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Purification of alpha-glucosidase inhibitors from grape extract

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PURIFICATION OF ALPHA-GLUCOSIDASE INHIBITORS FROM GRAPE EXTRACT

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THESIS

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DEDICATION

This thesis is dedicated to my parents; Gwendolyn and George, whose love and support has always been with me, my siblings; Chuck, Brian, Kendell, and Jimel who I love all dearly. My achievement is also dedicated to my closest and best friends; Brittney, Sateara, Ryan, and Christina. And last but not least, to a very special friend; Marcel who has been a great source of motivation, inspiration, and support.

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1. Introduction

Diabetes as a major public health problem

Diabetes is a chronic disease marked by uncontrolled blood glucose, which is the fifth leading cause of death by disease in the U.S [1]. Prevalence of diabetes in the general US population is steadily increasing with recent data indicating that 7.7% of adults have diagnosed diabetes and 5.1% is undiagnosed with diabetes [2]. Another 29.5% of the population is at risk of diabetes based on having pre-diabetes. Overall, approximately 40% of the U.S. population has some hyperglycemic condition [2]. Diabetes and its complications remain major causes of morbidity and mortality in the U.S.

The effects of diabetes can have a life-long impact on patients. Diabetes is associated with higher rates of lost work time, disability, and premature mortality. People with diabetes are at higher risk for heart disease, blindness, kidney failure, extremity amputations, and other chronic conditions [1]. People with diabetes have higher use of hospital inpatient care, outpatient and physician office visits, emergency visits, nursing facility stays, home health visits, visits with other health professionals, and prescription drug and medical supply use than their peers without diabetes [3].

The economic impact of diabetes is extensive. A significant portion of health care expenditures is attributed to diabetes and its complications [3]. The national cost of diabetes has grown to $156 billion and will increase to $192 billion by 2020 [1]. The actual future costs are likely to be higher than these projected amounts if the prevalence of diabetes continues to grow, which is

correlated to the growing problem of obesity. Because of these complications, diabetes exacts great personal and societal costs.

With the prevalence of diabetes growing, one reason behind it is the lifestyle that Americans live. With many not understanding or knowing what is involved in leading a healthy, nutritious lifestyle; that ultimately impacts causing unhealthy eating and living habits. Diabetes can lead to serious complications and premature death, but people with diabetes can take steps to control the disease and lower the risk of complications.

Type 2 diabetes mellitus is a progressive and complex disorder that is difficult to manage effectively. Majority of patients are overweight or obese at diagnosis and will be unable to achieve or sustain near normal glycemia without oral anti diabetic agents [6]; a sizeable proportion of patients will eventually require insulin therapy to maintain long-term glycemic control, either as mono-therapy or in conjunction with oral anti-diabetic therapy. The frequent need for escalating therapy is held to reflect progressive loss of islet β-cell function, usually in the presence of obesity-related insulin resistance. [4]

Alpha-glucosidase inhibition for diabetes management

Today's clinicians are presented with an extensive range of oral anti-diabetic drugs for management of type 2 diabetes. The main classes are heterogeneous in their modes of action, safety profiles, and tolerability. According to the Anti-diabetic Agents Study, “These main classes include agents that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues),

reduce hepatic glucose production (biguanides), delay digestion and intestinal absorption of carbohydrate, α-glucosidase inhibitors, (AGI's) or improve insulin action (thiazolidinediones)" [4].

Digestion of dietary carbohydrates primarily occurs in the small intestine by α-amylase to yield both linear maltose and branched isomaltose oligosaccharides, neither of which can be absorbed into the bloodstream without further hydrolysis by α-glucosidases to release glucose. Therefore, intestinal α-glucosidases play a crucial role in controlling postprandial blood glucose. For this reason, one attractive prevention and/or treatment strategy for type-2 diabetes is the inhibition of α-glucosidase.

The effectiveness of AGI's for diabetes treatment is well documented in numerous animal and human clinical studies. For instance, Acarbose has been shown to reduce postprandial serum glucose and insulin concentrations [5], improve metabolic control and produce a dose-dependent amelioration of diabetic nephropathy in the diabetic (*db/db*) mouse [6]. Acarbose also exerts beneficial effects on ischemia/reperfusion damage [7] and pancreatic islet damage by attenuating hyperglycemia [8]. In addition, it has been shown that α-glucosidase inhibitor prevents age-related glucose intolerance [5] and improves insulin sensitivity in fructose-fed rats [9], thus, providing additional evidence for an anti-diabetic effect of α-glucosidase inhibitors.

Therefore, AGI's have been used as anti-diabetic drugs to delay the breakdown of carbohydrate in the gut, and consequently slow the absorption of sugars, which will consequently lower postprandial insulin levels [10].

Alpha-glucosidase inhibitors and their side effects

Typical examples of current AGI's are Acarbose, Miglitol, and Voglibose. They decrease both postprandial hyperglycemia and hyperinsulinemia, and thereby may improve sensitivity to insulin and release the stress on β-cells [11]. These compounds do not induce hypoglycemia and have a good safety profile, although gastrointestinal adverse effects may limit long-term compliance to therapy [10]. Acarbose is currently the only oral anti-diabetes agent approved for the treatment of both pre-diabetes and type 2 diabetes [12]. However, it has been associated with significant side effects. For instance, Acarbose is a pseudotetrasaccharide that inhibits intestinal α-glucosidase reversibly at the brush border of intestinal mucosa [13] as well as the pancreatic alpha amylase. Acarbose being a non-specific inhibitor, inhibits both α-glucosidase and α-amylase. The inhibition of α-amylase produces serious gastrointestinal (GI) side effects, which can increase complications. Pancreatic alpha-amylases hydrolyze complex starches to oligosaccharides in the lumen of the small intestine. These are then acted on by α-glucosidases and further degraded to glucose, which is absorbed into the blood-stream [14]. Inhibition of alpha-amylase results in large amount of undigested carbohydrates entering into the colon [15]. In the colon, bacteria digest the complex carbohydrates causing gastrointestinal side effects such as diarrhea, flatulence, abdominal discomfort, and bloating [16]. These symptoms are commonly observed and significantly restrict the use of AGIs. GI events caused a high rate of patient withdrawal in clinical trials involving these compounds [17]. In particular, the use of Acarbose has been associated with adverse GI side effects [10, 18] such as bloating, abdominal discomfort, diarrhea, and flatulence, which occur in about 20% of patients taking this drug [19]. With

respect to preventing type 2 diabetes, these potential side effects are of particular importance in medication used by persons with impaired glucose. Therefore, specific inhibitors of alpha-glucosidases are preferable in order to reduce GI side effects associated with current AGI's that also inhibit alpha-amylase.

Grape Pomace

In the past three decades, considerable research effort has been devoted to the discovery and development of novel active AGI's since they are potential therapeutic agents for diabetes. A variety of natural compounds and extracts have already been identified as being beneficial in the treatment of diabetes [20-25]. In particular, phenolic compounds found in plants (i.e. tea, raspberries, strawberries, blueberries, and blackcurrants) have been found to be involved in the inhibition of α-glucosidase [26, 27]. For instance, green tea extract has been shown to inhibit rat intestinal α-glucosidases with an IC50 of 0.735mg/ml [25]. The oral administration of green tea extract (300mg/kg bw for 4 weeks) remarkably reduced the fasting blood glucose (by 54%) in STZ-induced diabetic rats [28]. A procyanidins-rich pine bark extract (Pycnogenol®) was recently shown to potentially inhibit α-glucosidases, which has been associated with its anti-diabetic effect in patients with type 2 diabetes [20, 29]. A group of natural acylated anthocyanins also show α-glucosidase inhibitory activity [26].

Grape pomace extract (GPE) was recently reported to selectively inhibit alpha-glucosidases without inhibiting alpha-amylase, leading to inhibition of postprandial hyperglycemia in diabetic animals [30], suggesting that grape pomace components may have a novel anti-diabetic effect.

Grape, *Vitis vinifera,* is one of the most popular and widely cultivated fruits in the world. Grape contains several active components including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidins, and the stilbene derivatives resveratrol [31]. There is growing interests on phenolic compounds found within grapes focusing on their biological activities linking to human health benefits such as antioxidant, cardio-protective, anticancer, anti-inflammation, anti-aging and antimicrobial properties [31].

These bioactive components of grape mainly exist in grape skin and seeds. Grape pomace, which is made up of the skins, seeds and stems are discarded during winemaking [32]. These wastes contain bioactive components with potent antioxidant and free radical scavenging activity. These chemical compounds have been linked to various health benefits. GPE and components have been widely studied for their antioxidant and other health-promoting properties [17]. However, very few studies have investigated the potential of grape pomace as an alternative bio-resource for diabetes management [30]. Currently the only research, conducted by our laboratory has reported that GPE has an inhibitory activity, exerting a significant anti-postprandial hyperglycemic effect, suggesting that grape pomace could be a valuable food derived bio-resource that is rich in antioxidants and anti-hyperglycemic compounds [30].

2. Objectives of this Study

Our long-term goal is to develop a potential dietary intervention for diabetes management from GPE. The specific objective of this research is to prepare more effective GPE by fractionating and isolating active compounds that inhibit alpha-glucosidases in a specific GPE. The data obtained from this study will help identify the perimeters/ conditions that optimize the preparation of GPE and its components will lead to future animal and/or human studies to further determine their potential in preventing and treating Type-2 diabetes.

3. Materials and Methods

Materials and reagents

Yeast type I α-glucosidase (EC 3.2.1.20, G5003), rat intestinal acetone powder (N1377-5G), *p*-nitrophenyl α-D-glucoside (pNPG), silica gel and C-18 were all purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Acarbose (A0802) was purchased from LKT Laboratories Inc,(St. Paul, MN).The HTS 7000 Bio Assay Reader was acquired from MTX Lab Systems Inc Plate, Perkin Elmer (Norwalk, Ct). The BUCHI Roto-evaporator R-124 and water bath B-481was acquired from GoIndustry DoveBid, (Westbury, NY).The HPLC Hitachi Elite LaChrom Autosampler L-2200 was purchased from HITACHI, Japan. The glassware 45/50 Synthware, Chemglass 24/40 1000 ml were purchased from Fisher Scientific Co (Hampton, New Hampshire). The butanol, ethanol and methanol solvent was HPLC grade (Fisher Scientific Co.)

Experimental protocols

1. GPE preparation

Air dried red grape pomace supplied Dr. John Parry at Virginia State University was grounded to fine powder. The powder (50g) was extracted with 500 ml of 70% ethanol under stirring overnight. The supernatant was filtered on a Büchner funnel and collected. The supernatant was concentrated by the BUCHI Roto-evaporator (Westbury, NY). The rotary evaporator was used to remove the organic solvents from the extract without excessive heating of the sample (set at 60°C). The concentrated GPE was collected and placed into a weighed vial. A small amount of methanol or ethanol was added to rinse and collect the residue. The concentrated GPE was placed under the hood to completely dry.

2. GPE fractionation and purification

2.1 Liquid-liquid separation

The separatory funnel was used in liquid-liquid extractions to separate (*partition*) the components of a mixture between two immiscible solvent phases of different densities. The concentrated GPE was poured into a separator funnel then Butanol and water were added to the funnel. The funnel was vigorously inverted several times to mechanically mix up the two solvents. The funnel was settled for the phases to separate. If it was difficult to distinguish between the layers more butanol was added as needed. Butanol and water are incompatible so the water will settle at bottom. Once the layers are distinguished, water was released out of the funnel. The partition was repeated six more times to ensure a more thorough separation of compounds into butanol fraction.

2.2 Open column chromatography

Column chromatography was used to separate compounds from the active fraction (identified by alpha-glucosidase assay) in. An open glass column (Chemglass 24x40) was used. Cotton was placed at the bottom of the column to prevent the stationary phase from being washed out. The column was originally packed using silica gel. Separation was unsatisfactory using this method. The column was then packed with 100mg of C18 (10 micron) via the dry method and activated with ethanol. The GPE fraction was reconstituted with 15% methanol and loaded into the column. It was eluted with aqueous methanol system. A range of ratios were used for the column: 30% MEOH 70% H20, 50% MEOH 50 % H20, and 70% MEOH 30 % H20.

Twenty fractions were collected based on different affinity between stationary and mobile phases, all listed in **Figure 1**. All the collected fractions

were subjected to thin layer chromatography test and those with the same components were combined for alpha-glucosidase assay.

2.3 Thin layer chromatography (TLC)

TLC was performed to determine whether the fractions collected were different or the same. After comparison, fractions with a similar pattern were combined. Thin layer chromatography was performed on a sheet of aluminum foil coated with a thin layer of absorbent material usually silica gel. This layer of adsorbent is known as the stationary phase. A portion of the fractions were dissolved in methanol and a small drop was placed on the base line of the plate about 1.5 cm from the bottom of the edge. Allow the sample to completely dry using a blow-dryer. A small amount of appropriate solvent; ethyl acetate, methanol, water in the ratio of 60/40/2 with 2 drops of acetic acid, was poured into a glass beaker. The TLC plate was placed into the beaker, making sure the spots of the sample don't touch the solvent and the lid is closed. The solvent was drawn up the plate by capillary action. The mobile phase flowed through the stationary phase and carried The solvent was drawn up the plate by capillary action. The mobile phase flowed through the stationary phase and carries the components of the mixture with it. the components of the mixture with it. When the solvent reaches no higher than top of the TLC plate, the plate was removed using tweezers. The TLC plate was placed on the side to dry. Different components traveled at different rates and the dried plate was scanned under UV light to detect spots that contain separated components.

2.4 Rotary evaporation of GPE fractions

The fractions collected from open column chromatography with the same TLC pattern were combined and further concentrated using a BUCHI Rotary Evaporator R-124, (Westbury, NY). The concentrated fractions were then air-dried in a fume hood and immediately stored in a -20°C freezer.

3. Prepare reagents for enzyme assay

Preparing stock and working solutions for enzymatic experiments: After all GPE fractions have been collected and completely dried, 2 mg of each fraction was placed into a small centrifuge tube and methanol was added to get a concentration of 2 mg/ml. Serial dilutions were made to prepare working GPE solutions to test activity.

3.1 Prepare Acarbose stock and working solution

Ten mg of Acarbose was dissolved in 10 mL 0.1 M phosphate buffer (PB, pH 6.8) to achieve a 1 mg/mL stock solution. Fifty µL of above solution was mixed with 4.95 mL 0.1 M PB pH 6.8 to achieve a 10 µg/mL working solution. Use a vortex mixer to obtain a homogeneous mix

3.2 Prepare substrate PNPG solutions in PB buffer

P-nitrophenyl-α-d-glucopyranoside (pNPG) is a universe substrate of α-glucosidases, which was used as the substrate for intestinal α-glucosidases in our experiments Sixty milligram of pNPG powder was dissolved in 50 mL 0.1 M PB pH 6.8 to achieve a 4 mM concentration.

3.3 Prepare rat intestinal α-glucosidases

Rat intestinal powder (2.5g) was suspended in 100 mL of 0.1 M PB (pH 6.8). The suspension was shaken overnight and the supernatant was collected

upon centrifugation and filtration using 0.45 µm of coarse filter paper. For experiments, the dried enzyme extract was weighed and dissolved in 0.1 M PB pH 6.8 to achieve a 25mg/ mL concentration.

4. Enzyme Assay

One hundred µl of each sample was loaded into each well of the 96 well plate. One hundred µl of Acarbose (10 µg/mL) and solvent used to dissolve GPE (5% Methanol and 95% H20) were used as positive and negative controls. The reaction was initiated by the addition of 100 µl of α-glucosidase enzyme and 50 µl of substrate (pNPG) into all wells. Absorbance readings of assay were recorded at 450nm with shaking using HTS 7000- Bio Assay Reader- Perkin Elmer (Norwalk, Ct). The α-glucosidase inhibitory activity was expressed as % inhibition.

5. High performance liquid chromatography (HPLC)

The HPLC (Hitachi, Schaumburg, IL) was used for the separation of the active fractions identified in the alpha-glucosidase assay for qualitative and quantitative analysis of potential active compounds. An analytical C18 (250x5mm) was used for separation and a diode array detector (DAD) was used to monitor fractions and compounds eluted from the column. Twenty mg of active fraction was dissolved in 2 ml methanol. The solution was filtered via 0.45 µm of filter paper and placed in a vial. A variety of mobile phases and programs (described below) were developed to optimize the separation. A gradient program was chosen since the isocratic separation was not satisfactory for separation. Methanol and water was used first for the mobile phase gradient. After series of tests, it was concluded that acetonitrile and water gradient method provided the

best results for extract separation. Using this program, two major peaks were separated and collected for identification and structure elucidation of unknown compounds.

4. Results and Discussion

Alpha-glucosidase inhibition by GPE and its fractions

GPE has been identified as an inhibitor of α- glucosidase in our previous studies. Our goal was to identify active fractions or components in GPE. The initial liquid-liquid partition of GPE led two fractions: water and butanol fractions. In our preliminary separation, we tried liquid-liquid separation into three phases: water, butanol, and ethyl acetate and found that the ethyl acetate fraction was the most effective fraction followed by the butanol fraction via the α -glucosidase assay. However, the ethyl acetate fraction contained very little amounts of compounds. In the following separation, we were only using water and butanol for partitioning. The butanol fraction was further subject to open column chromatography.

Open column chromatography

From the fractions assayed slight inhibition was recorded in sub fraction 1. The α-glucosidase inhibitory activity was expressed as % inhibition listed in **Figure 2**. In comparison to the other fractions from the column, fraction 1 had the highest inhibitory effects at 35% compared to the positive control 87.64% and negative control -.565% listed in **Figure 3**.

HPLC analysis on the fraction 1

Fraction 1 was further separated via HPLC to determine which compounds were within that fraction and more specifically, which compound possibly inhibited the enzyme alpha glucosidase. The sample was prepared and tested using a wide range of method systems and wavelengths. Methanol mixed

with water was first chosen as the mobile phase but displayed insufficient separation on C18 column since the peaks were crowded together. After a series of method development, we determined that the gradient system of acetonitrile (ACN) and water, wavelength 210 nm, and injection volume of 20 µl displayed optimal absorption and was used for collection of compounds. ACN was selected because it had low noise in UV detection and resulted in less ghost peaking for gradient baselines. The polarity is lower and it can interact with the molecules to provide better peak separation. The HPLC chromatograms and methods are shown in Figures 4-8.

Open column chromatography of GPE for scaling up

As above showed, we have successfully isolated and collected two compounds (peaks) from the active fraction 1. However, the amounts of the compounds were too little (less than 0.2 mg) to conduct structure elucidation. In order to obtain a sufficient amount of the compounds for activity and structure analysis, we tried to develop an alternative fractionation and separation procedure that could allow for scaling up. In this method development, GPE was recreated with the protocol slightly changed. The column was packed with Diaion Resin instead of C18. Diaion Resin (HP-20) was used because it has much larger sample capacity than C18 does. We collected the new fraction in hope of having a larger active sample amount for further testing. When the activity was tested on GPE using the assay there was no inhibition noticed in any of the fractions separated from the second column. Possible reasons why the second column produced no activity could be because the active compounds may work synergistically and they lose their activity once being fractionated by HP-20. The

method employing C18 is probably the best for purity of the fraction, but it would not be the best method quantitatively. We further tried with an open column loaded with coarse C18 resin to separate GPE and collected over 30 fractions. However, none of these fractions exerts significant inhibition on alpha-glucosidase.

Other reasons why inhibition could have been affected

The butanol fraction of GPE displayed inhibition on rat intestinal alpha-glucosidases. With the Grape pomace being mixed with other compounds this could have had a negative effect limiting its activity. The bioactivity of GPE could possibly be reduced once it is separated if the compounds have a synergistic effect. There is also a possibility of the fractions having an inhibitory effect for a limited amount of time. Subjecting the dried Grape Pomace to high temperatures, color extractions and distillation could have had a significant effect on the inhibitory activity of the alpha glucosidase enzyme. There is the possibility that concentrations could also be too low at this level and inhibition could not be observed. From this ongoing research there is activity noted in GPE but there could be many reasons to why it is difficult to notice higher levels of inhibition in the subsequent fractions of GPE. Fraction 1 showed significant inhibition of alpha glucosidase, but analyzing the results with the control, the absorbance levels were still low and not consistent enough to make a clear inference.

5. Conclusion

We identified an active fraction (F1) from GPE, through open column and HPLC, which had inhibitory effects to alpha glucosidase. The further HPLC separation yielded two major peaks. The two major peaks were collected and will undergo future structure identification and elucidation. After structure identification and elucidation is complete; testing will be conducted to the compounds, using the bio-assay reader, identifying their activity. If the compounds are active we will continue with the method listed above. We have tried open column chromatography using HP20 and coarse C18 for scale-up of GPE separation but the resulting fraction failed to show significant inhibition on alpha-glucosidases. Further method development is needed if the collected peaks are not active compounds.

Appendix A: Figures

Figure 1

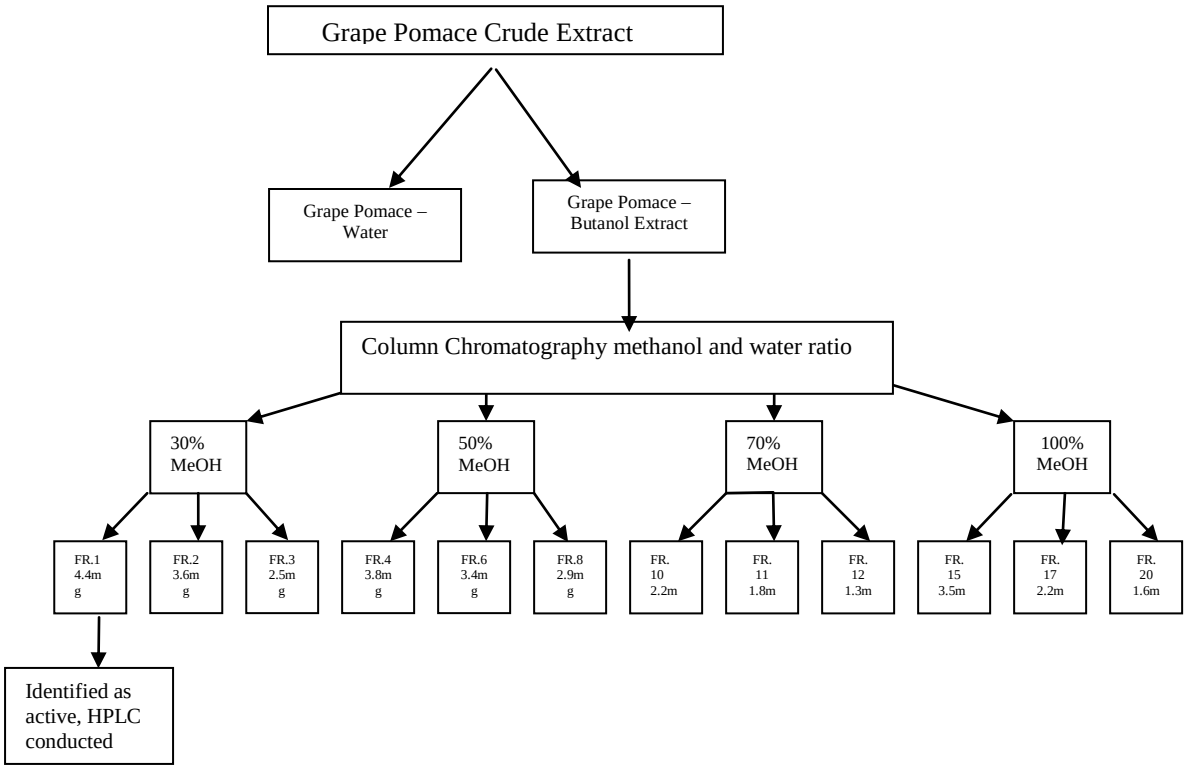

Figure 1- The process Grape pomace extract separation

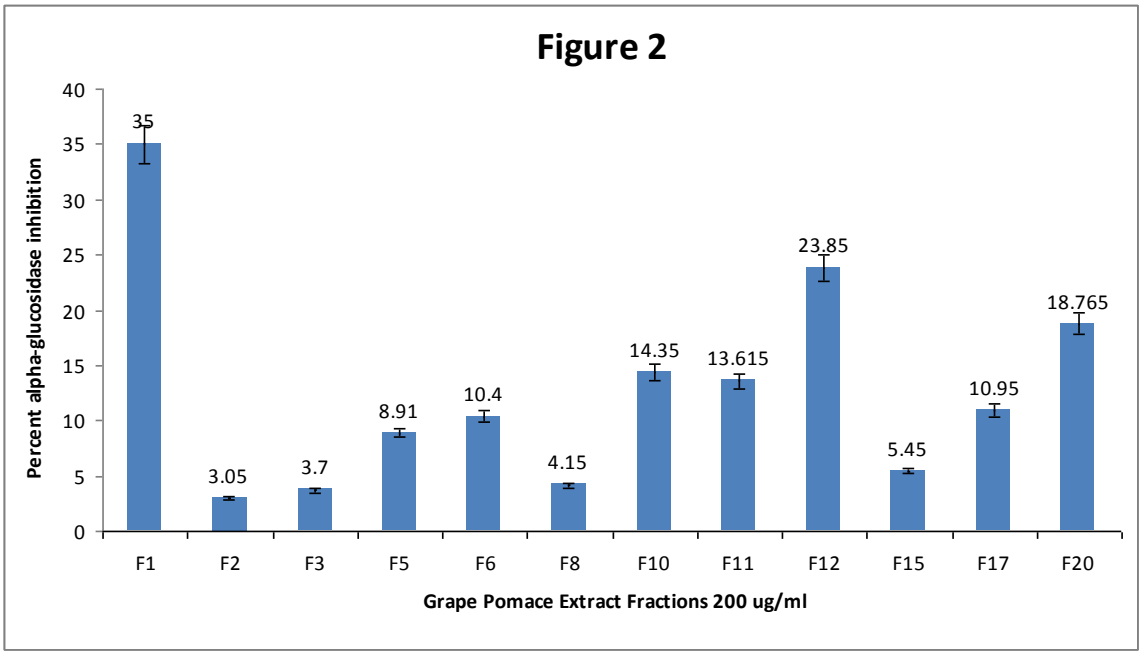

Figure 2 - **Percent Inhibition of Grape Pomace Fractions**
Percent inhibition of alpha- glucosidase from grape pomace fractions. Enzyme assay conducted with absorbance wavelength 450nm. F1 has the highest percent inhibition of 35% compared to other fractions. Bars with different superscript values are significantly different from each other ($p < 0.05$).

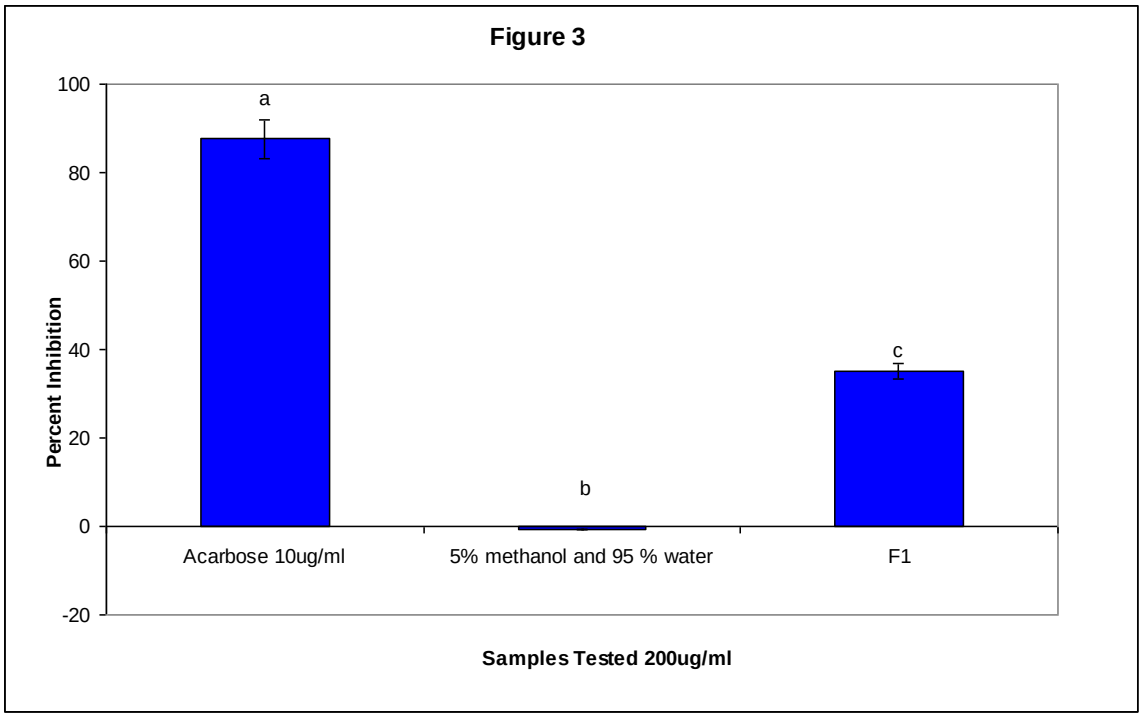

Figure 3- Comparison of Percent Inhibition to control

Enzyme assay conducted with wavelength 450nm. Percent inhibition of F1 compared to Acarbose (positive control) and 5% methanol and 95 % water (negative control). F1 showed positive inhibitory effects when compared to the positive and negative controls. Bars with different superscripts/subscripts values are significantly different from each other ($p < 0.05$).

Figure 4

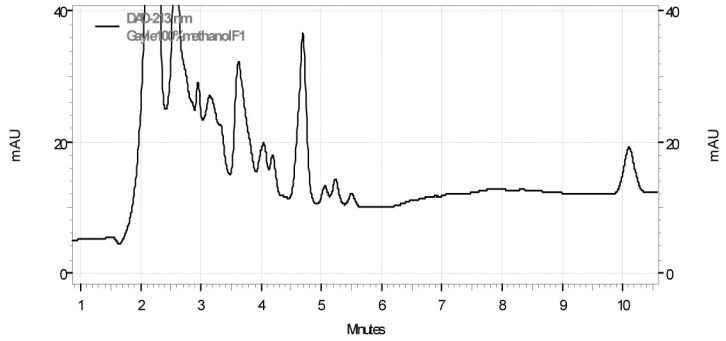

Figure4-Method 1

Based on the results provided in the chromatograph, the method used was insufficient. Methanol was used for the mobile phase. The peaks observed appeared before 5 minutes and were too crowded. The method needed to be altered to delay the appearance of the peaks. For method 1 Isocratic systems were used as follows: From 0-40min methanol concentration 100%.

Figure 5

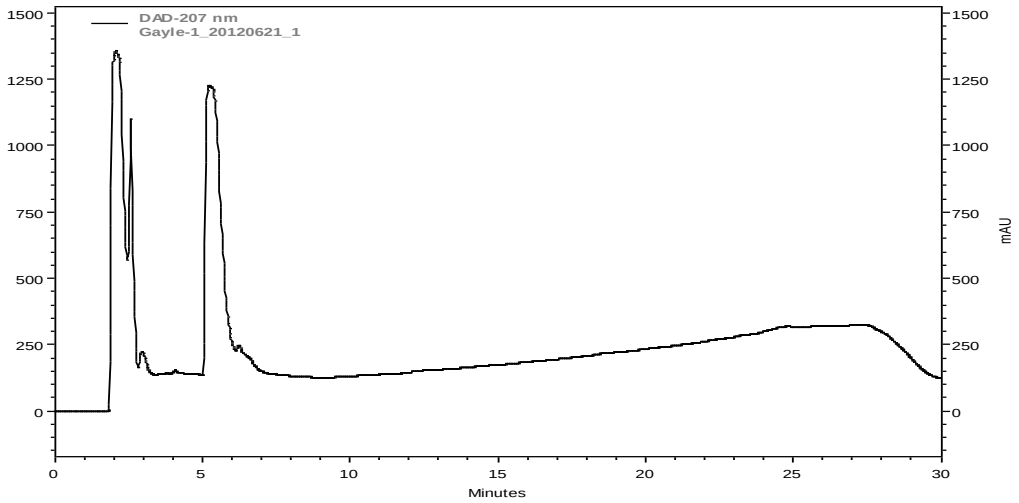

Figure 5- Method 2
A different method was employed using gradient system of methanol and water for HPLC testing. The chromatogram results still did not show possible compounds. The timing of the mobile phase needed to be down, to view the other compounds that could possibly be in the sample. Gradient systems were used as follows: 0-5 min 80% methanol, 20-23 min 100% methanol, 25-30 min 80 % methanol.

Figure 6

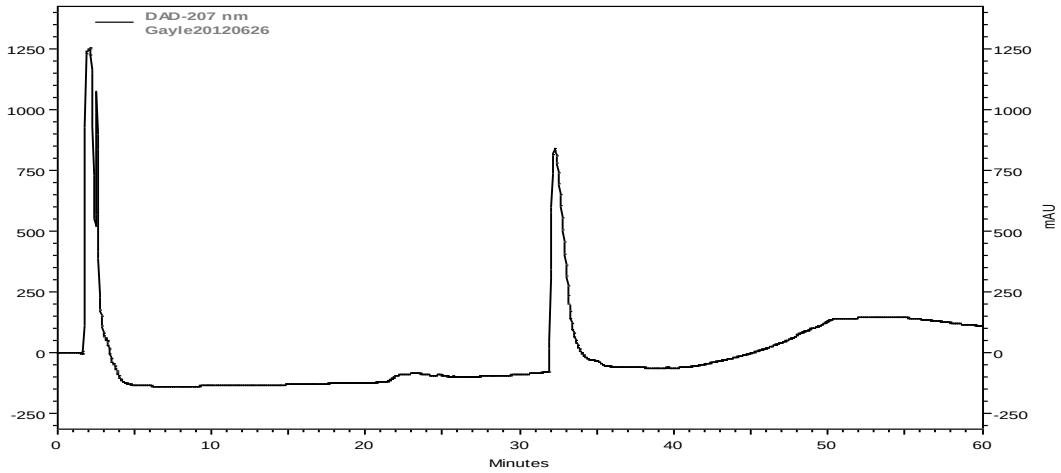

Figure 6- Method 3

This chromatogram was altered by increasing water in column to extend out the timing the peaks occurred. The time was also increased to observe the other possible compounds. Gradient systems were used as follows: 0-3min 10% methanol, 18-21min 30%methanol, 31-36 min 60% methanol, 46-50 min 95% methanol, 56-60 min 90% methanol.

Figure 7

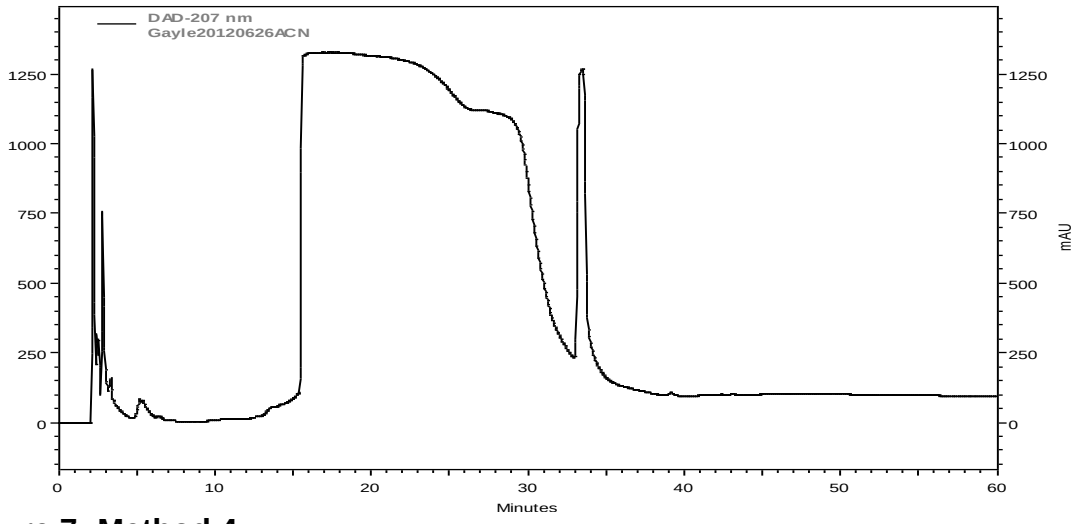

Figure 7- Method 4

ACN was introduced into the gradient system. The injection volume was increased to 30ul. Gradient systems were used as follows: 0-3min 10% acetonitrile, 18-21 min 30% acetonitrile, 31-36min 60% acetonitrile, 46-50 min 95% acetonitrile 56-60min 90% acetonitrile.

Figure 8

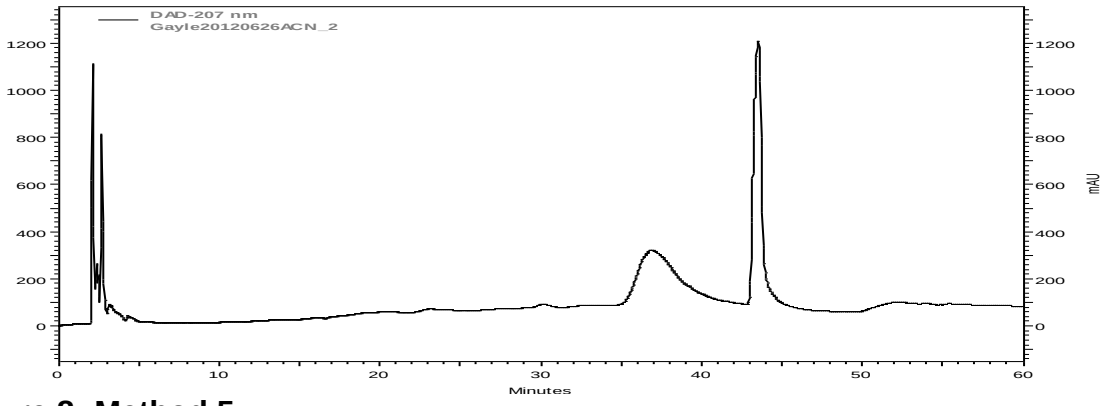

Figure 8- Method 5

We determined that for separtion ACN was a preferable solvent to separate the fraction 1. The injection volume was reduced to 20µl because the original injection made the peak too high. This chromatogram provided better peaks and 2 compounds were observed. We continued to adjust the method slightly to separate the peaks from each other and begin to collect the compound. Gradient systems were used as follows: 0-3 min 10% acetonitrile, 15-30 min 20% acetonitrile, 40-46 min 50% acetonitrile, 50 min 90 % acetonitrile, 54 min 95% acetonitrile, 57-60 min 90% acetonitrile.

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ABSTRACT

PURIFICATION OF ALPHA-GLUCOSIDASE INHIBITORS FROM GRAPE EXTRACT

by

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Degree: Master of Science

Diabetes and its complications are steadily growing and remain major causes of morbidity and mortality in the U.S. Intestinal α-glucosidases play a crucial role in controlling postprandial blood glucose. For this reason, one attractive prevention and/or treatment strategy for type-2 diabetes is the inhibition of α-glucosidase. The effectiveness of α-glucosidase inhibitors (AGI's) for diabetes treatment is well documented in numerous animal and human clinical studies. Grape pomace extract (GPE) has recently found in our laboratory to selectively inhibit alpha-glucosidases without inhibiting alpha-amylase, leading to inhibition of postprandial hyperglycemia in diabetic animals. The present study was designed to identify effective GPE by fractionating and isolating active compounds that inhibit alpha-glucosidases in a specific GPE. From enzyme assay testing, results revealed Fraction 1 active, showed 35% inhibition ($p < 0.05$) compared to the positive control 87.64% and negative control -.565%. HPLC was conducted on F1, which yielded two potential active compounds (peaks)

following ACN gradient system method. F1 will undergo future structure identification and elucidation.

Fractionation of GPE yielded a very small amount of active F1 for testing. Open column chromatography using HP20 and coarse C18 for scale-up of GPE separation but the resulting fraction failed to show significant inhibition on alpha-glucosidases. Further method development is needed if the collected peaks are not active compounds.

AUTOBIOGRAPHICAL STATEMENT

Gayle Melissa Shipp received a Bachelor of Science Degree in Nutrition from Michigan State University, in 2009. She then joined Wayne State University (WSU) where she has completed her graduate study towards the accomplishment of a Master of Science degree in Nutrition and Food Science and has recently decided to pursue her studies toward a Master in Public Health.

While completing her degree, Gayle also worked within the community at Wayne State with neighboring programs. She worked as a student advisor with Educational Talent Search and also worked as a teacher with the SEEMA program