and exercise are two important ways in preventing obesity (Hall et al., 2011). Starch is the primary source of the important energy dietary glucose, and starch digestion in humans relies on two types of enzymes, α-amylase and mucosal α-glucosidase. Therefore, manipulating the digestion process of starch and the digestion capacity of two types of enzymes are promising strategies in controlling the generation of glucose and the prevention of the development of obesity and Type II diabetes. Modifying starch, such as using heat treatment to alter starch structure can decrease the susceptibility of starch to starch degrading enzymes and manipulate the glycemic response. (Chung, Liu, & Hoover, 2009). Diets containing high amount of dietary fiber can increase gastric viscosity and decrease glucose absorption (Brennan, 2005; Lehmann & Robin, 2007). The reduction of hepatic glucose production, the enhancement of insulin secretion, and the manipulation of lipid metabolism could also affect postprandial glycemic response (Moller, 2001).

The direct inhibition of starch digestive enzymes is an alternative way of manipulating the postprandial glycemic response. For example, FDA approved three synthesized enzyme inhibitors including acarbose (also known as Precose, Bayer Pharmaceuticals LLC, Leverkusen, Germany), miglitol (known as Glyset, Pfizer Inc., New York City, NY), and voglibose (known as Voglib, Takeda Pharmaceutical Company Ltd, Osaka, Japan), which all interfere with the glucogenic activity of mucosal α-glucosidase through competitive inhibition (Chiasson et al., 2002). Acarbose, has an inhibition constant (Ki) of 9.9×10^{-7} M against

mucosal α-glucosidase (Samulitis, Goda, Lee, & Koldovský, 1987). In addition, acarbose can interfere with the hydrolytic activity of pancreatic α-amylase through non-competitive inhibition (Samulitis et al., 1987).

In addition to the synthesized inhibitors, many plants and fungi contain functional substances that have starch digestive enzyme inhibition activities. Rosmarinic acid, contained in the families of *Boraginaceae* and *Lamiaceae*, was found to have a non-competitive inhibition against the hydrolytic activity of pancreatic α-amylase (McCue & Shetty, 2004). A protein-based α-amylase inhibitor, α-A1, was discovered in kidney beans, which specifically inhibits pancreatic α-amylase through a non-competitive inhibition mechanism (Moreno, Altabella, & Chrispeels, 1990). For mucosal α-glucosidase inhibition, the aqueous extract of *Rhus chinensis* is presumed to inhibit mucosal α-glucosidase through non- competitive inhibition with an IC50 of 0.9 mg/mL (Shim et al., 2003). *Psidium guajava* leaf extract was found to have a mixed inhibition mechanism, of both competitive and non- competitive inhibition, against mucosal α-glucosidase with an IC_{50} of 1.0 g/L (B. Wang, Liu, Hong, Li, & Huang, 2007). Many plant extracts were found to have inhibition activities on both α-amylase and mucosal α-glucosidase activities. Clover contains luteolin, which had both α-amylase and α-glucosidase inhibitory activities (Kim, Kwon, & SoN, 2000). The methanolic extract of *Artocarpus heterophyllus* was shown to contain different kinds of phenolic compounds that inhibited both α-amylase and α-glucosidase inhibition activities (Nair, Kavrekar, & Mishra, 2013). Anthocyanins and ellagitannins, which can be found in raspberries and strawberries, have also been reported to inhibit both α-amylase and mucosal α-glucosidase activities through non- competitive inhibition (Mcdougall & Stewart, 2005).

Some functional molecules of potato peels can also interfere with the hydrolytic activity of either α-amylase or mucosal α-glucosidase. Calystegine, which is a nortropane alkaloid, is another functional molecule abundant in potato peels that have shown to inhibit mucosal α-glucosidase through competitive inhibition (Jocković, Fischer, Brandsch, Brandt, & Dräger, 2013). Phenolic acids, which are abundant in some potatoes, have a non-competitive inhibition on both α-amylase and mucosal α-glucosidase, which was, on average, ten times more concentrated in potato peels than potato flesh (Mäder, Rawel, & Kroh, 2009). Pectin, which is the primary water-soluble dietary fiber in potatoes, has never been reported to interfere with starch degrading enzymes either through the interaction with enzymes or

through a competition with starch molecules to bind with starch degrading enzymes. However, dietary fiber is known to be a physical barrier for the enzymatic digestion of starch. Other potential influences of starch digestion are minerals such as copper and nickel, which were reported to have the ability to interfere with mucosal α-glucosidase (Wang et al., 2004). In this study, the objective is to prove the concept that potato peels can interfere with starch digestive enzymes and manipulate the postprandial glycemic response. In addition, we examine the mechanism of inhibition and quantify the primary inhibitors in potato peels.

1.3 MATERIALS AND METHOD

1.3.1 Materials

Twelve potato varieties, which are popular for processing (i.e., producing frozen French fries) or fresh-used in the Northwest of the United States, were planted at the Parma Research and Extension Center at the University of Idaho (Parma, Idaho) in 2016 and 2017. All chemicals, reagents, and rat intestine acetone powder were purchased from Sigma-Aldrich Corp. (St. Louis, MO) unless specified otherwise. Maltose was purchased from Tokyo Chemical Industry (Tokyo, Japan). Phosphate buffered saline tablets, caffeic acid, and catechin were purchased from VWR International (Rendor, PA). Sulfuric acid and formic acid were purchased from T.J. Baker Chemical Company (Rendor, PA). Ferulic acid, methanol, and acetonitrile were purchased from Fisher Scientific Inc. (Huston, TX). Calystegine A3 and B2 were purchased from ChemFaces Inc. (Wuhan, Hubei province, China). Sieves were purchased from VWR International (Radnor, PA). Syringe filters with various membrane sizes, made of polytetrafluoroethylene (PTFE), were purchased from GE Healthcare (Chicago, IL) and Thermal Scientific Inc. (Rockwood, TN). The examinations and analyses described below were conducted in a laboratory with a humidity level of 10 ± 2 % and at room temperature in the range of 20 °C to 22 °C. The research was conducted at a location with atmospheric pressure at 29.68 Hg and an elevation of 2709 feet above sea level; thus, the temperature of the boiling water used in this study was 97.09 °C.

1.3.2 Potato peel extraction

Potato peels used in this study were obtained from J.R. Simplot (Caldwell, ID) and Parma Research and Extension Center at the University of Idaho (Parma, ID). The peels from J.R. Simplot were collected from their French fries production lines to represent the primary type of potato peel waste generated in the Northwest of the United States. Another common type of potato peel waste was studies and reported in the Chapter 2. The peels from J.R. Simplot were steam-peeled, immediately froze upon collection and shipped, with dry ice, to the laboratory located at the University of Idaho (Moscow, ID). The industrial peel wastes were mixed peels of various varieties including Russet Burbank, which is the primary potato cultivar used in the region. The twelve potato varieties were planted at the Parma Research and Extension Center in 2016 and the ten potato varieties were planted in 2017, were washed and then hand-peeled with a kitchen peeler (Farberware, Vallejo, CA). Some of the varities planted in 2016 were not available in 2017 that led to the difference of varieties in two years.

All of the peels, including industrial peel waste, were freeze-dried, ground with a coffee grinder (Krups, model F203; Solingen, Germany), passed through a sieve with a pore size of 0.04 mm, and then, stored in a freezer (-20°C) for further analyses.

Water was uesed to extract the enzyme inhibitors from potato peel powders according to our prelimary study (data not shown), which we found the water-soluble components had a significant inhibition power on starch digestive enzyme activities. We weighed 800 mg potato peel powder into a 15-mL centrifuge tube and suspended it with 20 mL deionized water with gentle stirring at room temperature for 30 min. The suspension was centrifuged at 4,000 ×g at room temperature for 10 min, and the supernatant was further filtered through a syringe filter with a 5-µm pore-size. Potato peel extracts were freeze-dried, weighed, and then solubilized with phosphate-buffered saline (137 mM sodium chloride, 2.7 mM potassium chloride, and 10 mM phosphate buffer, pH 7.4, prepared by dissolving one tablet of PBS saline buffer into 100 mL of deionized water) prior to the final concentration of 18 mg/mL (concentration of the water-soluble portion of potato peel extract). Each potato peel was extracted once.

1.3.3 α-Amylase and mucosal α-glucosidase extraction and purification

α-Amylase and mucosal α-glucosidase were extracted from rat intestine acetone powder as described in Toda, Kawabata, and Kasai (2001) with modifications. Rat intestine acetone powder (40 mg/mL) was homogenized in 0.1 M potassium phosphate buffer containing 5 mM Ethylenediaminetetraacetic acid (EDTA) and 1% Triton X-100 at 4 °C. The homogenate was centrifuged with a Sorvall RC-5B superspeed centrifuge at 15,000 *g* at 4 °C for 40 minutes. The supernatant was collected and dialyzed with a Pur-A-Lyzer mega dialysis kit (10 kDa weight-cut, Sigma Aldrich, St. Louis, MO) against 0.05 M potassium phosphate buffer and 1.5 mM EDTA for 12 hours at 4 °C. The dialyzed sample was filtered and concentrated by a membrane filter unit (100 kDa weight-cut, Millipore, Billerica, MA). The concentrated fraction, which contained mucosal α-glucosidase, and the filtrate, which contained α-amylase, were loaded separately into a gel permeation column packed with Sephadex G200 resin (2.5 × 50 cm, GE Healthcare Life Sciences, Marlborough, MA) and were eluted with 0.01M potassium phosphate buffer and 2.5 mM EDTA at a flow rate of 0.6 mL/min. Fractions were collected with 1.8 mL per fraction using a fraction collector FR-920 (GE Healthcare Life Sciences, Marlborough, MA) for a total of 180 minutes.

To identifying the fractions contaning α-glucosidase, the maltase activity of each fractions were examined. Maltose (100 mM) was prepared with phosphate saline buffer (PH7.4). The maltose solution (10 μL) was mixed with each collected fraction (10 μL) and react in a shaking water bath controlled at 37 °C for 30 minutes. The production of glucose was quantified using the a $_{D}$-glucose assay kit (Megazym). The fractions with detectable maltase activity were collected and pulled into a tube, then, concentrated through the membrane filtration (10 kDa weight-cut, Millipore, Billerica, MA). Then, the maltase, isomaltase and sucrase activity of the concentrated extract were quantified using maltose, isomaltose, and sucrose as the substrate at 25 mM in phosphate saline buffer (pH 7.4). The hydrolysis was conducted at 37 °C for 0, 3, 5, 7, and 9 minutes. The enzyme hydrolytic activity was inactivated by heating the mixture of substrates and extracts in a boiling water bath for 10 min, and then, glucose was quantified using a $_{D}$-glucose kit following the manufactor's procedure.

To identifying the fractions contaning α-amylase, we added gelatinized soluble potato starch (0.5% w/v; 10 μL) into each fraction (20 μL) and quantified the reducing power of the

hydrolysates after performing the hydrolysis at 37 °C for 15 min. The analysis of reducing power was performed following Shao and Lin (2018). The fractions with detectable α-amylase activity were pulled into a tube, then, concentrated through membrane filtration (10 kDa weight-cut, Millipore, Billerica, MA). Then, the hydrolytic activity was quantified again using souble potato starch as the substrate as described above.

The purity of α-glucosidase and α-amylase was examined with SDS-PAGE according to the method of Laemmli (1970). Purified α-glucosidase (20 µL), purified α-amylase (20 µL) and rat intestine powder extract (10 µL) were mixed with 3X Laemmli buffer (150 mM Tris-HCl buffer at pH 6.8, 6% SDS, 30% glycerol, 0.06% bromophenol blue) with a 2:1 ratio and heated in a boiling water bath for five minutes. Samples and protein standards (10 – 250 kDa) were loaded into acrylamide gel (6%). Tris-glycine buffer (25 mM Tris-HCl at pH 8.8, 250 mM glycine and 0.01% SDS) was loaded into an electrophoresis chamber. The electrophoresis was carried with Bio-Rad Mini protein electrophoresis at 110V, 0.03 mA for 80 minutes. The gel was stained with Coomassie stain (50 mL) for 60 minutes. The molecular weight of the visible bands in the samples was determined by the comparison with the protein standard.

1.3.4 Inhibition of potato peel extract on α-amylase and mucosal α-glucosidase

The inhibition of potato peel extract on α-amylase and mucosal α-glucosidase were examiend. In this experiement, potato peel extracts were prepared using 12 potato varieties planted in 2016 and ten varieties planted in 2017. The inhibition on α-amylase was investigated using an α-amylase assay kit (McCleary, McNally, Monaghan, & Mugford, 2002). The reaction rates of α-amylase with and without the addition of 9 mg/mL potato peel extract were compared, and the inhibition was represented by inhibition power, which was the percentage of reaction rate decreased with the addition of potato peel extract. The inhibition of potato peel extract on mucosal α-glucosidase was investigated using 25 mM maltose as the substrate. The reaction was incubated at 37 °C for 0, 3, 5, 7 and 9 minutes. The enzyme hydrolytic activity was inactivated by heating with a boiling water bath, and the glucose produced through enzymatic hydrolysis was quantified using a *D*-glucose kit. The reaction rate

of mucosal α-glucosidase with or without the addition of 9 mg/mL potato peel extract were compared and the inhibition was represented by inhibition power.

1.3.5 Inhibition mechanism of potato peel extract on α-amylase and mucosal α-glucosidase

Kinetic parameters of the inhibition of α-amylase and mucosal α-glucosidase was studied by the double-reciprocal-plot method of Lineweaver-Burk plot (Lineweaver & Burk, 1934). In this experiment, the potato peel extract was prepared using the potato peels obtained from the indutry. To examining the inhbition mechansim of potato peel extract on α-amylase, soluble potato starch with five concentrations (1 mg/mL to 5 mg/mL) was used as the substrate, and each treatment was mixed with three different quantities of potato peel extract solution with the concentrations at 0, 4.5 and 9 mg/mL. The hydrolysis was determined based on the increase of reducing power following Shao and Lin (2018). To examining the inhibition mechanism of potato peel exract on mucosal α-glucosidase, maltose with six different concentrations (5 mM - 80 mM), and each treatment was mixed with three different quantities of potato peel extract solution with the concentrations at 0, 4.5, and 9 mg/mL. The hydrolysis was determined absed on the increase of glucose concentration. The maximum velocity (V_{max}) and Michaelis constant (K_m) were calculated from Linerweaver-Burk plots from the y-axis and x-axis intercepts; the maximum velocity is the y-axis intercept of the plot, and the Michaelis constant is the x-axis intercept of the plot.

1.3.6 Inhibition of potato peel extract on maltase-glucoamylase, sucrase, and isomaltase

Mucosal α-glucosidase is comprised of maltase-glucoamylase and sucrase-isomatlase. In order to examine the inhibition on maltase-glucoamylase, a heat treatment was performed to inactivate sucrase-isomaltase as described by Dahlqvist (1959). Purified α-glucosidase was heated in water bath at 62 °C for 15 minutes. Maltase, sucrase and isomaltase activity was measured before and after heat inactivation. To measure the maltase activity of maltase-glucoamylase after heating, 10 µL of potato peel extract (4.5 and 9 mg/mL) was prepared using the peels obtained from the industry and mixed with heat treated α-glucosidase solution (5 µL) and maltose (25 mM, 5 µL). The reaction was kept in 37 °C water bath for 0, 3, 5, 7, and 9 minutes. The reaction was stopped by heating the microtubes in a boiling water bath for

10 minutes. Glucose was quantified using a glucose oxidase/peroxidase kit. The absorbance was read at 510 nm with a microplate reader.

The inhibition of potato peel extract on sucrase-isomaltase subunits of mucosal α-glucosidase was studied using sucrose and isomaltose (25 mM) as the substrates. Three different doses of potato peel extract at 0, 4.5 and 9 mg/mL were used to study inhibition. Glucose was quantified as the product from isomaltose and sucrose hydrolysis by a $_{D}$-glucose kit with the measurement of absorbance at 510 nm. The production of glucose was plotted against reaction time to determine the inhibition of potato peels on sucrase and isomaltase activity.

1.3.7 Quantifying pectin in potato peel extract

Pectin was quantified by a uronic acid assay following Blumenkrantz and Asboe-Hansen (1973). Potato peel extracts-saline buffer solution (18 mg/mL dehydrated potato peel extract, 40 µL) and galacturonic acid solutions with various concentrations (0, 40, 80, 120, 160, 200 µg/mL; 40µL of each) were added into a 96-well microplate. Then, sulphamic acid (4M, 5 µL), prepared by dissolving sulphamic acid crystals in deionized tap water, was added into the wells and mixed with potato peel extracts or galacturonic acid solutions to prevent the interference of neutral sugars on the quantification of galacturonic acid (Filisetti-Cozzi & Carpita, 1991). Acid/borax solution (200 µL), which consisted of 12.5 mM disodium tetraborate borax (4.7 g) in concentrated sulfuric acid (1L, 18 M), was added into the wells containing potato peel extracts, galacturonic acids, and the previously added reagents. The microplate was covered and heated at 95 °C in a convection oven for 60 min and then chilled in an ice bath for 20 min. Diphenyl reagent (2.9 mM, 10 µL), prepared by dissolving 3-phenyl phenol (50 mg) and sodium hydroxide (0.5 g) in deionized tap water (100 mL), was added into those cells and then, the microplate sat at room temperature (20-22 °C) for 5 min to allow for color development. The quantity of galacturonic acid was determined by reading the absorbance at 520 nm using a microplate reader (Spectra Max 190 Microplate Reader, Molecular Devices, CA). The galacturonic acid was applied to this analysis to generate a calibration curve of absorbance and concentration, and the pectin quantity in the potato peel

extracts was calculated based on the calibration curve and presented as µg of galacturonic acid per mL of peel extracts.

1.3.8 Quantifying phenolic acids in potato peel extract

The quantification of chlorogenic, ferulic, caffeic acid, as well as catechin, followed the procedure reported by Singh and Saldaña (2011) with modifications. Potato peel extract-saline phosphate buffer (200 µL) was mixed with methanol (800 µL, HPLC grade) in a microcentrifuge tube (2 mL) and vortexed. Then, this potato peel extract-methanol mixture was centrifuged at 10,000 ×g for 5 min at 4 °C. The supernatant was further filtered through a syringe filter with a 0.45-µm membrane pore size before injection into a High-Performance Liquid Chromatography (HPLC) system, which consisted of Waters 996 HPLC system (Waters Corporation, Milford, MA) equipped with Waters 2489 UV/Vis detector (Waters Corporation, Milford, MA), and a Waters Symmetry C18 column (4.6 x 150 mm, 3.5 µm). The mobile phase consisted of eluent A: 0.5% formic acid (ACS grade) in water (HPLC grade) and eluent B: 0.5% formic acid (ACS grade) in methanol (HPLC grade). The mobile phase was eluted with an eight-step linear gradient starting from 84% eluent A and 16% eluent B; then, eluent B rose to 19% by 15min, to 27% by 25 min, to 41% by 26 min, to 65% by 36 min, and reached 100% of eluent B by 44 min. After reaching 100%, the proportion of eluent B decreased to where it started by 45 min. The total elution time was 60 min with a flow rate at 0.5 mL/min. The loop size was 20 µL, and the signals were detected at 280 nm. The calibration curve was established by injecting six different concentrations of chlorogenic acid, caffeic acid, ferulic acid, and catechin ranging from 0.005 mM to 0.8 mM. The quantification of phenolic acids was determined by plotting the corresponding peak area and superimposing it onto the calibration curve. The total amount of phenolic acids was the sum of the quantity of chlorogenic acid, caffeic acid, ferulic acid, and catechin.

1.3.9 Quantifying calystegine in potato peel extract

Calystegine in potato peel extract was quantified using a high-performance liquid chromatography/tandem mass spectrometry analysis following Petersson et al. (2013). The potato peel extract-saline phosphate buffer (44 uL) was mixed with acetonitrile (HPLC grade,

320 uL) and ammonium acetate (HPLC grade, 80 uL). The sample and calystegine standard (2 uL) were injected into an Agilent LCMS Infinity 1200 (Aligent Technologies Inc., Santa Clara, CA) with a 100 mm X 3.0µm Atlantis Hilic column (Waters Corporation, Milford, MA) and ESI ion source at a flow rate of 0.45 mL/min at 35 ºC. Mobile phase A consisted of 0.02M ammonium acetate (HPLC grade, pH 5.3) in water (HPLC grade), and the mobile phase B consisted of 100% acetonitrile (HPLC grade). The gradient started with 10% A and 90% B, and from 0.5 min to 4.5 min, changed linearly to 60% A and 40 % B. The gradient finally held at 60% A and 40% B for 1 min. The LC system was connected to a 5500 QTRAP tandem mass spectrometer (AB SCIEX, Concord, ON, Canada), equipped with a Turbo VTM ion source operated in positive ion mode. The ion source parameters were as follows: needle voltage 4300 V, curtain gas 275 kPa, nebulizer and Turbo gas 410 kPa, a 600 °C Turbo gas temperature. Delustering potential, collision potential, and collision cell exit potential were optimized during infusion of a mixture of the analytes (10−100 ng/mL) employing the Analyst 1.5 software (AB SCIEX, Concord, ON, Canada). The concentration of calystegine isomers was calculated based on the calibration curve generated by the standards, and the concentration of calystegine isomers were combined and reported as calystegine content.

1.3.10 Quantifying copper, nickel, and zinc in potato peel extract

The mineral content of potato peel extract was quantified using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Potato peel extract-saline phosphate buffer was filtered through a syringe filter with a membrane pore size of 0.45-µm before injecting into an Agilent 7500cx ICP-MScx (Aligent Technologies Inc., Santa Clara, CA). Following a published method, the system was set as follows: nebulizer at 41 psi, an auxiliary flow of 0.20, a pump rate of 1.0 mL/min, a scan integration time of 0.25 s, a Mn peaking wavelength, and acid flexible tubing with 0.030 mm internal diameter (Anderson, 1996). Element calibration standard was prepared at concentrations of 100, 10, 1, and 0.1 ug/mL and injected to generate a calibration curve. The copper, zinc, and nickel in the potato peel extract, which in the literature showed the most significant inhibition on α-amylase and mucosal α-glucosidase (Wang et al., 2004), were quantified and the concentration was combined and represented as total mineral content.

1.3.11 Assessment of the effect of potato peel extract in in vivo starch digestion

Six male Wistar rats (250 g) were acclimatized for seven days after arriving at a certified animal research facility. The rats were fed a balanced rice-based diet and fasted overnight before the feeding study. The control feeding study was conducted with gelatinized maize starch. Maize starch (5% w/v) was gelatinized and mixed with water at a 3:1 ratio. The mixture was fed to rats through oral gavage with the a dosage of 10 mL/kg of body weight. The blood (20 µL) was collected from the tail vein at 0, 30, 60, 90, 120, and 150 minutes after feeding. The blood glucose concentration was measured using an AlphaTRAK 2 glucometer (Zoetis, Parsippany, NJ). The treatment feeding study, with the addition of potato peel extract, was conducted seven days after the control feeding study. Gelatinized maize starch (5 % w/v) was mixed with concentrated potato peel extract (65 mg/mL) at a 3:1 ratio and fed to the rats at a dosage of 10 mL/kg of body weight. Blood collection and blood glucose concentration measurement was the same as the control feeding study. The collected blood was further treated for C13 analysis. The blood was mixed with acetone (300 µL) immediately after collection. The sample was then centrifuged at 3,000 g for 10 minutes. The supernatant, which contained plasma glucose, was collected and dried under nitrogen. The dried samples were solubilized in 100 µL of the mixture of pyridine and acetic anhydrate at the ratio of 1:2 for glucose derivatization. The derivatization was kept at room temperature for 12 hours and the samples were dried again using nitrogen. The dried samples were solubilized in 300 µL ethyl acetate prior to GC/MS injection.

1.3.12 Data analysis

Statistical analysis was conducted using IBM SPSS statistical software. Mean and the standard deviation was calculated in different assays. Statistical significance among the control and treated groups were assessed using paired sample t-tests. Differences were considered significant at $p<.05$. A multiple linear correlation study was conducted between the measured inhibition and quantified inhibitors to identify the significance of these inhibitors on the overall inhibition.

1.4 RESULTS

1.4.1 Inhibition mechanism of potato peel extract on α-amylase

A Lineweaver-Burk plot was used to study the inhibition mechanism of potato peel extract on α-amylase (Fig. 1.1). The y-axis intercept showed the reciprocal of the maximum enzyme reaction rate (V_{max}) while the x-axis intercept showed the reciprocal of the Michaelis constant (K_m), indicating when the reaction of the substrate concentration reached 50% of its maximum reaction rate. When comparing the Vmax, three treatments (control, 4.5 mg/mL peel extract and 9 mg/mL peel extract) showed different intercepts at 2.0, 2.9 and 4.7, respectively. All three treatments had similar Km values at - 0.2. The high similarities of the Km values and different Vmax values indicated the non-competitive inhibition mechanism of potato peel extract on α-amylase activity.

1.4.2 Inhibition mechanism of potato peel extract on mucosal α-glucosidase

The Lineweaver-Burk plot was used to identify the inhibition mechanism of peel extract on mucosal α-glucosidase (Fig. 1.2). The application of peel extract at three different doses of 0, 4.5 and 9 mg/mL showed different y-axis intercepts at 1.7, 1.8 and 2.1, and x-axis intercepts at -0.26, -0.2 and -1.5, which indicated different Vmax and Km values. Since the Vmax and Km values of the three peel extract dosages were significantly different, the inhibition mechanism of potato peel extract on mucosal α-glucosidase was shown to be a mixed inhibition mechanism.

1.4.3 Inhibition of potato peel extract on individual subunits of mucosal α-glucosidase

Heat inactivation was applied to partially inactivated sucrase-isomaltase subunits in mucosal α-glucosidase and the maltase, isomaltase and sucrase activity was measured before and after the heat inactivation of sucrase-isomaltase (Table 1.1). 0.53 U/mg of maltase activity was measured before heat inactivation and 0.33 U/mg of maltase activity remained after heat inactivation. Thus, heat treatment reduced the maltase activity by 40%. Both sucrase and isomaltase activities were measured at 0.06 U/mg and 0.04 U/mg before heat inactivation, while only 0.003 U/mg and 0.001 U/mg remained after heat inactivation. The

95% reduction of the isomaltase and sucrase activity indicated the inactivation of sucrase-isomaltase subunit activity. Sixty-one percentage of maltase activity was remained after heating, which indicated the maltase-glucoamylase protein complex was active while sucrase-isomaltase protein complex was inactive.

The heat-treated mucosal α-glucosidase, which only contained maltase-glucoamylase subunit activity, was then used to study the inhibition by potato peel extract (Fig. 1.3A). The enzyme reaction rates, with 0 mg/mL, 4.5 mg/mL and 9 mg/mL of potato peel extract added, were 0.091 mM/min, 0.072 mM/min and 0.056 mM/min, respectively which indicated a significant reduction of enzymatic activity by 28% and 45%.

Sucrose and isomaltose were used as the substrate to study the inhibition on sucrase-isomaltase activity (fig 1.3B, C). For sucrase activity, the enzyme reaction rates, with 0 mg/mL, 4.5 mg/mL and 9 mg/mL of potato peel extract added, were 0.0176 mM/min, 0.0171 mM/min and 0.0132 mM/min, respectively. The addition of 4.5 mg/mL of peel extract decreased the sucrase activity by 18%, and the reduction was not statistically significant. The addition of 9 mg/mL peel extract further reduced sucrase activity by 32%, and the inhibition on sucrase activity was statistically significant ($p = 0.000$). For isomaltase activity, the enzyme reaction rates with 0 mg/mL, 4.5 mg/mL and 9 mg/mL of potato peel extract added were 0.40 mM/min, 0.032 mM/min and 0.027 mM/min, respectively. The isomaltase activity was significantly reduced by both of the dosages of potato peel extract by 26% and 41%. The study, therefore, showed the dose-response inhibition of potato peel extract on both maltase-glucoamylase and sucrase-isomaltase subunits of mucosal α-glucosidase.

1.4.4 Correlation and multiple linear regression of inhibitor concentration and α-amylase inhibition

A statistical analysis was used to identify the significance of each quantified inhibitor including phenolic acids, mineral content, and pectin, on the inhibition of α-amylase using the inhibition study conducted with the peel extract from 12 potato varieties from 2016 and 10 potato varieties from 2017. The inhibition power of these potato peel extracts on α-amylase ranged from 0.3% to 26%, with the majority of the inhibition observed between 10% to 19%. The quantification of inhibitors showed the content of phenolic acids in potato peel extract

ranged from 0.03 mM to 0.1 mM. The pectin content in the peel extract ranged from 173 mg/mL to 917 mg/mL, and the mineral content ranged from 2.58 ug/mL to 10.4 ug/mL. The correlation study between the inhibition power and inhibitor concentration (Fig. 1.4) showed the highest correlation between phenolic acid concentration and α-amylase inhibition with a coefficient of determination (R^2) at 0.62. Both pectin content and mineral content showed a low degree of correlation with α-amylase inhibition with the R^2 value measuring at 0.11 and 0.001, respectively.

A multiple linear correlation analysis was then conducted to predict the contribution of each source of inhibitors in the overall inhibition (Table 1.2). The model generated by a multiple linear correlation analysis showed different levels of impact of each quantified inhibitor on α-amylase inhibition. Phenolic acid content showed the highest coefficient at 334.62, followed by mineral content at 1.622 and pectin content at 0.004. When comparing the level of significance at $p < .05$, only phenolic acid showed a significant impact on α-amylase activity. The model with all three inhibitor contents showed the overall coefficient of significance (R^2) at 0.68, which was not a significant improvement over the correlation with phenolic acid concentration alone, which was 0.62.

1.4.5 Correlation and multiple linear regression of inhibitor concentration and mucosal α-glucosidase inhibition

A correlation and a multiple linear regression model were applied between quantified inhibitor concentration and measured mucosal α-glucosidase inhibition of potato peel extract from different potato varieties (12 varieties in 2016 and ten varieties in 2017) to identify the main sources of inhibition. The correlation study of four quantified inhibitors: calystegine (three isoforms), phenolic acids (four identified phenolic acids), pectin and minerals (copper, nickel, and zinc) along with mucosal α-glucosidase inhibition power, showed that all four inhibitor sources have a positive correlation with inhibition (Fig. 1.5). Among the quantified inhibitors, calystegine and phenolic acid had the highest correlation to mucosal α-glucosidase inhibition with a significance of correlation at 0.65 and 0.51. Pectin and mineral content had the lowest correlation to inhibition with a significance of correlation at 0.21 and 0.12. The combined multiple linear regression model indicated that different inhibitors had different

levels of impact on the overall inhibition (Table 1.3). When comparing the coefficients in the model, phenolic acid had the highest coefficient at 146.46 followed by minerals and calystegine. Pectin contained the lowest coefficient at 0.015. When comparing the significance level at a confidence level of 0.05, calystegine, phenolic acid and pectin are considered significant factors while minerals, with a significance higher than 0.05, which should not be considered a significant factor for the overall inhibition. The combined multiple linear regression model had a coefficient of significance of 0.748, which was higher than any of the inhibitors when considered individually, which demonstrates the overall improvement of inhibition prediction when considering all four factors at the same time.

1.4.6 Potato peel extract delayed the in vivo glucose response and increased glucose absorption rate after starch ingestion in rats

The glucose response curve showed differences between the control feeding and the feeding with the addition of potato peel extract (Fig. 1.6). The control feeding reached the peak blood glucose concentration 30 minutes after feeding at 128 mg/L while the feeding with the added potato peel extract showed the peak blood glucose concentration reached 138 mg/mL at 60 minutes after feeding. The net change of blood glucose concentration from 0-minute baseline to peak concentration was 37 mg/L for the control feeding and 33 mg/L with the potato peel extract added showing a slight decrease in the net blood glucose concentration change. A faster glucose absorption rate was also observed. Compared with the control group, the group fed with starch and potato peel extract showed more significant decreases in their blood glucose concentration after reaching peak blood glucose concentration. Between 60 minutes to 120 minutes, the treated group showed the blood glucose concentration decreased by 27 mg/L, while the control group showed the blood glucose concentration decreased by15 mg/L between 30 minutes to 120 minutes after feeding. Large variations were observed in the blood glucose response of different individual rats with the statistical analysis showing no significant differences between the two feeding experiments.

1.5 DISCUSSION

The digestion of starch is an enzymatic process which breaks down the intricately structured starch molecules into glucose. The digestion process is aided by two main groups of enzymes, α-amylase and mucosal α-glucosidase following a sequential hydrolysis process (Lin, Hamaker, & Nichols Jr, 2012). The breakdown of starch begins with the hydrolytic activity of salivary α-amylase in the oral cavity and esophagus (Ramasubbu, Paloth, Luo, Brayer, & Levine, 1996). Digestion is followed by pancreatic α-amylase, which is secreted into the small intestine. The digestion by two types of α-amylases largely reduces the size of starch molecules into short linear oligomers linked by α-1,4 glycosidic linkages and branched glucans which contained α-1,6 glycosidic linkages (A. H.-M. Lin et al., 2012). These small molecules are then digested sequentially by mucosal α-glucosidases into glucose (A. H. M. Lin et al., 2012).

The inhibition study of potato peel extract on α-amylase and mucosal α-glucosidase showed a significant reduction of enzymatic activity with the addition of potato peel extract. For α-amylase, the Lineweaver-Burk plot showed similar Km values but significantly different Vmax value, suggesting the domination of non-competitivenon-competitive inhibition. The finding suggests that α-amylase inhibition is primarily caused by non-competitive inhibitors. The inhibition mechanism of potato peel extract on mucosal α-glucosidase resulted in different km and Vmax values, which consequently, was identified as mixed inhibition. This indicated that the inhibition could come from both competitive and non-competitivenon-competitive inhibition.

Maltase-glucoamylase exhibited a distinctly different hydrolytic function and capacity during starch digestion compared with sucrase-isomaltase had much higher α-1,4 glycosidic linkage hydrolytic efficiency and capacity compared with sucrase-isomaltase (A. H. M. Lin et al., 2012); while sucrase-isomaltase subunits carried major α-1,6 linkage, and sucrose hydrolytic activities. Maltase-glucoamylase is thus, proposed to be primarily responsible for rapid glucose genesis while sucrase-isomaltase, with its slow activity and abundant quantity, is essential for maintaining a slow and stable glucose release after starch ingestion (Lin, Lee, & Chang, 2016). The inhibition study showed significant inhibition on both maltase-glucoamylase and sucrase-isomaltase subunits of mucosal α-glucosidase. Therefore, potato peel extract is proposed to have significant impacts throughout the different stages of mucosal

α-glucosidase digestion, and the findings indicate it positively impacts the modulation of the overall glucose response.

The correlation study between inhibitor concentration and α-amylase and mucosal α-glucosidase inhibition demonstrated that the content of phenolic acid and calystegine are highly correlated with mucosal α-glucosidase inhibition while phenolic acid content is also highly correlated with α-amylase inhibition. Potato contains three main types of phenolic acids, which includes chlorogenic acid, caffeic acid, and ferulic acid (Albishi, John, Al-Khalifa, & Shahidi, 2013). Multiple studies have suggested that phenolic acid inhibits α-amylase (Funke & Melzig, 2005; KWON, Apostolidis, & Shetty, 2008). The IC_{50} values of chlorogenic acid, ferulic acid, and caffeic acid on α-amylase were at 1.4 mM, 4.8 mM, and 5.0 mM, respectively, with a non-competitive inhibition mechanism (Funke & Melzig, 2005). Our analysis, confirming previously published studies, found that potato peel extract contains a significant quantity of phenolic acid ranging from 0.03 to 0.12 mM with chlorogenic acid as the most abundant phenolic acid. For the inhibition on mucosal α-glucosidase, phenolic acid had significant non-competitivenon-competitive inhibition on individual subunits of mucosal α-glucosidase (Simsek, Quezada-Calvillo, Ferruzzi, Nichols, & Hamaker, 2015). Chlorogenic acid had the highest inhibition against both Ct-maltase-glucoamylase and Ct-sucrase-isomaltase, while catechin showed the highest inhibition against Nt-maltase-glucoamylase and Nt-sucrase-isomaltase (Simsek et al., 2015). Our study confirmed the significant inhibitory effect of phenolic acid on both α-amylase and mucosal α-glucosidase. The high correlation between phenolic acid concentration and both α-amylase (R^2=0.62) and mucosal α-glucosidase (R^2=0.50) inhibition agreed with previously published literature that phenolic acid is the major non-competitivenon-competitive inhibitor in potato peel extract that inhibits both α-amylase and mucosal α-glucosidase.

Calystegines are nortropane alkaloids that exist in various types of plants such as Solanaceae (potato), Atropa, and Calystegia (Nash et al., 1993). Potato contains an abundant quantity of calystegines in the form of calystegine A_3 and B_2, which are concentrated in potato peels (Keiner & Dräger, 2000). Calystegine is a mucosal α-glucosidase inhibitor, and the reported inhibition mechanism study showed competitive inhibition on mucosal α-glucosidase with a Ki value of 227 μM (Jocković, Fischer, Brandsch, Brandt, & Dräger, 2013). The further silico docking studies confirmed calystegine binds to the active sties of α-glucosidase,

which verifies its function as a competitive inhibitor. Our data found significant content of calystegine in potato peel extract with concentrations ranging from 4.2 – 20.2 µg/mL when combining the concentrations of all detected isomers. The mass spectrometry results showed significant content of calystegine A_3, and calystegine B_2 isomer as literature reported (Keiner & Dräger, 2000). In addition, we identified another two types of calystegine A isomer and one other type of calystegine B isomer. It is unknown if these newly identified isomers alone can interfere with enzymatic hydrolysis. However, our data showed that the total amount of three calystegine isomers is higly correlcted with the inibhtion on mucosal α-glucosidase inhibition (R^2=0.65). Our findings suggests, in potato pleels, calystegine is the primary mucosal α-glucosidase competitive inhibitor.

Some researchers have suggested that copper, nickel, and zinc have non-competitive inhibition on both α-amylase (Cordeiro, Martins, & Luciano, 2002) and mucosal α-glucosidase (Wang et al., 2004). However, in our study, the total amount of copper, nickel, and zinc, which ranged from 1.8 µg/mL to 20.2 µg/mL, had a weak correlation with either α-amylase or mucosal α-glucosidase inhibition in potato peel extract. A potential explanation could be the availability of the minerals causing the inhibition. Some components in potato peels such as soluble dietary fiber were shown to bind with polyvalent metal ions (Torre, Rodriguez, & Saura-Calixto, 1991), resulting in the reduction of "free" metal ions available for interaction with enzymes. Another explanation could be the unknown quantity of "charge-carrying" minerals. The mineral analysis quantified both the charged and uncharged minerals while the inhibition study demonstrated that only the "charge-carrying" metal ions such as Cu^{2+} showed significant inhibition on enzyme activity (Cordeiro et al., 2002). Thus, the current analysis on mineral content could not explain the actual contribution of metal ions on the overall effect of α-amylase and mucosal α-glucosidase inhibition.

Soluble dietary fiber interferes with pancreatic enzymes in both protein and starch digestion (Dunaif & Schneeman, 1981). It is proposed that the mechanism of interference increases viscosity and functions as a physical barrier for enzyme-substrate interaction. Pectin is the major soluble dietary fiber in potato peels (Camire, Violette, Dougherty, & McLaughlin, 1997) and our data found 161.3 to 920.2 µg/mL of pectin in potato peel extract. The correlation study indicated there is only a weak correlation between pectin content and inhibition with an R^2 value of 0.11 for α-amylase and 0.2 for mucosal α-glucosidase with no

statistical significance. Our preliminary study (Fig. 1.7), which used commercial apple pectin, did not show significant inhibition of either α-amylase or mucosal α-glucosidase at a dosage of 2.8 mg/mL. Since, at 920 ug/mL, the highest quantified pectin content in potato peel extract was much lower than the dosage applied in our preliminary study, our finding suggested that pectin was not a significant factor that directly impacted both enzymes' activities. However, the largest dosage of commercial pectin applied at 5.6 mg/mL showed significant inhibition on α-amylase activity, which suggests pectin still carries inhibitory functions when present at high dosages. Besides direct inhibition on starch digestive enzymes, soluble dietary fiber could still impact starch digestion through its interference with glucose transporters and glucose absorption (Hannan et al., 2007) Thus, pectin in potato peel extract could still have a potential effect on in vivo starch digestion and glucose absorption.

An animal feeding study, using a rat model, was established to evaluate the effect of potato peel extract in an in vivo system. The glucose response curve showed a delay of peak glucose response from 30 minutes to 60 minutes which indicates a delay of starch digestion. The results agreed with the in vitro inhibition study suggesting potato peel extract could delay and reduce starch digestion due to its inhibition function on both α-amylase and α-glucosidase activities. A faster blood glucose absorption rate was also observed as the decrease rate of blood glucose concentration after reaching peak blood glucose concentration was higher when rats were fed starch with potato peel extract. Literature has shown that an increase in the glucose absorption rate could be beneficial to those with Type II diabetes as it helps to prevent high blood glucose levels and related symptoms (Knowler et al., 2002). Some medication such as metformin was developed based on this concept (Bailey & Turner, 1996). Our finding suggests that potato peel extract might contain other functional molecules that impact starch digestion and absorption beyond the direct inhibition on starch digestive enzymes. Due to large individual variances, the data did not show statistically significant differences between the control feeding group and the treatment group.

1.6 CONCLUSION

In conclusion, our study demonstrated potato peel extract inhibited both α-amylase and mucosal α-glucosidase, as well as two individual subunits of mucosal α-glucosidase. The

inhibition on α-amylase was predominantly non-competitive inhibition as a result of phenolic acid while the inhibition on mucosal α-glucosidase was a mixed inhibition caused by calystegine and phenolic acid. The inhibition effect was verified in an in vivo system with a delayed glucose response from starch digestion. Our findings, therefore, suggest the potential for potato peel extract to be used as a dietary supplement to modulate glycemic response through its direct inhibition on starch digestive enzymes.

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1.8 TABLES AND FIGURES

Table 1. 1 Heat inactivation of the sucrase-isomaltase subunit of mucosal α-glucosidase

Disaccharidase activity	Activity before inactivation (U/mg)	Activity after heat inactivation (U/mg)	% of activity remained
Maltase	0.53 ± 0.003	0.33 ± 0.006	61.654
Sucrase	0.06 ± 0.0004	0.003 ± 0.000	5.556
Isomaltase	0.04 ± 0.0003	0.001 ± 0.000	4.375

Note: Maltase specific activity is defined as μmol of glucose released per minute per mg of protein with 25 mM maltose at 37 °C in pH 7.4 PBS buffer. Isomaltase specific activity is defined as μmol of glucose released per minute per mg of protein with 25 mM isomaltose at 37 °C in pH 7.4 PBS buffer. Sucrase specific activity is defined as μmol of glucose released per minute per mg of protein with 25 mM sucrose at 37 °C in pH 7.4 PBS buffer. Heat inactivation is performed by incubating mucosal α-glucosidase in 61 °C water bath for 15 minutes. Data were reported as mean ± standard deviation. of three replicates.

Table 1. 2 Statistical models from multiple linear regressions between inhibitor concentration and α-amylase inhibition

Coefficients

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Significance
(Constant)	-13.179	5.077	N/A	-2.596	.018
Pectin	.004	.006	.099	.692	.498
Phenolic Acids	334.616	59.142	.822	5.658	.000
Minerals	1.622	1.141	.205	1.421	.172

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.826	.682	.629	4.85176

Note: A multiple linear correlation was conducted between inhibitor concentration and α-amylase inhibition. A linear regression coefficient of each inhibitor and the significance levels were presented. The inhibitor was considered a significant factor with a significance level at $p < .05$. The linear model was summarized with the overall coefficient of determination presented. NA refers to non-detectable.

Table 1. 3 Statistical model from multiple linear regressions between inhibitor concentration and mucosal α-glucosidase inhibition

Coefficient

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Significance
(Constant)	-17.441	5.158	N/A	-3.382	.004
Calystegine	.807	.263	.465	3.066	.007
Phenolic Acids	146.680	67.176	.326	2.184	.044
Mineral	1.866	1.115	.233	1.674	.114
Pectin	.015	.005	.367	2.871	.011

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.865[a]	.748	.685	4.46735

Note: A multiple linear correlation was conducted between inhibitor concentration and inhibition. A linear regression coefficient of each inhibitor and the significance levels were presented. The inhibitor was considered a significant factor with a significance level at $p < .05$. The linear model was summarized with the overall coefficient of determination presented.

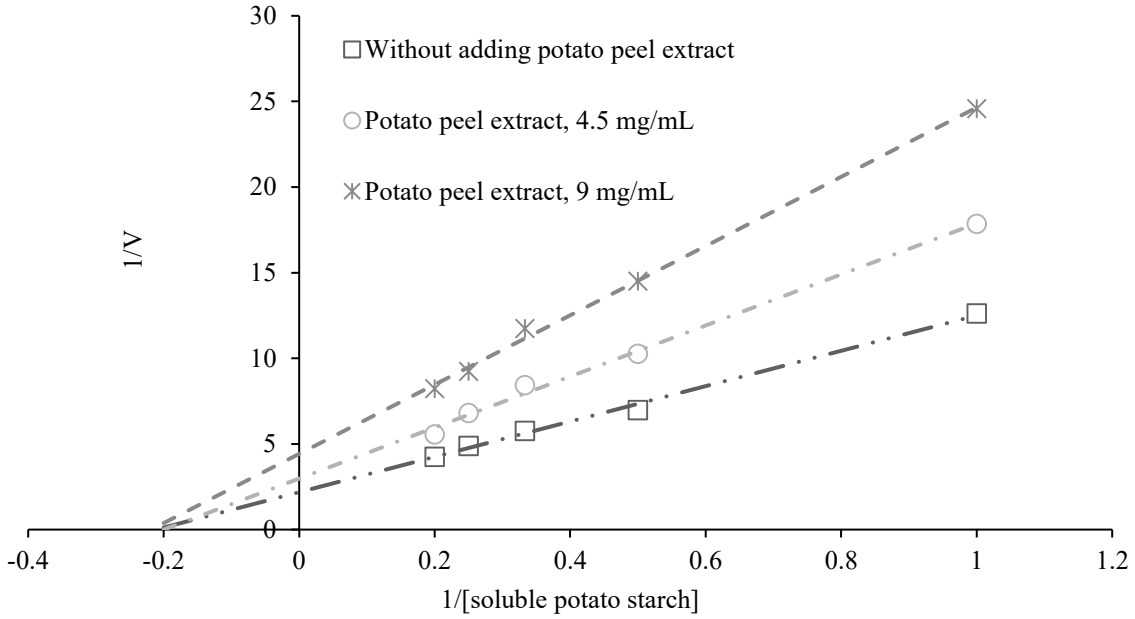

Figure 1. 1 Lineweaver-Burk plot of potato peel extract inhibition on α-amylase.

Increased concentrations of soluble potato starch (1.0 – 5.0 mg/mL) were used to determine the K_m and V_{max} values of mucosal α-glucosidase with three doses of peel extract at 0, 4.5 and 9 mg/mL. The data were plotted as 1/V against 1/[S] with the y-axis intercept representing maximum velocity (V_{max}) and the x-axis intercepts representing Michaelis constant (K_m).

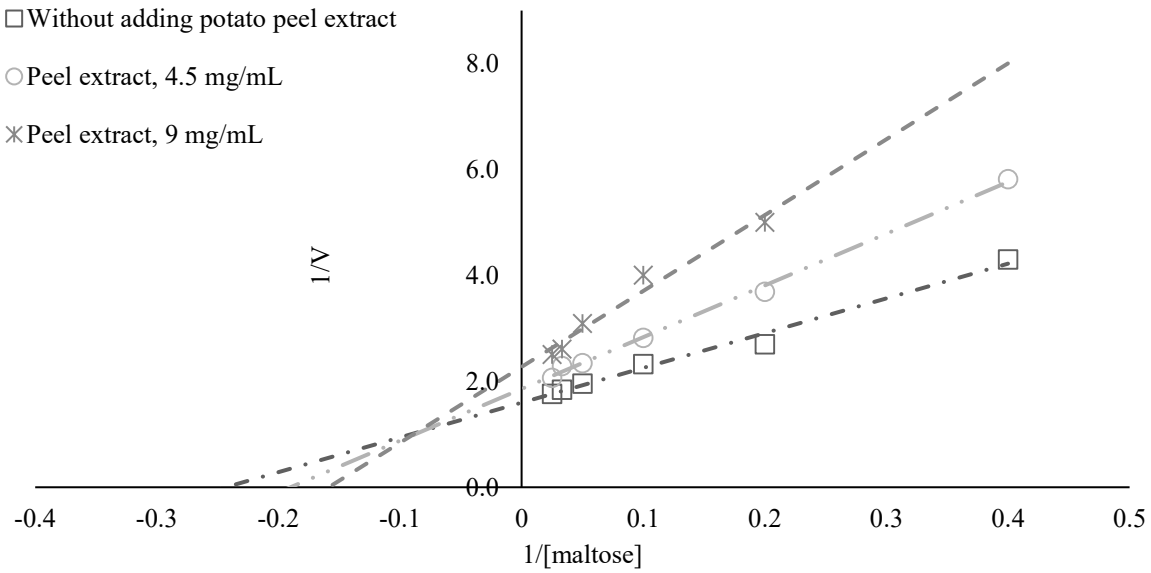

Figure 1. 2 Lineweaver-Burk plot of potato peel extract inhibition on mucosal α-glucosidase. Increased concentrations of maltose (10-80 mM) were used to determine the Km and Vmax values of mucosal α-glucosidase with three doses of peel extract at 0, 4.5 and 9 mg/mL. The data were plotted as 1/V against 1/[S] with the y-axis intercept representing maximum velocity (V_{max}) and x-axis intercepts representing Michaelis constant (K_m).

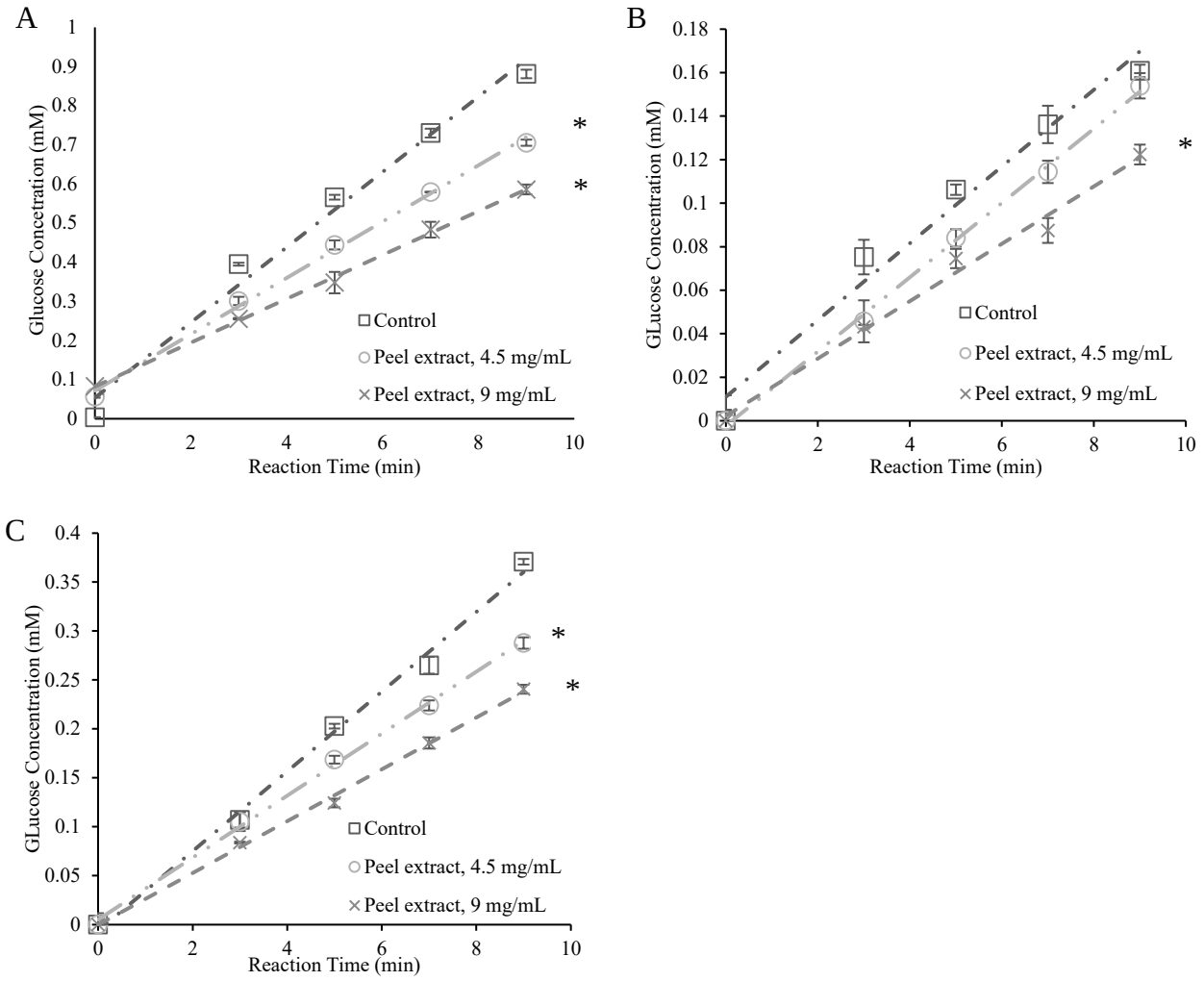

Figure 1. 3 Inhibition of potato peel extract on separate subunits of mucosal α-glucosidase. A. Represents the inhibition of maltase-glucoamylase, a subunit of mucosal α-glucosidase, by potato peel extract. The enzyme activity curve was constructed with the increase in glucose concentration through maltose (25 mM) hydrolysis. B. Represents the inhibition of isomaltase activity in mucosal α-glucosidase potato peel extract. The enzyme activity curve was constructed with the increase in glucose concentration through isomaltose (25 mM) hydrolysis. C. Represents the inhibition of sucrase activity in mucosal α-glucosidase by potato peel extract. The enzyme activity curve was constructed with the increase in glucose concentration through sucrose (25 mM) hydrolysis. Data were the mean ± standard deviation of three independent experiments. Pair sample t-tests were conducted between the control and treatments, and differences were considered significant at * $p < .05$.

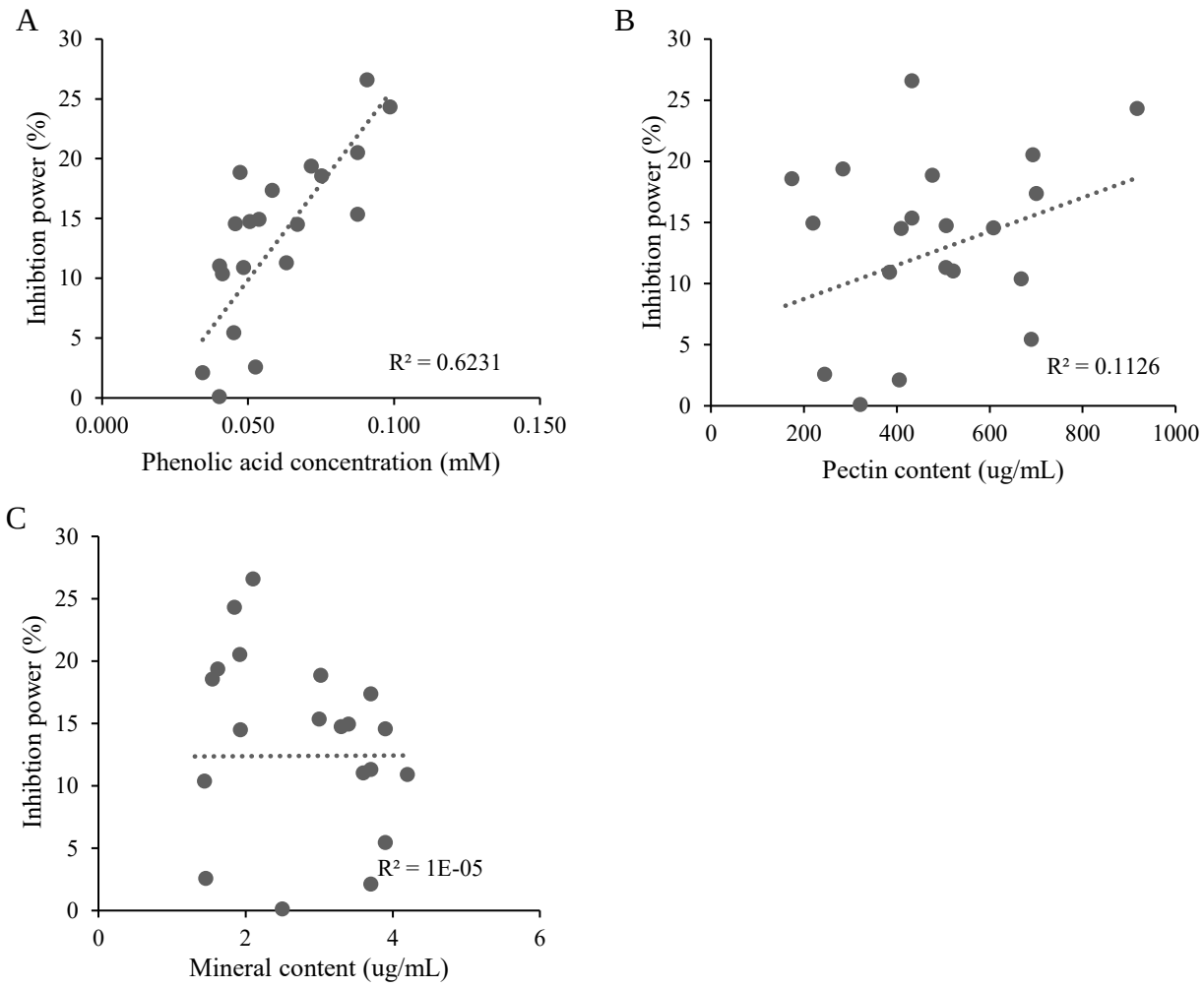

Figure 1.4 Correlation between inhibitor concentration and the inhibition of α-amylase.

Inhibition power was defined as the percentage of α-amylase activity reduced with the addition of potato peel extract. A**.** The phenolic acid concentration was the total amount of chlorogenic, caffic acid, and ferulic acid and catechin. B. Pectin content was quantified through the uronic acid assay C. Mineral content was the total amount of copper, zinc, and nickel.

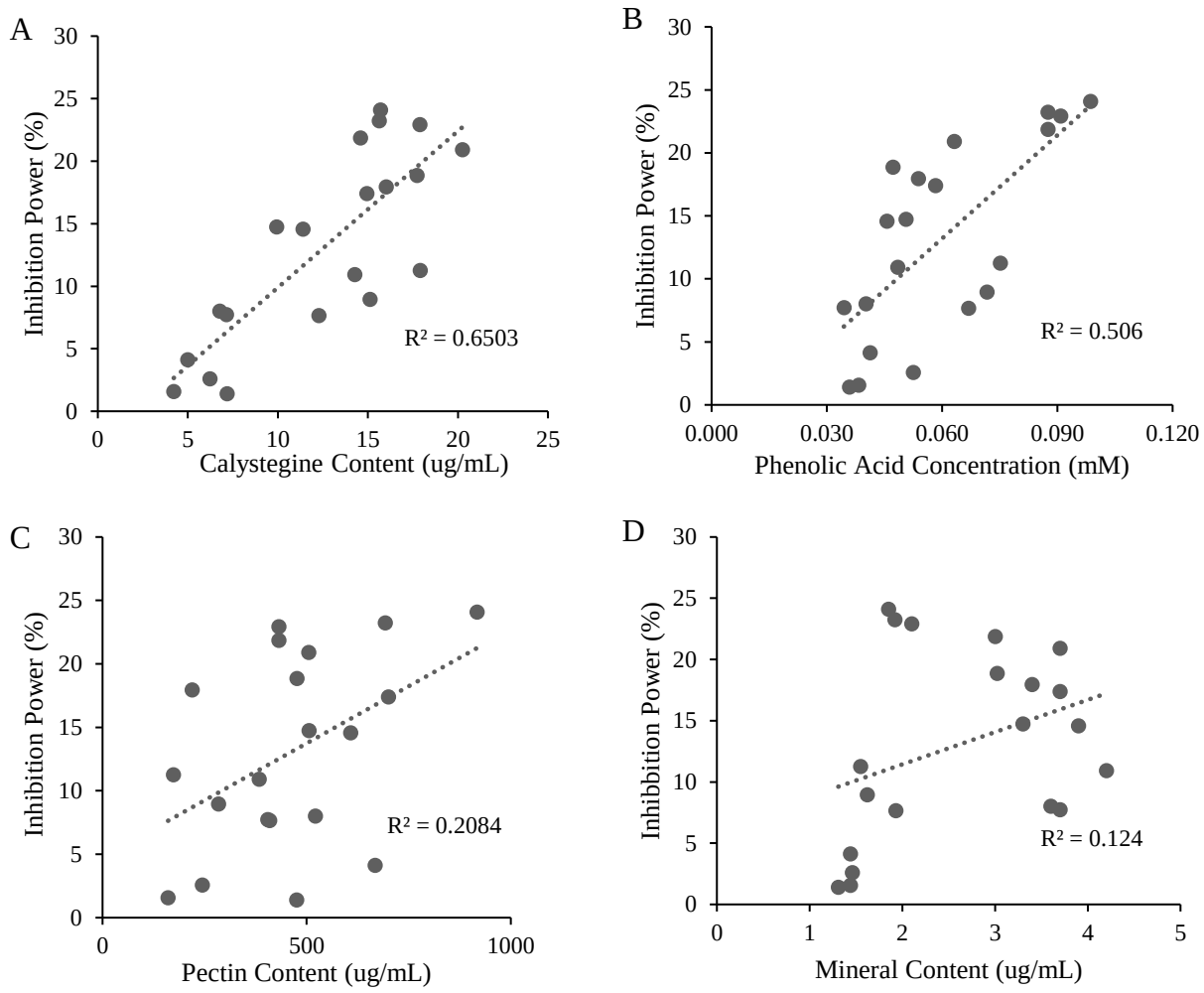

Figure 1.5 Correlation between inhibitor concentration and the inhibition of mucosal α-glucosidase. Inhibition power was defined as the percentage of mucosal α-glucosidase activity reduced with the addition of potato peel extract. A. Calystegine content was the combined concentration of Calystegine A and B isomers. B. The phenolic acid concentration was the combined concentration of chlorogenic acid, caffeic acid, and ferulic acid and catechin. C. Pectin content was quantified through uronic acid assay. D. Mineral content was the combined concentration of copper, zinc, and nickel.

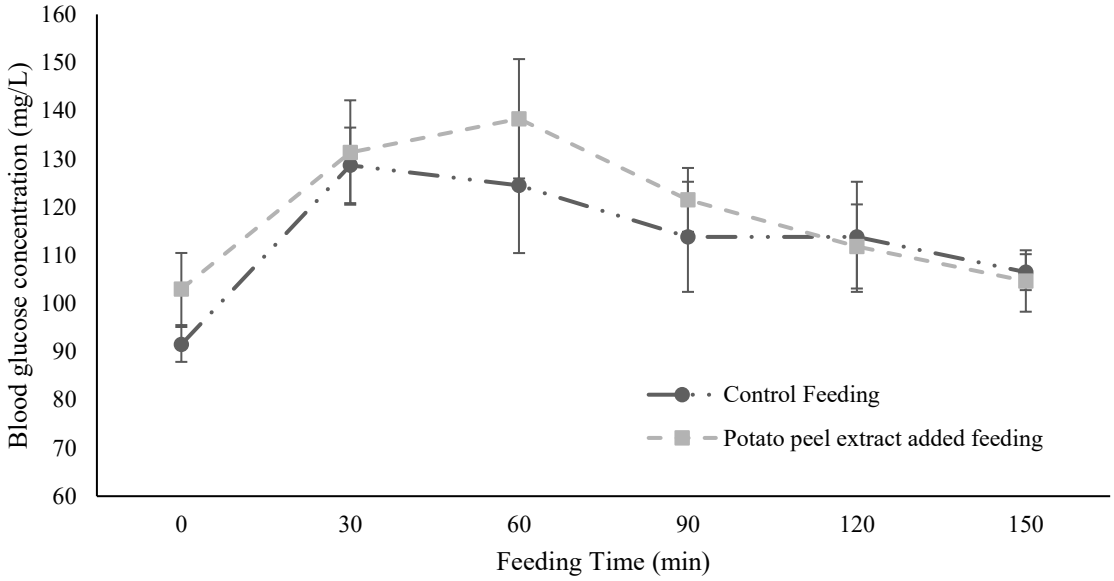

Figure 1.6 Glucose response curve comparison between control feeding and feeding with potato peel extract added. Control feeding (10 mL/ kg of body weight) was conducted with a mixture of 5% (w/v) gelatinized maize starch and water at a 3:1 ratio. Treatment feeding (10 mL/ kg of body weight) was conducted with a mixture of 5% (w/v) gelatinized maize starch and concentrated potato peel extract (65 mg/mL) at a 3:1 ratio. Data were presented as the mean ± standard deviation of blood glucose concentration from six male Wistar rats.

1.9 APPENDIX

Description of the varieties and sources of potato

All the potatoes of the study were planted in Parma, Idaho. Russet Burbank, Payette Russet, Premier Russet, Ranger Russet, Clearwater Russet, Umatilla Russet, A06021-1, and A03921-2 were planted and harvested in two consecutive years 2016 and 2017. Some varieties were not available in both years. Mountain Gem Russet, Targhee Russet, Russet Norkotah were planted only in 2016. Huckleberry Gold, Pomerelle Russet were planted only in 2017.

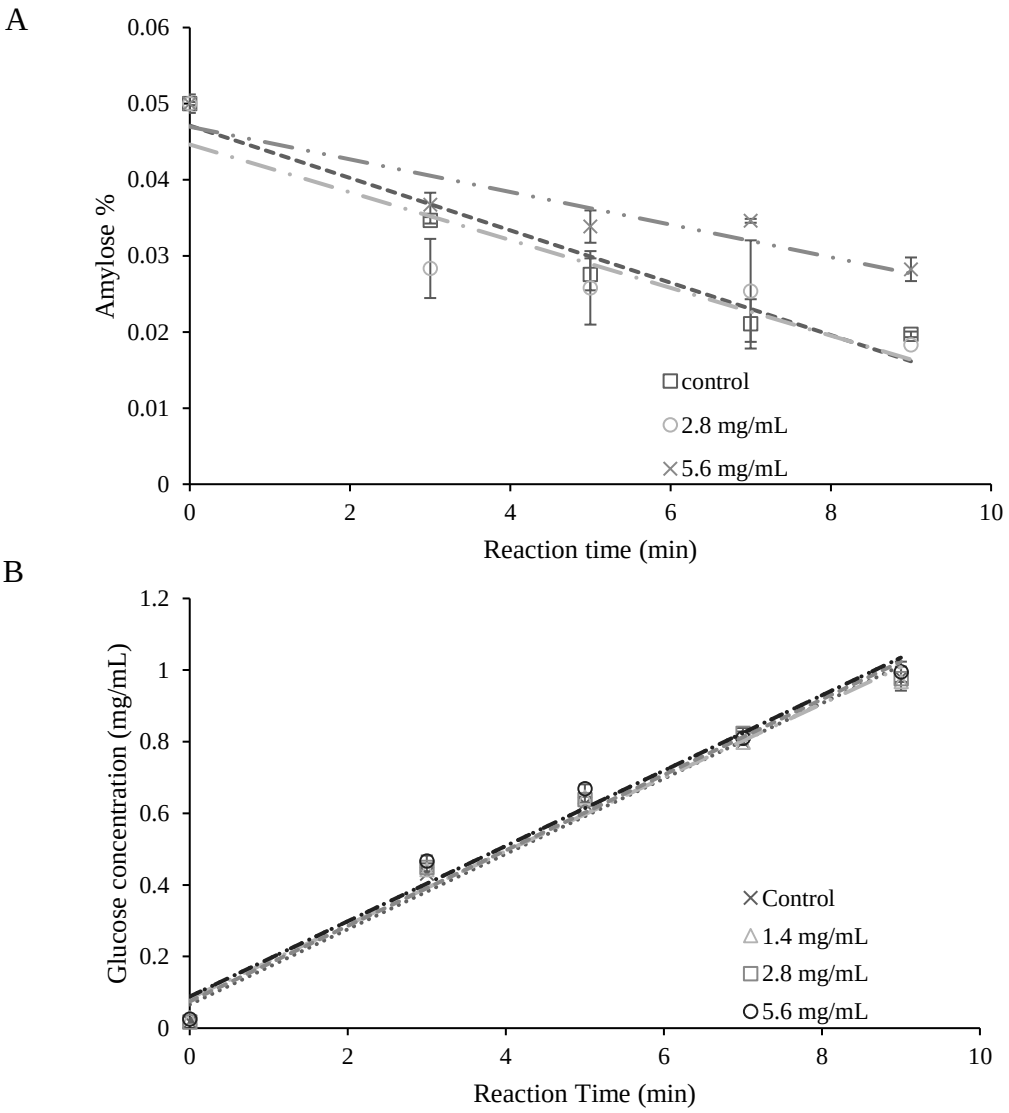

Figure 1.7 Inhibition of pectin on α-amylase and mucosal α-glucosidase activity.

A. Inhibition of pectin on α-amylase was studied with 0.5% amylose used as the substrate and the decrease of amylose percentage during hydrolysis was measured using iodine binding. Pectin was added at concentrations of 0, 2.8 and 5.6 mg/mL. B. Inhibition of pectin on mucosal α-glucosidase activity was studied using 25 mM maltose as the substrate. The generation of glucose during hydrolysis was measured using a D-glucose assay kit. Pectin was added at concentrations of 0, 1.4, 2.8 and 5.6 mg/mL. Data were presented with the mean and standard deviation of triplicate experiments.

CHAPTER 2

IMPACT OF PEELING METHOD AND CULTIVAR OF POTATO PEELS ON THE INHIBITION OF STARCH DIGESTIVE ENZYMES

2.1 ABSTRACT

The lack of sufficient peel waste utilization has burdened the potato industry. Potato peels contain multiple functional substances such as calystegines and phenolic compounds, which inhibit starch digestive enzymes and thus, may be able to be used to treat obesity and Type II diabetes. To further apply the findings to the industry, potato varieties and the effect of peeling method on inhibition performance and inhibitor content was studied. Potato peels from five common processing varieties from two different growing years were hand-peeled or industrial steam-peeled. An inhibition study was performed using the aqueous extract from potato peels and rat pancreatic α-amylase and mucosal α-glucosidase. Calystegine, minerals, phenolic acids and pectin were quantified as major sources of inhibitors. The results showed different potato varieties and the peeling method demonstrated significantly different inhibitor content and inhibitory effects with Ranger Russet and Payette Russet having the highest phenolic acid content and the greatest overall inhibition. Steam peeling, compared with hand peeling and abrasion peeling, showed a significantly higher inhibitory effect with high phenolic acid content. The results demonstrated that certain processing varieties and the steam peeling method could generate peel waste with higher effectiveness in treating obesity and Type II diabetes.

2.2 INTRODUCTION

Potato peels Potato peels contain starch (21 - 52% w/w dw), non-starch polysaccharides (25 - 60% w/w dw) such as pectin, cellulose, lignin and hemicelluloses, protein (16 - 19% w/w dw), lipids (0.6 - 1.1% w/w, dw), and ash (< 1% w/w, dw) (Liang, McDonald, & Coats, 2014). The potato industry is greatly interested in enhancing the value of potato peels in order to improve the sustainability and profitability of the potato industry. Researchers investigating

applications for potato peel waste have focused on three major components: carbohydrates, polyphenolic compounds, and glycoalkaloids (dos Santos, Ventura, Bordado, & Mateus, 2016). Carbohydrates are the primary component of potato peel waste and account for 60 – 80% of its weight. In order to utilize non-starch polysaccharides, Liang et al. (2014) applied a mixture of cultures from a wastewater treatment plant to fermented potato peel waste and produced up to 0.25 g of lactic acid per gram of potato peel waste, which is a valuable probiotic ingredient. Kumar et al. (2016) heated potato peels with an acid catalyst using a microwave to hydrolyze the remaining starch in peel waste, which generated glucose for producing ethanol. Researchers are particularly interested in the phenolic compounds found in potato peels due to their free radical scavenging properties. Farvin et al. (2012) studied the effects of phenolic acids in potato peels, including gallic acid, protocatechuic acid, and chlorogenic acid, for fish preservation and found potato peel extract significantly reduced the production of carbonyl compounds, and volatiles, which are the main products of oxidation, during storage. Glycoalkaloids in potatoes are well known for their adverse effects in humans; however, a study by Kenny et al. (2013) found that glycoalkaloids in potato peels possess anti-inflammatory properties by suppressing the secretion of pro-inflammatory factors in mice. This study indicates that potato peel waste may have pharmaceutical applications. However, using fermented carbohydrates from potato peel waste is questionable because the profit margin may be too small due to the necessary expenses of purchasing bacterial sources and fermentation equipment. The extraction and purification of the targeted phenolic and glycoalkaloid compounds are complicated, and therefore, could limit their wide application in the industry. To date, the potato industry still lacks sufficient ways of utilizing potato peel waste and continues to search for reliable and efficient applications.

Our research, reported in Chapter 1, has shown that potato peels contain functional molecules such as phenolic acids, calystegine, and pectin that inhibit the activity of starch digestive enzymes, i.e., α-amylase and α-glucosidase, and consequently interfere with glucogenesis. Therefore, we proposed that potato peels may be used to modulate glycemic response. To apply our findings to industry, it is necessary to consider what potato varieties are used and how potatoes are peeled in the industry as peeling methods may affect the inhibitor content and inhibition performance of potato peels.

In the United States, the major processing varieties include Russet Burbank, Ranger Russet, and Clearwater Russet (Keijbets, 2008). Potato varieties critically determine the content of each component, such as phenolic compounds and calystegine in the peel. For example, Russet Nekota contains a significantly higher quantity of phenolic compounds compared with Russet Burbank (Albishi, John, Al-Khalifa, & Shahidi, 2013). In a previous study, we also observed that different potato varieties contained significantly different quantities of calystegine, phenolic acids, pectin, and minerals, which resulted in differences in their inhibitory effects on α-amylase and mucosal α-glucosidase. In the United States, potatoes are processed differently based on product types. Steam peeling is widely used for making French fries while abrasion peeling is widely used for making potato chips and dry potato powder (Camire, Violette, Dougherty, & McLaughlin, 1997). Peeling methods can also result in differences in peel composition. Compared to steam peeling, the abrasion peeling method generates less dietary fiber and more starch (Camire et al., 1997). The compositional differences that arise from peeling methods, therefore, could further affect inhibitor content and digestive enzyme inhibition.

In this study, we compared the inhibition differences among common processing potato varieties and peeling methods. Our research findings could help the industry develop a strategy for potato peel waste utilization by focusing on its nutritional benefits in controlling glycemic response.

2.3 MATERIALS AND METHODS

2.3.1 Materials

Five potato varieties often used for processing (i.e. producing frozen French fries) including Russet Burbank, Ranger Russet, Clearwater Russet, Payette Russet, and Umatilla Russet, were planted at the Parma Research and Extension Center (Parma, Idaho) in 2016 and 2017. All chemicals, reagents and rat intestine acetone powder were purchased from Sigma-Aldrich Corp. (St. Louis, MO) unless specified otherwise. Maltose was purchased from Tokyo Chemical Industry (Tokyo, Japan). Phosphate buffered saline tablets, caffeic acid and catechin were purchased from VWR International (Rendor, PA). Sulfuric acid and formic acid were purchased from T.J. Baker Chemical Company (Rendor, PA). Ferulic acid, methanol,

and acetonitrile were purchased from Fisher Scientific Inc. (Huston, TX). Calystegine A3 and B2 were purchased from ChemFaces Inc. (Wuhan, China). The sieves with various pore sizes were purchased from VWR International (Radnor, PA). Syringe filters with various membranes sizes, made of polytetrafluoroethylene (PTFE), were purchased from GE Healthcare (Chicago, IL) and Thermal Scientific Inc. (Rockwood, TN). The examinations and analyses described below were conducted in a laboratory with a humidity level of 10 ± 2% and at room temperature in the range of 20 °C to 22 °C. The research was conducted at a location with atmospheric pressure at 29.68 Hg and an elevation of 2709 feet above sea level; thus, the boiling water used in this study was 97.09 °C.

2.3.2 Potato peel preparation

The potato peels used in this study originated from three sources: J.R. Simplot (Caldwell, ID), Basic American Foods (Blackfoot, ID), and potatoes grown at the Parma Research and Extension Center (Parma, ID). The peels from J.R. Simplot and Basic American Foods were collected from their plant sites to represent the two primary types of potato peel wastes generated in the industry in the United States. The peels from J.R. Simplot were steam-peeled, immediately frozen upon collection and shipped, with dry ice, to the laboratory located at the University of Idaho (Moscow, ID). The peel waste from Basic American Foods was abrasion peeled, resulting in dry peel waste, and they were shipped to the laboratory without a specified shipping temperature. The industrial peel wastes were a collection of peels from various varieties including Russet Burbank, which is the primary potato cultivar used in the region. The five potato varieties grown at the Parma Research and Extension Center in 2016 and 2017, were washed and then hand-peeled with a kitchen peeler (Farberware, Vallejo, CA). A portion (200 lbs.) of potatoes grown at the Parma Research and Extension Center, in 2017, was steam-peeled at the pilot plant of J.R. Simplot (Caldwell, ID). The steam-peeled peels were placed on a screen and washed with water, air-dried, frozen (-20°C), and then shipped to the laboratory.

All of the peels, including the industrial peel wastes, were freeze-dried, ground with a coffee grinder (Krups, model F203; Solingen, Germany), and passed through a sieve with a pore size of 0.04 mm, and stored in a freezer (-20°C) for further analyses.

For extracting the functional components in potato peels, we used water to extract the enzyme inhibitors, which were water-soluble according to our preliminary study (data not shown). We weighed 800 mg potato peel powder into a 15-mL centrifuge tube and suspended the potato peel powder with 20 mL of deionized tap water with gentle stirring at room temperature for 30 min. The suspension was centrifuged at 4,000 ×g at room temperature for 10 min and the supernatant was further filtered through a syringe filter with a 5-µm pore-size. Potato peel extracts were freeze-dried and then solubilized with phosphate-buffered saline (5 mL, 137 mM sodium chloride, 2.7 mM potassium chloride, and 10 mM phosphate buffer, pH 7.4, prepared by dissolving one tablet of PBS saline buffer into 100 mL of deionized tap water) prior to analysis. The potato peels were extracted in triplicate.

2.3.3 Influence of potato peels in the α-amylase hydrolytic activity

The influence of potato peels in mammalian α-amylase hydrolytic activity was examined using blocked p-nitrophenyl α-D-maltoheptaoside as the substrate, which is part of the α-amylase assay kit (Megazyme u.c., Wicklow, Ireland). We measured the change of the hydrolysis products with and without the presence of potato peel extract in this in vitro hydrolytic reaction. The functional components in potato peels were extracted by water and converted into a saline phosphate buffer as described above. Alpha-amylase was extracted from the commercial rat intestine acetone powder as described in Chapter 1. An aliquot (20 µL) of the substrate with glucoamylase was transferred to a 2-mL microcentrifuge tube; then, the α-amylase solution (20 µL) was added into the microtube. The control groups of this study used saline phosphate buffer to replace the potato peel extract; for the treatment groups, an aliquot (40 µL) of potato peel extract-saline phosphate buffer was added into the tube while adding enzymes described below. The in vitro hydrolytic reaction was conducted in a water bath controlled at 37 °C. After 0, 5, 10, 15 and 20 min, the enzymatic activity was inactivated by adding 300 µL stopping reagent (20% (w/v) tri-sodium phosphate solution, part of the α-amylase kit). After cooling to room temperature, the hydrolysate was transferred to a 96-well microplate to assess the absorbance at 400 nm (Spectra Max 190 Microplate Reader, Molecular Devices, CA). The change of hydrolytic product p-nitrophenol was expressed as a change in absorbance, and the reduction of p-nitrophenol concentration in the treatment

groups was due to the presence of the potato peel extracts. The inhibition power of potato peels on α-amylase was calculated following the equation below. Each hydrolysis reaction was performed in triplicate, and p-nitrophenol quantification was also performed in triplicate.

2.3.4 Influence of potato peels in the mucosal α-glucosidase hydrolytic activity

The influence of potato peels in mammalian α-amylase hydrolytic activity was examined using maltose as the substrate. We measured the change of the hydrolysis products with and without the presence of potato peel extract in this in vitro hydrolytic reaction. The functional components in potato peels were extracted by water and converted into a saline phosphate buffer as described above. Mucosal α-glucosidase was extracted from the commercial rat intestine acetone powder as described in Chapter 1. An aliquot (5 µL, 100 mM) of the maltose solution was transferred to a 2-mL microcentrifuge tube; then, the mucosal α-glucosidase solution (5 µL) was added into the microtube. The control groups of this study used a saline phosphate buffer to replace potato peel extract; for the treatment groups, an aliquot (10 µL) of potato peel extract-saline phosphate buffer was added into the tube while adding mucosal α-glucosidase. The in vitro hydrolytic reaction was conducted in a water bath controlled at 37 °C. After 0, 3, 5, 7 and 9 min, the enzyme activity was inactivated by heating in boiling water for 10 min. After cooling to room temperature, the glucose was quantified by a D-glucose assay kit (Megazyme u.c., Wicklow, Ireland). GOPOD reagent (600 µL) was added into the hydrolysate, and the sample was kept in a 45 ºC water bath for 20 minutes for color development. The aliquot of the sample (300 µL) and glucose standard (1 mg/mL) was transferred to a 96-well microplate to assess the absorbance at 510 nm (Spectra Max 190 Microplate Reader, Molecular Devices, CA). The change of hydrolytic product glucose was expressed as a change in absorbance, and the reduction of glucose concentration in the treatment groups was due to the presence of the potato peel extracts. The inhibition power of the potato peels on mucosal α-glucosidase was calculated as the percentage of enzyme activity diminished with the addition of potato peel extract. Each hydrolysis reaction was performed in triplicate, and glucose quantification was also performed in triplicate.

2.3.5 Quantifying pectin in potato peels

Pectin was quantified through a uronic acid assay following the method developed by Blumenkrantz and Asboe-Hansen (1973). Potato peel extract-saline buffer solution (40 µL) and galacturonic acid solutions with various concentrations (0, 40, 80, 120, 160, 200 µg/mL; 40µL of each) were added into a 96-well microplate. Then, sulphamic acid (4M, 5 µL), prepared by dissolving sulphamic acid crystal in deionized tap water, was added into the wells and mixed with potato peel extracts or galacturonic acid solutions to prevent the interference of neutral sugars on the quantification of galacturonic acid (Filisetti-Cozzi & Carpita, 1991). Acid/borax solution (200 µL), which consisted of 12.5 mM disodium tetraborate borax (4.7 g) in concentrated sulfuric acid (1L, 18 M), was added into the wells containing potato peel extracts, galacturonic acids, and the previously added reagents. The microplate was covered and heated at 95 °C in a convection oven for 60 min, and then chilled in an ice bath for 20 min. Diphenyl reagent (2.9 mM, 10 µL), prepared by dissolving 3-phenyl phenol (50 mg) and sodium hydroxide (0.5 g) in deionized tap water (100 mL), was added into those cells and then, the microplate was set at room temperature (20-22 °C) for 5 min to allow for color development. The quantity of galacturonic acid was determined by reading the absorbance at 520 nm using a microplate reader (Spectra Max 190 Microplate Reader, Molecular Devices, CA). The galacturonic acid was applied to this analysis to generate a calibration curve of absorbance and concentration, and the pectin quantity in the potato peel extracts was calculated based on the calibration curve and presented as µg of galacturonic acid per mL of peel extracts.

2.3.6 Quantifying phenolic acids in potato peels

The quantification of chlorogenic, ferulic, and caffeic acid, as well as catechin, followed the procedure reported by Singh and Saldaña (2011) with modifications. Potato peel extract-saline phosphate buffer (200 µL) was mixed with methanol (800 µL, HPLC grade) in a microcentrifuge tube (2 mL) and vortexed. Then, this potato peel extract-methanol mixture was centrifuged at 10,000 ×g for 5 min at 4 °C. The supernatant was further filtered through a syringe filter with a membrane of 0.45-µm pore size before injection into a High-Performance Liquid Chromatography (HPLC) system, which consisted of Waters 996 HPLC system

(Waters Corporation, Milford, MA) equipped with Waters 2489 UV/Vis detector (Waters Corporation, Milford, MA), and a Waters Symmetry C18 column (4.6 x 150 mm, 3.5 µm). The mobile phase consisted of eluent A: 0.5% formic acid (ACS grade) in water (HPLC grade) and eluent B: 0.5% formic acid (ACS grade) in methanol (HPLC grade). The mobile phase was eluted with an eight-step linear-gradient initially starting at 84% eluent A and 16% eluent B; then eluent B rose to 19% by 15 min, to 27% by 25 min, to 41% by 26 min, to 65% by 36 min, and reached 100% of eluent B by 44 min. Afterward, eluent B decreased to 16% of the mobile phase composition by 45 min. The total elution time was 60 min with a flow rate at 0.5 mL/min. The sample loading loop size was 20 µL, and the signals were detected at 280 nm. The calibration curve was established by injecting six different concentrations of chlorogenic, caffeic acid, and ferulic acid as well as catechin ranging from 0.005 mM to 0.8 mM. The quantification of phenolic acids was determined by plotting the corresponding peak area and superimposing it onto the calibration curve. The quantified chlorogenic, caffeic acid, and ferulic acid, as well as the catechin concentrations, were combined and presented as the total phenolic acid content in the potato peel extract.

2.3.7 Quantifying calystegine in potato peels

The calystegine in the potato peel extract was quantified using high-performance liquid chromatography/tandem mass spectrometry analysis following Petersson et al. (2013). The Potato peel extract-saline phosphate buffer (44 µL) was mixed with acetonitrile (HPLC grade, 320 µL) and ammonium acetate (HPLC grade, 80 µL). The sample and calystegine standard (2 µL) were injected into an Agilent LCMS Infinity 1200 (Aligent Technologies Inc., Santa Clara, CA) with a 100 mm X 3.0µm Atlantis Hilic column (Waters Corporation, Milford, MA) and ESI ion source at a flow rate of 0.45 mL/min at 35 ºC. Mobile phase A consisted of 0.02M ammonium acetate (HPLC grade, pH 5.3) in water (HPLC grade), and mobile phase B consisted of 100% acetonitrile (HPLC grade). The gradient started with 10% A and 90% B, and changed linearly over 0.5 min and 4.5 min to 60% A and 40% B, respectively. The gradient was finally held at 60% A and 40% B for 1 min. C. The LC system was connected to a 5500 QTRAP tandem mass spectrometer (AB SCIEX, Concord, ON, Canada), equipped with a Turbo VTM ion source operated in positive ion mode. The ion source parameters were as follows: a needle voltage of 4300 V, curtain gas at 275 kPa, nebulizer and Turbo gas at 410

kPa, and Turbo gas at a temperature of 600 °C. Delustering potential, collision potential, and collision cell exit potential were optimized during the infusion of a mixture of the analytes (10−100 ng/mL) employing Analyst 1.5 software (AB SCIEX, Concord, ON, Canada). The concentration of calystegine isomers was calculated based on the calibration curve generated by the standards, and the concentration of calystegine isomers was combined and reported as calystegine content.

2.3.8 Quantifying copper, nickel, and zinc in potato peels

The mineral content of potato peel extract was quantified using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Potato peel extract-saline phosphate buffer was filtered through a syringe filter with a membrane pore size of 0.45-µm before injecting into an Agilent 7500cx ICP-MScx (Aligent Technologies Inc., Santa Clara, CA). Following a published method, the system was set as follows: nebulizer at 41 psi, an auxiliary flow of 0.20, a pump rate of 1.0 mL/min, a scan integration time of 0.25 s, a Mn peaking wavelength, and acid flexible tubing with 0.030 mm internal diameter (Anderson, 1996). The element calibration standard was prepared at concentrations of 100, 10, 1 and 0.1 µg/mL and injected to generate a calibration curve. The copper, zinc, and nickel in potato peel extract, which in the literature showed the most significant inhibition on α-amylase and mucosal α-glucosidase (Wang et al., 2004), were quantified and the concentrations were combined and represented as total mineral content.

2.3.9 Data analysis

The results were presented as mean (M) and ± standard deviation (SD). The significant effect of treatment was determined by one-way analysis of variance (ANOVA), and Tukey's multiple comparison test was performed to identify which mean amongst a set of means differed from the rest. SPSS statistic software was used to analyze the data (Version 25, IBM Corporation, Armonk, NY). We used an alpha level of .05 for all statistical tests, and statistically significant differences were considered significant at $p < .05$.

2.4 RESULTS

2.4.1 The influence of potato peels in α-amylase hydrolytic activity

The influence of potato varieties - The impact of potato peels on α-amylase hydrolytic activity is presented as inhibition power, which is based on the reduction of the concentration of the hydrolytic product, p-nitrophenol, in an in vitro hydrolysis system with the presence of potato peels compared with the control group without potato peels. There was a statistically significant difference between the control group (without potato peels) and treatment group (with potato peels) as determined by one-way ANOVA (F (4, 1) = 56.3, $p = .000$). The significance of cultivar differences was determined by two-way ANOVA, and the results indicated significant differences among five varieties for α-amylase inhibition (F (4, 1) = 125.8, $p = .000$). Among the five varieties grown in 2016, Ranger Russet had the highest inhibition power (M = 30.9%, SD = 1.2%) followed by Payette Russet (M = 24.9%, SD = 2.4%), Russet Burbank (M = 24.2%, SD = 0.87%), Umatilla Russet (M = 8.58%, SD = 5.1%), and Clearwater Russet (M = 0.76%, SD = 1.1%). A Tukey post hoc test revealed that Ranger Russet had a statistically significant higher inhibition power than Clearwater Russet (p = 0.000), and Umatilla Russet ($p = .000$), while there was not a statistical significance when comparing Ranger Russet with Russet Burbank ($p = .076$), and Payette Russet ($p = .125$). Russet Burbank had a statistically significant higher inhibition power than Umatilla Russet ($p = .000$) and Clearwater Russet ($p = .000$), while Umatilla Russet had a significantly higher inhibition power than Clearwater Russet ($p = .035$). In 2017, similar to the trend of 2016, Ranger Russet had the highest inhibition power (M = 22.4%, SD = 1.2%) followed by Russet Burbank (M = 16.5%, SD = 0.60%), Payette Russet (M = 14.8%, SD = 2.5%), Umatilla Russet (M = 6.23%, SD = 0.71%), and Clearwater Russet (M = 5.52%, SD = 0.71%) The statistical significance between the varieties was the same in 2016 and 2017. As in 2016, Clearwater Russet and Umatilla Russet had the lowest inhibition power among the five varieties.

The influence of growing years - There was a statistically significant difference between growing years as determined by two-way ANOVA (F (1, 4) = 37.558, $p = .000$). For the individual varieties, we also observed a significant difference between the two growing years in Ranger Russet ($p = .010$), Russet Burbank ($p = .012$), Payette Russet ($p = .021$), and

Clearwater Russet ($p = .000$); there was no significant difference between 2016 and 2017 in Umatilla Russet ($p = .063$).

The influence of peeling methods - The five varieties, which were planted in 2017, were hand-peeled and steam-peeled. The data presented in the sections "The influence of potato varieties" and "The influence of growing years" were the results of how the hand- peeling affected potato peels. The inhibition power of the five varieties using industrial-scale steam-peeling had a similar trend in which Ranger Russet had the highest inhibition power (M = 57.5%, SD = 3.0%) followed by Russet Burbank (M = 53.1%, SD = 1.9%), Umatilla Russet (M = 49.7%, SD = 4.7%), Payette Russet (M = 42.4%, SD = 2.2%), and Clearwater Russet (M = 34.8%, SD = 1.6%). There was a statistically significant difference in the hand-peeled and steam-peeled potato peels as determined by one-way ANOVA (F (1, 4) = 1713.73, $p = .000$). The increased inhibition power in each cultivar was different in which the increase in Umatilla Russet was 43.4%, followed by Russet Burbank (36.5%), Ranger Russet (35.1%), Clearwater Russet (29.3%), and Payette Russet (27.6%).

In the industry, abrasion-peeling and steam-peeling are two common peeling techniques. We collected industrial peel waste in the Northwest of the United States to demonstrate the inhibition power of peels obtained from these two techniques. The peel waste was collected from the potato processors' manufacturing lines, which was generated from a number of different varieties with different processing parameters that can potentially influence the inhibition power. The selected potato varieties used in this study were the primary varieties planted in the Northwest in the United States. There was a statistically significant difference between abrasion-peeled waste (M = 6.94%, SD = 0.87%) and steam-peeled waste (M = 41.26%, SD = 2.87%) as determined by a Student's t-test (t (4) = 19.821, $p = .000$).

2.4.2 The influence of potato peels in mucosal α-glucosidase hydrolytic activity

The influence of potato varieties - The impact of potato peels on mucosal α-glucosidase hydrolytic activity is presented as inhibition power, which is based on the reduction of the concentration of the hydrolytic product, glucose, in an in vitro hydrolysis system with the presence of potato peels compared with the control without potato peels. There was a statistically significant difference between the control group (without potato peels) and treatment group (with potato peels) as determined by two-way ANOVA (F (1, 4) = 7.49, p

= *.001*). Among the five varieties grown in 2016, Ranger Russet had the highest inhibition power (*M* = 32.6%, *SD* = 2.3%) followed by Clearwater Russet (*M* = 29.1%, *SD* = 2.9%), Russet Burbank (*M* = 27.8%, *SD* = 2.6%), Payette Russet (*M* = 27.3%, *SD* = 1.9%), and Umatilla Russet (*M* = 25.7%, *SD* = 1.9%). A Tukey post hoc test revealed that Ranger Russet had a statistically significant higher inhibition power (*p = .029*) than Umatilla Russet, and there was not a statistical significance among Ranger Russet, Russet Burbank, Clearwater Russet and Payette Russet. In 2017, the trend was different than in 2016; Ranger Russet still had the highest inhibition power (*M* = 20.54%, *SD* = 0.62%) followed by Payette Russet (*M* = 18.9%, *SD* = 2.9%), Clearwater Russet (*M* = 15.8%, *SD* = 2.9%), Russet Burbank (*M* = 14.6%, *SD* = 3.2%), and Umatilla Russet (*M* = 12.9%, *SD* = 1.7%) The statistical significance between the varieties was the same in 2016 and 2017. As in 2016, Ranger Russet had a significantly higher inhibition power than Umatilla Russet (*p = .024*).

The influence of growing years - There was a statistically significant difference between growing years as determined by one-way ANOVA (F (1, 4) = 184.7, *p = .000*). For the individual varieties, we also observed a significant difference between the two growing years in Ranger Russet (*p = .022*), Russet Burbank (*p = .034*), Umatilla Russet (*p = .000*), and Clearwater Russet (*p = .011*); there was no significant difference between 2016 and 2017 in Payette Russet (*p = .054*).

The influence of peeling methods - The inhibition power of the five varieties using industrial-scale steam-peeling had a different trend compared with hand peeling in which Ranger Russet still had the highest inhibition power (*M* = 67.7%, *SD* = 1.2%) followed by Russet Burbank (*M* = 57.5%, *SD* = 1.7%), Umatilla Russet (*M* = 54.4%, *SD* = 2.6%) and Payette Russet (*M* = 53.4%, *SD* = 2.2%). Clearwater Russet (*M* = 27.2%, *SD* = 1.4%) did not show significant increases in inhibition power when comparing hand peeling to steam peeling. There was a statistically significant difference in hand-peeled potato peels and steam-peeled potato peels as determined by one-way ANOVA (F (1, 4) = 1845.27, *p = .000*.) The increased inhibition power in each cultivar was different in which the highest increase was in Ranger Russet at 43.4%, followed by Russet Burbank (42.8%), Umatilla Russet (41.5%), Payette Russet (34.5%), and Clearwater Russet (11.4%).

For the comparison between abrasion peeled peel waste and steam peeled peel waste, there was a statistically significant difference between abrasion-peeled waste (*M* = 18.4%, *SD*

= 1.97%) and steam-peeled waste (*M* = 48.72%, *SD* = 2.23%) as determined by a Student's t test (t (4) = 17.758, *p* = *.000*).

2.4.3 The quantity of inhibitor pectin in potato peels

Pectin quantity in the peels from different potato varieties - The pectin content of potato peel extract ranged from 190.56 µg/mL to 410.3 µg/mL.There were significant differences of pectin content among the five varieties as determined by two-way ANOVA (F (4, 1) = 91.28, *p* = *.000*). Among the five varieties grown in 2016, Clearwater Russet contained the highest pectin content (*M* = 409.6 µg/mL, *SD* = 29.2 µg/mL), followed by Ranger Russet (*M* = 352.5 µg/mL, *SD* = 14.3 µg/mL), Umatilla Russet (*M* = 321.7 µg/mL, *SD* = 15.3 µg/mL), Russet Burbank (*M* = 283.0 µg/mL, *SD* = 10.8 µg/mL), and Payette Russet (*M* = 190.56 µg/mL, *SD* = 5.67 µg/mL). A Tukey post hoc test revealed that Clearwater Russet had significantly higher pectin content compared with the other four varieties (*p =.000, .000, .014, .001* for Russet Burbank, Clearwater Russet, Ranger Russet, and Umatilla Russet, respectively). Ranger Russet did not have significant differences compared to Umatilla Russet (*p =.252*), while both had significantly higher pectin content compared with Clearwater Russet (*p =.014, .001*).

The pectin content of potato peel extract from 2017 followed a similar trend as 2016. Clearwater Russet contained the highest pectin content (*M* = 410.3 µg /mL, *SD* = 32.1 µg /mL), followed by Umatilla Russet (*M* = 347.6 µg /mL, *SD* = 14.2 µg mL), Ranger Russet (*M* = 342.2 µg /mL, *SD* = 33.1 µg/mL), Russet Burbank (*M* = 295.7 µg /mL, *SD* = 9.84 µg /mL), and Payette Russet (*M* = 210.3 µg /mL, *SD* = 9.72 µg /mL). Clearwater Russet had significantly higher pectin content (*p = .000, .001, .026, 0.041* for Payette Russet, Russet Burbank, Ranger Russet and Umatilla Russet, respectively) compared with the four other varieties, while the pectin content among Clearwater Russet, Umatilla Russet, Ranger Russet, and Russet Burbank did not have significant differences. Payette Russet had a significantly lower pectin content (*p =.006, .000, .000, .000* for Russet Burbank, Clearwater Russet, Ranger Russet, and Umatilla Russet, respectively).

Pectin quantity in the peels from potatoes grown in different years - There were no significant differences when comparing the pectin content of potato peel extract from 2016 and 2017 as determined by one-way ANOVA (F (4, 1) = 1.782, *p* = *.197*).

Pectin quantity in the peels processed from different methods - The pectin content of the five varieties peeled by industrial scale steam-peeling had a different trend compared with those peeled by hand peeling in which Russet Burbank had the highest pectin content (*M* = 1608.5 ug/mL, *SD* = 95.0 ug/mL) followed by Umatilla Russet (*M* = 1549.8 ug/mL, *SD* = 22.98 ug/mL), Payette Russet (*M* = 1429.8 ug/mL, *SD* = 59.5 ug/mL) and Ranger Russet (*M* = 965.5 ug/mL, *SD* = 16.0 ug/mL). There was a statistically significant difference in the pectin content of hand-peeled and steam-peeled potato peel extract from the five varieties as determined by two-way ANOVA (F (4, 1) = 4762.7, *p =.000*).

For the comparison between abrasion-peeled peel waste and steam-peeled peel waste, there was a statistically significant difference in pectin content between abrasion-peeled waste (*M* = 1430.3 ug/mL, *SD* = 22.2 ug/mL) and steam-peeled waste (*M* = 1523.3 ug/mL, *SD* = 33.53 ug/mL) as determined by a Student's t test (t (4) = -4.005, *p =.016*).

2.4.4 The quantity of inhibitor phenolic acids in potato peels

Phenolic acid quantity in peels from different potato varieties - Chlorogenic acid, ferulic acid, caffeic acid and catechin were quantified and combined as phenolic acid content of potato peel extract. There was a statistical significance in phenolic acid content among five varieties as determined by two-way ANOVA (F (4, 1) = 24.4, *p =.000*). The phenolic acid content of the potato peel extract ranged from 0.0525 mM to 0.0776 mM. Among the five varieties grown in 2016, Ranger Russet contained the highest phenolic acid content (*M* = 0.0776 mM, *SD* = 0.002 mM), followed by Russet Burbank (*M* = 0.0766 mM, *SD* = 0.002 mM), Payette Russet (*M* = 0.0619 mM, *SD* = 0.0004 mM), Umatilla Russet (*M* = 0.0587 mM, *SD* = 0.001 mM), and Clearwater Russet (*M* = 0.0506 mM, *SD* = 0.002 mM). A Tukey post hoc test revealed that Ranger Russet and Russet Burbank did not contain significantly different phenolic acid content (*p = .875*), but both had significantly higher phenolic acid content than Clearwater Russet (*p =.001, .004*). Ranger Russet also had significantly higher phenolic acid content compared with Umatilla Russet (*p =.014*) and Payette Russet (*p = .042*).

The phenolic acid content of potato peel extract from 2017 followed a different trend than that of 2016. Russet Burbank contained the highest phenolic acid content (*M* = 0.0745 mM, *SD* = 0.004 mM), followed by Ranger Russet (*M* = 0.0651 mM, *SD* = 0.004 mM),

Clearwater Russet (*M* = 0.0581 mM, *SD* = 0.001 mM), Payette Russet (*M* = 0.0565 mM, *SD* = 0.003 mM), and Umatilla Russet (*M* = 0.0525 mM, *SD* = 0.002 mM). Russet Burbank had significantly higher phenolic acid content (*p = .000, .000, .021, .000* for Payette Russet, Clearwater Russet, Ranger Russet, and Umatilla Russet, respectively) compared with all of the other varieties. While Ranger Russet had significantly higher phenolic acid content compared with Payette Russet (*p = .036*) and Umatilla Russet (*p = .003*).

Phenolic acid quantity in the peels from potatoes grown in different years - There was a statistically significant difference between growing years as determined by two-way ANOVA (F (1, 4) = 6.43, *p = .023*). For the individual varieties, we also observed a significant difference between the two growing years in Ranger Russet (*p = .012*), Clearwater Russet (*p = .008*). There was no significant difference between 2016 and 2017 in Payette Russet (*p = .234*), Umatilla Russet (*p = .067*), and Russet Burbank (*p = .102*).

Phenolic acid quantity in the peels processed from different methods - The phenolic acid content in the five varieties using industrial scale steam-peeling had a similar trend with hand peeling in which Ranger Russet contained the highest phenolic acid content (*M* = 2.95 mM, *SD* = 0.08 mM), followed by Russet Burbank (*M* = 2.21 mM, *SD* = 0.08 mM), Umatilla Russet (*M* = 1.96 mM, *SD* = 0.02 mM), Payette Russet (*M* = 1.81 mM, *SD* = 0.1 mM), and Clearwater Russet (*M* = 0.21 mM, *SD* = 0.08 mM). There was a statistically significant difference in hand-peeled versus steam-peeled potato peel extract phenolic acid content as determined by two-way ANOVA (F (1, 4) = 9943.54, *p =.000*).

For the comparison between abrasion peeled peel waste and steam peeled peel waste, there was a statistically significant difference in phenolic acid content between abrasion-peeled waste (*M* = 0.089 mM, *SD* = 0.003 mM) and steam-peeled waste (*M* = 1.61 mM, *SD* = 0.02 mM) as determined by a Student's t test (t (4) = 111.345, *p =.000*).

2.4.5 The quantity of inhibitor calystegine in potato peels

Calystegine quantity in the peels from different potato varieties - Three identified calystegine A isomers and two identified claystegine B isomers were quantified and combined as calystegine content of potato peel extract. There was a statistical significance among the five varieties in calystegine content as determined by two-way ANOVA (F (4, 1) = 67.1, *p =.000*). The calystegine content of the potato peel extract ranged from 7.70 µg/mL to 12.31

µg/mL. Among the five varieties grown in 2016, Clearwater Russet contained the highest calystegine content (*M* = 12.31 µg/mL, *SD* = 0.52 µg/mL), followed by Russet Burbank (*M* = 12.07 µg/mL, *SD* = 0.21 µg/mL), Umatilla Russet (*M* = 11.00 µg/mL, *SD* = 0.33 µg/mL), Ranger Russet (*M* = 10.93 µg/mL, *SD* = 0.52 µg/mL), and Payette Russet (*M* = 7.87 µg/mL, *SD* = 0.43 µg/mL). A Tukey post hoc test revealed that Clearwater Russet and Russet Burbank did not have significant differences in calystegine content (*p = .949*), while both had significantly higher calystegine content than Ranger Russet (*p = .015, .044*) and Payette Russet (*p = .000, .000*). Ranger Russet did not have significant differences with Umatilla Russet (*p* = 1.000), while both had significantly higher calystegine content than Payette Russet (*p = .000, .000*).

The calystegine content of the potato peel extract from 2017 followed a different trend than 2016. Ranger Russet contained the highest calystegine content (*M* = 10.41 µg/mL, *SD* = 0.44 µg/mL), followed by Clearwater Russet (*M* = 8.76 µg/mL, *SD* = 0.44 µg/mL), Russet Burbank (*M* = 8.07 µg/mL, *SD* = 0.56 µg/mL), Payette Russet (*M* = 7.70 µg/mL, *SD* = 0.32 µg/mL) and Umatilla Russet (*M* = 4.60 µg/mL, *SD* = 0.49 µg/mL). Ranger Russet had significantly higher calystegine content (*p = .000, .001, .009, .000* for Payette Russet, Russet Burbank, Clearwater Russet, and Umatilla Russet, respectively) compared with all of the other varieties. There was not a significant difference between Clearwater Russet and Russet Burbank (*p =.401*), but both were significantly higher than Umatilla Russet (*p =.000, .000*).

Calystegine quantity in the peels from potatoes grown in different years - There was a statistically significant difference between growing years as determined by two-way ANOVA (F (1, 4) = 338.03, *p =.000*). For the individual varieties, we also observed a significant difference between the two growing years in Russet Burbank (*p =.000*), Clearwater Russet (*p =.000*) and Umatilla Russet (*p =.000*). There was no significant difference between 2016 and 2017 in Ranger Russet (p =*.834*), and Payette Russet (*p =.953*).

Calystegine quantity in the peels processed from different methods - There was a statistically significant difference in the calystegine content of the hand-peeled and steam-peeled potato peel extract as determined by two-way ANOVA (F (1, 4) = 489.37, *p =.000*). Among the calystegine content of the five varieties, Russet Burbank contained the highest calystegine content (*M* = 6.92 µg/mL, *SD* = 0.24 µg/mL), followed by Payette Russet (*M* = 5.36 µg/mL, *SD* = 0.22 µg/mL), Ranger Russet (*M* = 4.78 µg/mL, *SD* = 0.20 µg/mL),

Clearwater Russet (*M* = 4.62 µg/mL, *SD* = 0.18 µg/mL), and Umatilla Russet (*M* = 3.52 µg/mL, *SD* = 0.18 µg/mL). Compared with hand-peeled extract, steam-peeled extract contained significantly less calystegine content in Ranger Russet (*p =.000*), Clearwater Russet (*p = .000*), and Payette Russet (*p =.009*).

There was not a statistically significant difference in calystegine content between abrasion-peeled waste (*M* = 5.04 µg/mL, *SD* = 0.34 µg/mL) and steam-peeled waste (*M* = 5.53 µg/mL, *SD* = 0.25 µg/mL) as determined by a Student's t test (t (4) = -2.090, *p* = 0.105).

2.4.6 The quantity of inhibitor copper, nickel, and zinc in potato peels

Mineral quantity in the peels from different potato varieties - Copper, nickel and zinc were quantified and combined as mineral content of potato peel extract. There was a statistical significance in calystegine content among the five varieties as determined by two-way ANOVA (F (4, 1) = 13.8, *p =.000*). The mineral content of the potato peel extract ranged from 2.67 µg/mL to 1.99 µg/mL. Among the five varieties grown in 2016, Clearwater Russet contained the highest mineral content (*M* = 2.73 µg/mL, *SD* = 0.12 µg/mL), followed by Payette Russet (*M* = 2.47 µg/mL, *SD* = 0.06 µg/mL), Umatilla Russet (*M* = 2.50 µg/mL, *SD* = 0.00 µg/mL), Ranger Russet (*M* = 2.20 µg/mL, *SD* = 0.10 µg/mL), and Russet Burbank (*M* = 1.99 µg/mL, *SD* = 0.10 µg/mL). A Tukey post hoc test revealed that Clearwater Russet and Umatilla Russet did not have significant differences in mineral content (p =*.056*), while both had significantly higher calystegine content than Ranger Russet (p =*.000, .012*) and Russet Burbank (p =*.000, .000*). Ranger Russet did not have significant differences with Russet Burbank (*p =.085*).

The mineral content of potato peel extract from 2017 showed a different trend than 2016. Payette Russet contained the highest calystegine content (*M* = 2.71 µg/mL, *SD* = 0.09 µg/mL), followed by Umatilla Russet (*M* = 2.67 µg/mL, *SD* = 0.12 µg/mL), Clearwater Russet (*M* = 2.47 µg/mL, *SD* = 0.06 µg/mL), Russet Burbank (*M* = 2.40 µg/mL, *SD* = 0.36 µg/mL), and Ranger Russet (*M* = 2.20 µg/mL, *SD* = 0.10 µg/mL). Payette Russet had significantly higher mineral content (*p =.040*) than Ranger Russet, while no other significant differences were observed among the five varieties.

Mineral quantity in the peels from potatoes grown in different years - There was a statistically significant difference between growing years as determined by two-way ANOVA

(F (1, 4) = 4.622, *p =.044*). For the individual varieties, we also observed a significant difference between the two growing years in Payette Russet (*p =.020*), Russet Burbank (*p =.010*), Clearwater Russet (p =*.009*) and Umatilla Russet *(p =.030*). There were no significant growing year differences in Ranger Russet (*p = 1.000*).

Mineral quantity in the peels processed from different methods - There was a statistically significant difference in the mineral content of the hand-peeled and steam-peeled potato peel extract as determined by two-way ANOVA (F (1, 4) = 716.50, *p =.000*). Among the mineral content of the five varieties, Clearwater Russet contained the highest mineral content (*M* = 25.97 µg/mL, *SD* = 1.59 µg/mL), followed by Umatilla Russet (*M* = 24.60 µg/mL, *SD* = 2.86 µg/mL), Ranger Russet (*M* = 16.63 µg/mL, *SD* = 0.58 µg/mL), Payette Russet (*M* = 5.87 µg/mL, *SD* = 2.55 µg/mL), and Russet Burbank (*M* = 4.67 µg/mL, *SD* = 0.38 µg/mL). Compared with hand-peeled extract, steam-peeled extract from all five varieties contained significantly higher mineral content at *p =.000*.

For the comparison between abrasion peeled peel waste and steam peeled peel waste, there was not a statistically significant difference in the mineral content between abrasion-peeled waste (*M* = 5.70 µg/mL, *SD* = 0.46 µg/mL) and steam-peeled waste (*M* = 4.60 µg/mL, *SD* = 0.63 µg/mL) as determined by a Student's t test (t (4) = 2.44, *p =.071*).

2.5 DISCUSSION

2.5.1 Cultivar differences on inhibitor content and α-amylase and mucosal α-glucosidase inhibition

In this study, we found that the peel extract from five common processing potato varieties had significant differences in α-amylase and mucosal α-glucosidase inhibition function, which correlates closely with the inhibitor content. Ranger Russet, with the higher concentrations of phenolic acid and calystegine content in two consecutive growing years, showed significantly higher inhibition on both α-amylase and mucosal α-glucosidase compared to Clearwater Russet and Umatilla Russet. The selective inhibition of calystegine on mucosal α-glucosidase was also observed. Clearwater Russet which had the highest content of calystegine and low phenolic acid content showed similar inhibition performances

as Ranger Russet for mucosal α-glucosidase inhibition. However, the potato peel extract from Clearwater Russet showed significantly lower inhibition on α-amylase compared with Ranger Russet due to its lower phenolic acid content. This agreed with our previous study that calystegine and phenolic acid had a joint effect on mucosal α-glucosidase inhibition while phenolic acid was the key inhibitor for α-amylase. Mineral content and pectin content did not have a strong correlation with α-amylase and mucosal α-glucosidase inhibition. Ranger Russet, for example, had comparably lower pectin and mineral content compared with Clearwater Russet while having a higher inhibition power. This finding also agreed with our previous study that minerals and soluble dietary fiber could be minor factors that affect the inhibition power on α-amylase and mucosal α-glucosidase. We, therefore, suggest that due to their high content of starch digestive enzyme inhibitors such as phenolic acid and calystegine, certain potato varieties such as Ranger Russet might be more beneficial to modulate glycemic response compared with other varieties such as Umatilla Russet.

2.5.2 Potential environmental effect on α-amylase and mucosal α-glucosidase inhibition

The inhibition power and inhibitor content of potato peel extract was also highly associated with environmental factors, and we observed significant differences between the two consecutive growing years. In most of the varieties, potato peel extract from potatoes harvested in 2016 showed significantly higher content of phenolic acid and calystegine compared with the 2017 harvest year. This was also reflected in the inhibition performances as all five varieties, which showed significantly higher α-amylase and mucosal α-glucosidase inhibition in the 2016 growing year compared with 2017. The differences in environmental conditions in the two growing years might explain the large differences in inhibitor concentrations and inhibition performances. It has been reported that potatoes grown during a period of extended daylight and cooler temperatures contain about 1.4 times higher total phenolic concentration (Reyes, Miller, & Cisneros-Zevallos, 2004). In 2017, the potatoes grown in Idaho experienced unusually hot weather that resulted in a major yield reduction compared with the previous 2016 growing year. According to weather records at Parma, Idaho, the temperatures in August, the month when potato tuber growth is greatest, was significantly higher in 2017 at 79.04 °C than in 2016 at 73.48 °C (Climate Parma - Idaho, 2019). The hot weather and other environmental stresses might be the factor that contributed

to the major reduction of phenolic acid and calystegine content in potato peels that further affected their inhibition performances.

2.5.3 Effect of peeling method on α-amylase and mucosal α-glucosidase inhibition

Peeling method also played a critical role in determining inhibitor content and potato peel extracts' inhibition on starch digestive enzymes. In this study, we compared three major peeling methods: hand-peeling, steam-peeling, and abrasion-peeling. Comparing with hand-peeled potato peel extract, the industrial steam-peeled extract showed significantly higher inhibition power on both α-amylase and mucosal α-glucosidase. The high inhibition power was primarily a result of the significantly higher phenolic acid content in the steam-peeled extract which was more than 30 times higher than the content in hand-peeled extract. The reason for the differences could be due to the peel composition differences from the two peeling methods. The potato peel is the most abundant site for phenolic compounds with concentrations, on average, more than ten times higher than potato flesh (Mäder et al., 2009). The peel is also known for its high concentrations of calystegine, mineral, and dietary fiber compared with the flesh. Steam-peeling compared with hand-peeling had a significantly lower quantity of flesh attached to the peel, which thus, resulted in the major reduction of starch and protein proportion in the peel waste and significantly increased the proportion of phenolic acid, mineral and dietary fiber in the potato peel extract (Toma et al., 1979). As a result, the removal of potato peel through steam peeling resulted in significantly higher inhibition power. However, though the potato peel is where calystegine content is primarily located, the calystegine content in potato peel extract processed with steam peeling was significantly less than hand-peeling. The significant reduction of calystegine content from steam peeling might be a result of the combination of high temperatures and extended washing during the peeling process.

We also compared the inhibition differences between steam-peeling and abrasion-peeling by directly collecting potato peel waste from processing plants. Our inhibition study indicated that the potato peel extract from steam-peeling had significantly higher α-amylase and mucosal α-glucosidase inhibition power compared with the potato peel extract from abrasion peeling. The quantification of major inhibitors indicated a significantly higher quantity of phenolic acid in the peel extract processed with steam peeling while other major

inhibitor concentrations were not significantly different. Therefore, the high concentration of phenolic acid in steam peeling peel extract could be the major factor that resulted in the significantly higher inhibition on both starch digestive enzymes. The differences in mechanisms between two peeling methods might be the major cause of peel extract composition and inhibition differences. During abrasion peeling, the potato peel was mechanically shredded which resulted in more starch and less dietary fiber and smaller peel sizes compared with steam peeling, which heated the peels using high-pressure steam. The higher starch and lower dietary fiber content indicated more potato flesh was attached to the peel waste generated by abrasion peeling (Camire et al., 1997). At the same time, the smaller peel sizes might result in a major loss of phenolic acid during the washing and cleaning stage after peeling. Other factors such as potato cultivar differences used by chip and potato powder processing (abrasion peeling) and by French Fries processing (steam peeling) might also affect potato peel extract composition and inhibition power (Al-Weshahy & Rao, 2012).

2.6 CONCLUSION

As a conclusion, our current study showed that potato peel extract from different varieties had different inhibitor concentrations which further affected the inhibition power on α-amylase and mucosal α-glucosidase. Varieties such as Ranger Russet had higher inhibitor concentration and inhibition power compared with varieties such as Umatilla Russet and Clearwater Russet, which showed that Ranger Russet might be an optimal cultivar to collect peels during processing for inhibitor extraction. Other than cultivar differences, growing temperature and peeling method also had major effects on peel extract composition and inhibition. The higher growing temperature during tuber development had the potential to cause the major reduction of inhibition power. Steam peeling, compared with hand peeling and abrasion showed major increases in phenolic acid content and lower calystegine content, which resulted in significantly higher α-amylase and mucosal α-glucosidase inhibition. Our study, therefore, could be used to help the potato processing industry to further develop the utilization of potato peel as natural enzyme inhibitors to modulate glycemic response.

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2.8 TABLES AND FIGURES

Table 2. 1 **Quantification of potential inhibitors from potato peel extract from hand peeling and steam peeling of the 2016 and 2017 potatoes and industrial waste.**

Year/ peeling method	Varieties[1]	Phenolic acid[2] (mM)	Calystegine[3] (µg/mL)	Minerals[4] (µg/mL)	Pectin (µg/mL)
2016 Hand-peeled	Payette Russet	0.0619 ± 0.0004^{a}	7.87 ± 0.43^{a}	2.47 ± 0.06^{a}	190.56 ± 5.67^{a}
	Russet Burbank	0.0766 ± 0.0021^{ab}	12.07 ± 0.2^{b}	1.89 ± 0.10^{b}	283.02 ± 10.84^{b}
	Clearwater Russet	0.0506 ± 0.0027^{ac}	12.31 ± 0.5^{b}	2.73 ± 0.12^{c}	409.57 ± 29.23^{c}
	Ranger Russet	0.0776 ± 0.0019^{b}	10.93 ± 0.52^{c}	2.20 ± 0.10^{b}	352.54 ± 14.35^{d}
	Umatilla Russet	0.0587 ± 0.0105^{a}	11.00 ± 0.33^{bc}	2.50 ± 0.00^{ac}	321.79 ± 15.67^{bd}
2017 Hand- peeled	Payette Russet	0.0565 ± 0.0029^{a}	7.70 ± 0.32^{a}	2.71 ± 0.09^{a}	210.32 ± 9.72^{a}
	Russet Burbank	0.0745 ± 0.0038^{b}	8.07 ± 0.56^{a}	2.40 ± 0.36^{ab}	295.67 ± 9.84^{b}
	Clearwater Russet	0.0581 ± 0.0009^{ac}	8.76 ± 0.44^{a}	2.47 ± 0.06^{ab}	410.30 ± 32.12^{c}
	Ranger Russet	0.0651 ± 0.0041^{c}	10.41 ± 0.44^{b}	2.20 ± 0.10^{b}	342.19 ± 33.16^{b}
	Umatilla Russet	0.0525 ± 0.0022^{a}	4.60 ± 0.49^{c}	2.67 ± 0.12^{ab}	347.62 ± 14.23^{b}
2017 Steam-peeled	Payette Russet	1.8086 ± 0.1003^{a}	5.36 ± 0.22^{a}	5.87 ± 2.55^{a}	1429.79 ± 59.53^{a}
	Russet Burbank	2.2076 ± 0.0839^{b}	6.92 ± 0.24^{b}	4.67 ± 0.38^{b}	1608.53 ± 95.03^{a}
	Clearwater Russet	0.2063 ± 0.0029^{c}	4.62 ± 0.18^{a}	25.9 ± 1.59^{c}	1109.64 ± 15.05^{b}
	Ranger Russet	2.9484 ± 0.0761^{b}	4.78 ± 0.20^{a}	16.6 ± 0.58^{d}	965.52 ± 15.97^{b}
	Umatilla Russet	1.9560 ± 0.023^{ab}	3.52 ± 0.18^{c}	24.6 ± 2.86^{cd}	1549.76 ± 22.98^{a}
Steam-peeled	Industrial Waste	1.6083 ± 0.0239^{a}	5.04 ± 0.32^{a}	5.70 ± 0.46^{a}	1430.29 ± 22.21^{a}
Abrasion-peeled	Industrial Waste	0.0893 ± 0.0034^{b}	5.53 ± 0.25^{a}	4.60 ± 0.63^{a}	1523.30 ± 33.53^{a}

Note: [1]All of the varieties were grown and harvested in Parma, ID. Industrial potato peel waste was collected from major potato processors. [2] Phenolic acid content was the combined concentration of chlorogenic acid, caffeic acid, ferulic acid, and catechin. [3] Calystegine content was the combined concentration of the identified calystegine A and B isomers. [4] Mineral content was the combined concentration of copper, nickel, and zinc. [5] The data were presented as mean ± standard deviation from triplicate experiments and different letters presented statistical differences among means within each group examined by one-way ANOVA and Tuckey's test with a significance level of $\alpha = .05$.

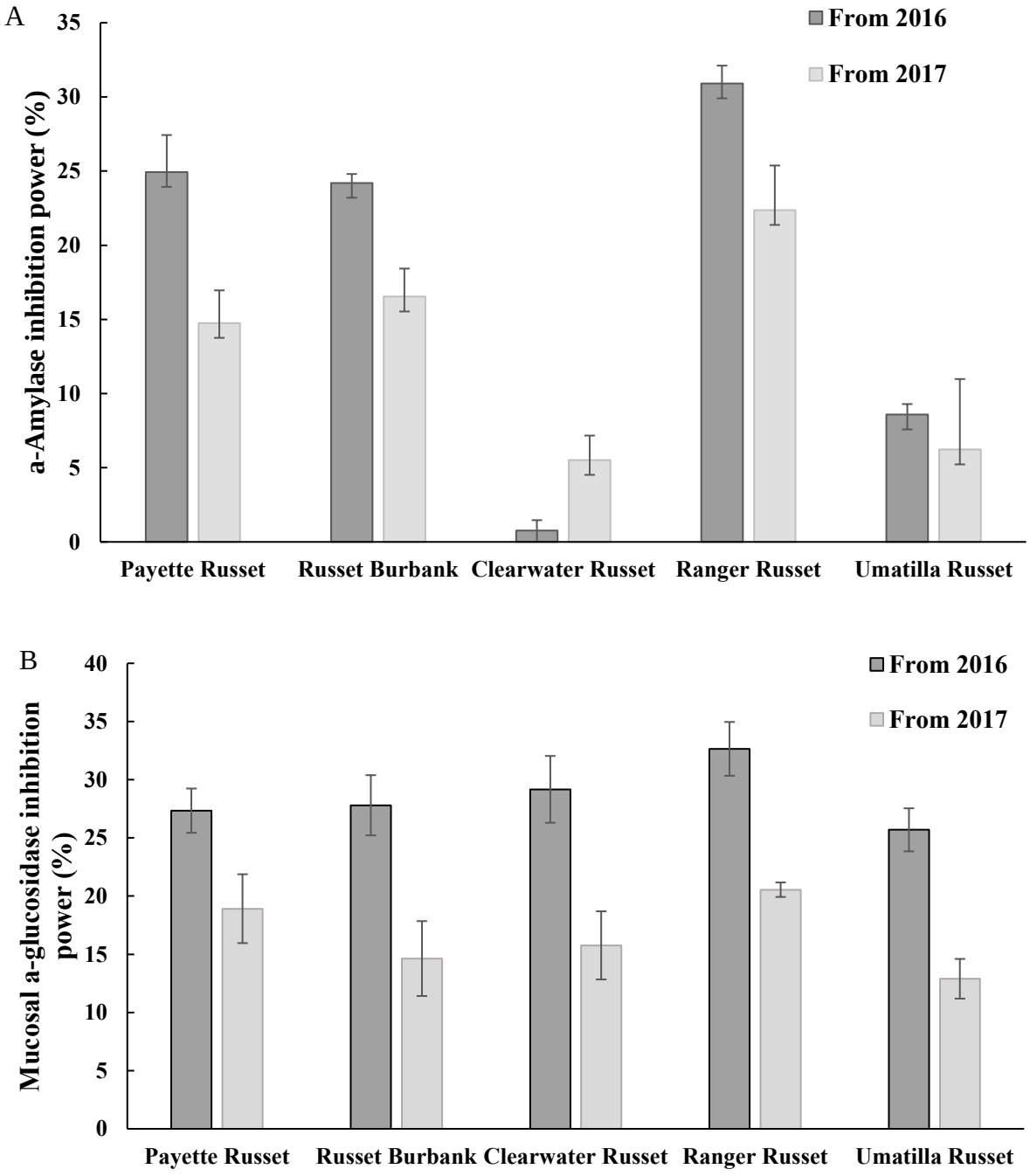

Figure 2. 1 Inhibition of potato peel extract on α-amylase (A) and mucosal α-glucosidase (B). Inhibition power was defined as the percentage of enzymatic activity diminished with the addition of potato peel extract. Values were presented as mean ± standard deviation from triplicate experiments.

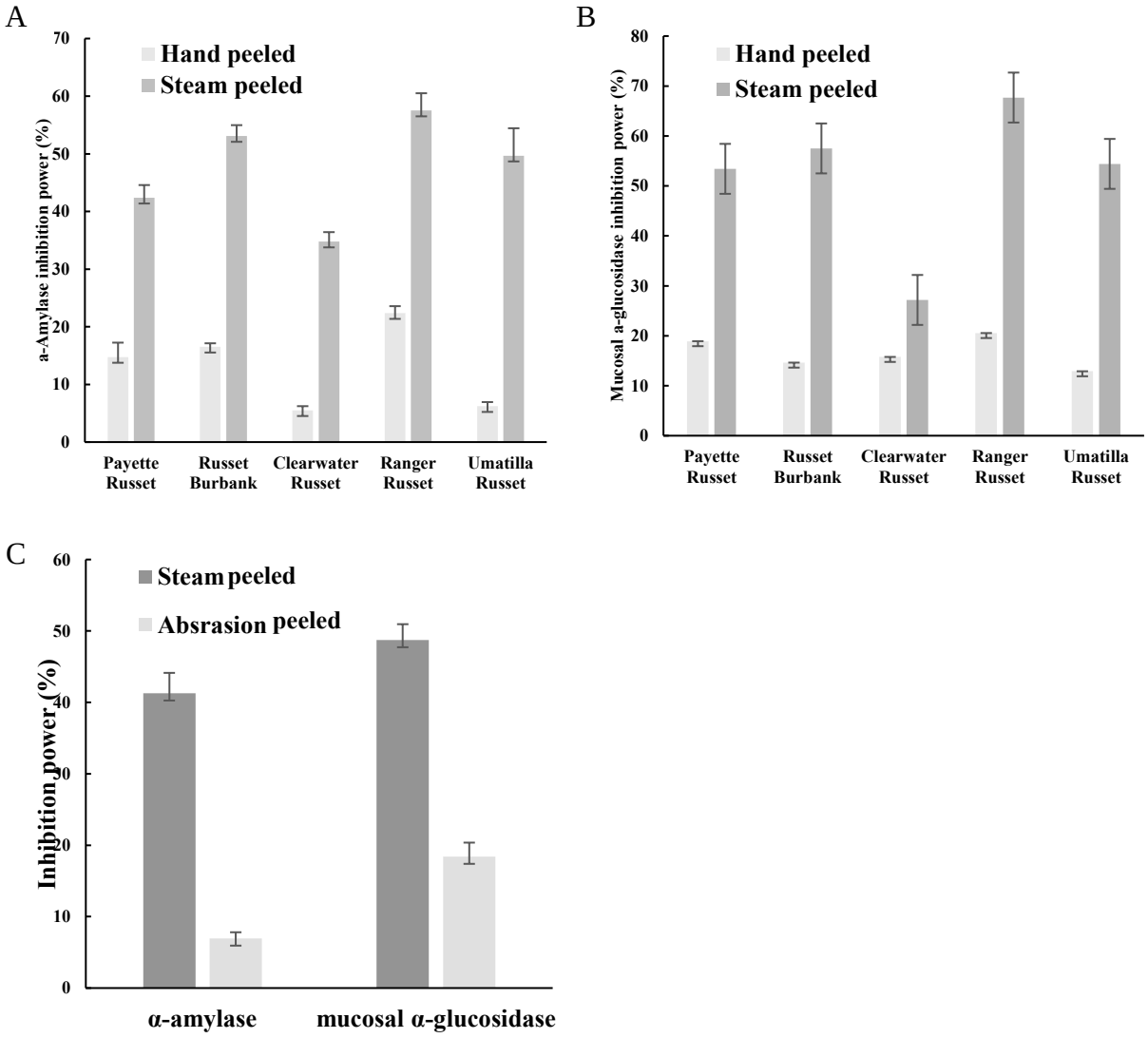

Figure 2. 2 The impact of peeling methods and varieties on the inhibition on α-amylase and mucosal α-glucosidase. A. Inhibition of hand-peeled and steam-peeled potato peel extract on α-amylase. B. Inhibition of hand-peeled and steam-peeled potato peel extract on mucosal α-glucosidase. C. Inhibition of potato peel extract from industrial waste on α-amylase and mucosal α-glucosidase. The inhibition power was defined as the percentage of enzymatic activity diminished with the addition of potato peel extract. Values were presented as mean ± standard deviation from triplicate experiments.