

## ABSTRACT

PATTERSON, KATHERINE WORTH. Effect of Pepsin Hydrolysis on Amylase Activity and Lack of Amylase Inhibitory Activity of Sweet Potato Protein. (Under the direction of Dr. Jonathan C. Allen).

Caiapo, an extract of Japanese white-skinned sweet potato, has been shown to possess antidiabetic properties. Caiapo treatment has been associated with a decrease in fasting plasma glucose, an increase in insulin sensitivity, and a decrease in H<sub>b</sub>A<sub>1c</sub>. A 22 kDa acidic glycoprotein has been isolated and deemed the active component of Caiapo. Beauregard sweet potatoes, one of the most common commercial cultivars in the United States, have been shown to elicit a low glycemic response and may possess antidiabetic properties similar to Caiapo. Protein band patterns of Caiapo and protein isolated from Beauregard sweet potato were found to be almost identical. Inhibition of pancreatic amylase by sweet potato protein was proposed as a possible mechanism for the low glycemic response to sweet potatoes because the addition of Caiapo to white potato decreased the glycemic response while the addition of Caiapo to glucose did not decrease the glycemic response.

The first objective of this study was to determine the most efficient method for extracting protein from Beauregard sweet potato. Protein in solution after homogenization with buffers of varying concentrations of sodium phosphate (0.05 M and 0.025 M) and sodium chloride (0.3 M, 0.2 M, and 0.1 M) was compared to homogenization with distilled water by BCA assay. The concentration of protein in solution was similar for all buffers. Protein in solution after precipitation of protein with calcium chloride and resuspension was also compared by BCA assay. The highest concentration of protein in solution was achieved with the use of the 0.05 M sodium phosphate and 0.1 M sodium chloride buffer, however, none of the protein concentrations were statistically different.

The second objective of this study was to determine the effect of digestive conditions on sweet potato protein. In order for a protein to inhibit pancreatic amylase in the small intestine the active portion of the protein must not be destroyed by the acidic conditions of the stomach or pepsin digestion. Sweet potato protein was subjected to pepsin digestion under acidic condition and then run on a gel next to protein not subjected to digestive conditions. Proteins resistant to pepsin digestion were found in Beauregard sweet potato flesh, peel, and leaf.

The third objective of this study was to quantify the native amylase activity of Beauregard sweet potato protein. Protein extracted from the flesh exhibited higher amylase activity ( $32.7 \pm 5.93$  units/mg protein) than protein extracted from the peel ( $13.0 \pm 1.12$  units/mg protein). Digestion with pepsin eliminated amylase activity in the flesh ( $0.013 \pm 0.812$  units/mg protein) and peel extracts ( $0.609 \pm 0.795$  units/mg protein). No amylase activity was found in the leaf extract ( $-1.40 \pm 0.469$  units/mg protein) or the leaf extract treated with pepsin ( $-0.625 \pm 0.179$  units/mg protein).

The fourth objective of this study was to determine if proteinaceous amylase inhibitors are present in Beauregard sweet potato. The amylase activity of pancreatic amylase and sweet potato protein were determined separately and then in combination by measuring the degradation of starch over time with a dinitrosalicylic acid (DNS) reagent. DNS reacts with sugar reducing ends to produce a compound that absorbs at 540 nm. The amylase inhibitory activity was calculated by subtracting the sum of the individually determined pancreatic and sweet potato amylase activities from the amylase activity of the combination determined experimentally. Amylase inhibitors were not apparent in any of the fractions.

Effect of Pepsin Hydrolysis on Amylase Activity and Lack of Amylase Inhibitory Activity of  
Sweet Potato Protein

by  
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## **BIOGRAPHY**

Katherine Worth Patterson was born in Raleigh, North Carolina on March 11, 1986. She graduated with a B.S. in Biological Sciences from North Carolina State University in May 2007 and plans to continue on to a Ph.D. program after completion of her M.S. degree.

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## **CHAPTER 1: LITERATURE REVIEW**

## **CARBOHYDRATE METABOLISM**

Digestion of carbohydrate begins in the mouth by the action of salivary  $\alpha$ -amylase. Only limited digestion of carbohydrate occurs, however, because salivary  $\alpha$ -amylase is denatured in the stomach due to the low pH. Digestion begins again in the small intestine when pancreatic  $\alpha$ -amylase is secreted. Starch is broken down into maltose, isomaltose, and maltotriose by  $\alpha$ -amylase through the hydrolysis of  $\alpha$ -1-4 glycosidic bonds. These products as well as any other disaccharides that were ingested must be further digested to their respective monosaccharide units by brush border enzymes (maltase, isomaltase, lactase, and sucrase) before absorption. Maltose is hydrolyzed to two glucose molecules by maltase. Isomaltose is hydrolyzed to two glucose molecules by isomaltase. Lactose is hydrolyzed to one molecule of glucose and one molecule of galactose by lactase. Sucrose is hydrolyzed to one molecule of fructose and one molecule of glucose by sucrase. After absorption, glucose, galactose, and fructose are transported to the liver via the portal blood. The liver can transform galactose and fructose into glucose (Gropper and others 2005).

Insulin is secreted by the beta cells of the pancreas in response to increased glucose metabolism by the cells. The metabolism of glucose by the beta cells increases the amount of ATP in the cell relative to the amount of ADP in the cell and  $K_{ATP}$  channels close. This causes a depolarization of the plasma membrane, activates  $Ca^{2+}$  channels, and allows  $Ca^{2+}$  to promote exocytosis of insulin granules (Muoio and Newgard 2008). Glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide-1 (GLP-1) are secreted by the gastrointestinal tract in response to glucose absorption and also help regulate insulin secretion. GIP and GLP-1 enhance insulin secretion by beta cells and inhibit glucagon

secretion by alpha cells (Holst and others 2008). Glucose transporters (GLUT) are required for cellular uptake of glucose. GLUT4, found in adipocytes and skeletal muscles, is susceptible to insulin regulation (Gropper and others 2006). Insulin binds 2 units of the tyrosine kinase receptor protein on the cell membrane and activation of the receptor ensues. The 2 units phosphorylate each other and phosphorylate signaling molecules to regulate the processes of the cell. The addition of intracellular GLUT4 to the plasma membrane and the initiation of glycogen synthesis are controlled indirectly by insulin binding. The addition of more GLUT4 to the plasma membrane allows for greater glucose uptake by the cell and the activation of glycogen synthetase allows for glucose storage in the form of glycogen (Fox 2006). When blood glucose levels become low, glucagon is secreted. Glucagon initiates glycogenolysis and gluconeogenesis to increase blood glucose levels and maintain homeostasis (Quesada and others 2008).

## **DIABETES**

Diabetes is characterized by abnormal glycemia. Diabetes can result from unbalanced insulin and glucagon production as well as cell receptor resistance to insulin. In the case of type I diabetes, little or no insulin is secreted due to destruction of the beta cells. In the case of type II diabetes, cells first become resistant to insulin then insulin production decreases (Turkoski 2006).

Over 14 million people in the United States have been diagnosed with diabetes and it is suspected that over 6 million more have diabetes but have not been diagnosed. Approximately 90% of cases are classified as type II and approximately 10% of cases are classified as type I. Diabetes increases risk of heart attack, stroke, kidney failure, nerve damage, and blindness (Turkoski 2006).

### **Diagnosis**

Diabetes is diagnosed based on the following guidelines: (1) Symptoms are present and a random glucose sample is  $\geq 200$  mg/dL. (2) Two fasting blood glucose samples are  $\geq 126$  mg/dL. (3) A blood glucose sample taken at either 30, 60, or 90 minutes and a blood glucose sample taken 2 hours after a glucose challenge are both  $\geq 200$  mg/dL (Hoogwerf and others 2006).

### **Treatment**

Insulin is required for treatment of type I diabetes, however, oral medications are commonly used in the treatment of type II diabetes when diet and exercise are not sufficient. Combinations of multiple oral medications or oral medications and insulin can be used in the treatment of type II diabetes. Since different classes of diabetes medications have different

mechanisms of action, multiple medications may provide greater relief than a single medication (Hoogwerf and others 2006).

Sulfonylureas, such as glimepiride, glipizide, and glyburide, change the method of release of insulin from beta cells, causing increased insulin secretion. They can only be used if the pancreas contains active beta cells (Turkoski 2006).

Insulin secretagogues, such as nateglinide and repaglinide, also change the insulin release method of the beta cells, causing more insulin to be secreted. Side effects include dizziness, headache, and weight gain (Turkoski 2006).

Biguanides, such as metformin, reduce the amount of glucose absorbed in the intestines, decrease the amount of glucose produced by the liver, and increase cell receptor sensitivity. Gastrointestinal upset is the main side effect of biguanides (Turkoski 2006).

Thiazolidinediones, such as pioglitazone and rosiglitazone, alter certain genes to increase cell receptor sensitivity to insulin. Side effects include fluid retention, weight gain, and muscle pain. Increased risk of liver failure is also associated with thiazolidinediones (Turkoski 2006).

Alpha-glucosidase inhibitors, such as acarbose, delay digestion and thus impair absorption of glucose in the intestines by inhibiting the enzyme responsible for breaking down maltose. The main side effect of alpha-glucosidase inhibitors is gastrointestinal upset (Turkoski 2006).

## **SWEET POTATOES**

Sweet potatoes (*Ipomoea batatas*) are an important crop for much of the world. The sweet potato is very drought tolerant, thrives in a wide variety of climates, and does not have to be harvested all at once. The entire plant is edible, the root, the stalk, and the leaves (Bovell-Benjamin 2007).

China leads the world in sweet potato production with approximately 65% of the market. In the United States, North Carolina and Louisiana are the top sweet potato growers with approximately 40% of the market share each. While hundreds of cultivars exist, Beauregard is a popular variety in North Carolina (Bovell-Benjamin 2007).

### **Nutrient Composition of Sweet Potato**

Sweet potato roots are high in  $\beta$ -carotene (approximately 3 mg/100 g) and thus help to meet vitamin A requirements. Sweet potatoes also contain other important nutrients such as protein, fiber, calcium, iron, potassium, vitamin C, folate, vitamin B<sub>6</sub>, and anthocyanins (Bovell-Benjamin 2007).

The root of the sweet potato can vary in protein content from 1.0% to 9.0% protein on a dry weight basis and the leaf of the sweet potato can vary from 4.0% to 27.0% protein on a dry weight basis. The root of the Beauregard cultivar was reported at  $4.7 \pm 0.5\%$  protein on a dry weight basis (Bovell-Benjamin 2007). Growing conditions can greatly affect protein content of sweet potatoes. Purcell and others (1978) measured the protein content of sweet potatoes from the same cultivar, Jewel, grown in different locations and found significant differences with one location producing sweet potatoes of 4.1% crude protein and another location of 8.8% crude protein (Walter and others 1984).

The distribution of protein in sweet potatoes is not consistent. Protein concentration is lower at the root end than the stem end and lower in the middle than the outer section of the sweet potato (Walter and others 1984). Sporamins A and B make up 80% of the protein found in sweet potatoes. These proteins are used by the plant for storage and have been shown to inhibit proteases and act as antioxidants (Bovell-Benjamin 2007).

### **Sweet Potato Extracts with Antidiabetic Properties**

#### ***Caiapo***

Caiapo, an extract from Japanese white skinned sweet potato, has been shown to possess antidiabetic properties. Caiapo has been shown to decrease fasting plasma glucose, increase insulin sensitivity, and decrease H<sub>b</sub>A<sub>1c</sub> (Ludvik and others 2004). It has been suggested that the active component of Caiapo is a 22 kDa acidic glycoprotein (Kusano and others 2001).

In a 6 week study, Ludvik and others (2003) compared the effects of a high dose Caiapo supplement (4 g/day), a low dose Caiapo supplement (2 g/day) and a placebo on fasting plasma glucose and insulin sensitivity in type II diabetic patients treated by diet alone (n=18). Fasting plasma glucose decreased in the high dose Caiapo group from  $8.3 \pm 0.6$  to  $7.2 \pm 0.4$  mmol/L ( $P < 0.05$ ) with no change in the low dose Caiapo group or the placebo group. Insulin sensitivity as measured by an oral glucose tolerance test increased only in the high dose Caiapo group from  $308 \pm 13$  to  $334 \pm 10$  mg/min/m<sup>2</sup> ( $P = 0.048$ ). Insulin sensitivity as measured by a frequently sampled intravenous glucose tolerance test, however, increased in both the high dose and low dose Caiapo groups from  $1.21 \pm 0.32$  to  $1.73 \pm 0.40 \times 10^4$  min<sup>-1</sup>

$^1/(\mu\text{U}/\text{mL})$  ( $P=0.021$ ) and  $2.02 \pm 0.7$  to  $2.76 \pm 0.89 \times 10^4 \text{ min}^{-1}/(\mu\text{U}/\text{mL})$  ( $P<0.05$ )

respectively. There was no change in C-peptide,  $\text{H}_b\text{A}_{1c}$ , or body weight in any of the groups.

The high dose Caiapo group and the placebo group had a higher BMI than the low dose Caiapo group. The initial average BMI of the high dose Caiapo group was  $28.6 \pm 1.3 \text{ kg}/\text{m}^2$  and the initial average BMI of the placebo group was  $28.9 \pm 0.9 \text{ kg}/\text{m}^2$  whereas the initial average BMI of the low dose Caiapo group was  $25.5 \pm 0.8 \text{ kg}/\text{m}^2$ . The high dose Caiapo group also had higher fasting plasma insulin than the low dose Caiapo group and the placebo group. The high dose Caiapo group had an initial average fasting plasma insulin of  $13.4 \pm 2.5 \text{ pmol}/\text{L}$  while the low dose Caiapo group had an initial average fasting plasma insulin of  $8.3 \pm 1.6 \text{ pmol}/\text{L}$  and the placebo group had an initial average fasting plasma insulin of  $8.7 \pm 1.7 \text{ pmol}/\text{L}$ . These differences may have influenced the results of the study.

Ludvik and others (2004) then conducted a 12 week human study in which type II diabetic patients treated by diet alone ( $n=61$ ) were randomly assigned to receive either a 4 g Caiapo supplement or a placebo each day. After 2 months of treatment, both the Caiapo group and the placebo group showed a decrease in fasting blood glucose from  $143.7 \pm 1.9$  to  $132.3 \pm 1.8 \text{ mg}/\text{dl}$  ( $P<0.001$ ) and  $144.4 \pm 1.9$  to  $138.8 \pm 2.2 \text{ mg}/\text{dl}$  ( $P<0.001$ ) respectively. After 3 months of treatment, however, only the Caiapo group maintained the decrease in fasting blood glucose of  $128.5 \pm 1.7 \text{ mg}/\text{dl}$  down from the initial  $143.7 \pm 1.9 \text{ mg}/\text{dl}$  ( $P<0.001$ ). Percent  $\text{H}_b\text{A}_{1c}$  decreased in the Caiapo group from  $7.21 \pm 0.15$  to  $6.94 \pm 0.14$  ( $P<0.001$ ) after 2 months of treatment and after 3 months was down to  $6.68 \pm 0.14$  ( $P<0.001$ ). Body weight decreased in both groups but the decrease was more significant in the Caiapo group (from  $78.9 \pm 1.7$  to  $75.2 \pm 1.4 \text{ kg}$  after 3 months) as compared to the

placebo group (from  $77.2 \pm 2.0$  to  $76.2 \pm 1.7$  kg after 3 months). Insulin sensitivity as measured by an oral glucose tolerance test increased in the Caiapo group throughout treatment (measured at 1 month, 2 months, and 3 months) while no significant change was reported in the placebo group.

The decrease in body weight observed in both the Caiapo group and the placebo group could have contributed to the decrease in fasting blood glucose and increase in insulin sensitivity observed. Weight loss in obese type II diabetic patients has been shown to improve glycemia and decrease insulin resistance (Bantle and others 2006).

Ludvik and others (2008) later performed a 5 month study to evaluate the long term efficacy of Caiapo. Type II diabetic patients treated by diet alone (n=27) were randomly assigned to receive either 4 g of Caiapo each day or a placebo. After 5 months of Caiapo treatment, H<sub>b</sub>A<sub>1c</sub> decreased from  $6.46 \pm 0.12\%$  to  $6.25 \pm 0.11\%$  (P=0.008), fasting blood glucose decreased from  $138 \pm 4$  mg/dl to  $128 \pm 5$  mg/dl (P=0.039), and oral glucose insulin sensitivity increased from  $293 \pm 15$  mL/m<sup>2</sup>/min to  $321$  mL/m<sup>2</sup>/min (P=0.0072). Fasting plasma insulin decreased but not significantly from  $20.7 \pm 2.2$  μU/mL to  $17.6 \pm 1.7$  μU/mL (P=0.06). Adiponectin increased from  $5.97 \pm 0.65$  μg/mL to  $6.63 \pm 0.70$  μg/mL (P=0.013) and fibrinogen decreased from  $3.83 \pm 0.16$  mg/mL to  $3.64 \pm 0.18$  mg/mL (P=0.02). There was no significant change in body weight in either group. The only significant change exhibited in the placebo group was an increase in H<sub>b</sub>A<sub>1c</sub> from  $6.25 \pm 0.10\%$  to  $6.50 \pm 0.12\%$  (P=0.0001).

Kusano and others (2001) attempted to isolate the component responsible for the observed antidiabetic effects of Caiapo. Dialysis, ethanol precipitation, trichloroacetic acid

(TCA) precipitation, ion exchange chromatography, and ultrafiltration were performed on the sweet potato extract. The active component was not removed by dialysis and was found in the ethanol soluble fraction and the TCA soluble fraction. The active component was isolated to one fraction by ion exchange chromatography and ultrafiltration showed the molecular weight to be less than 30 kDa. The active component was assumed to be a 22 kDa acidic glycoprotein based on gel filtration and QA column testing. When the isolated active component was fed to db/db mice in an amount of 20 mg/kg/day for 14 days fasting blood glucose and fasting blood insulin decreased compared to the control,  $652 \pm 88$  to  $525 \pm 73$  mg/dL ( $P < 0.05$ ) and  $81 \pm 9.2$  to  $62 \pm 12$   $\mu$ U/mL ( $P < 0.05$ ) respectively.

Sakuramata and others (2004) studied the effect of Caiapo in combination with mulberry leaf powder and loquat leaf powder on glucose tolerance. Mulberry leaf is an alpha-glucosidase inhibitor and loquat leaf has an insulin-like effect. Male KK-Ay mice were fed either Caiapo alone (357 mg/kg), Caiapo (357 mg/kg) mixed with mulberry leaf powder (143 mg/kg) or Caiapo (194 mg/kg) mixed with loquat leaf extract (6 mg/kg) for 28 days with a glucose load test performed every 7 days. After 1 week of feeding, blood glucose levels 60 minutes after a glucose load were lower in the group being fed Caiapo mixed with mulberry leaf extract and Caiapo mixed with loquat leaf extract than in the group being fed Caiapo alone ( $P < 0.05$ ). After 3 weeks of feeding, the group fed Caiapo alone showed similar reduction in blood glucose levels.

### ***Flavone from Sweet Potato Leaf***

Flavone extracted from sweet potato leaf has been shown to possess antidiabetic properties as well. A decrease in fasting plasma insulin, fasting blood glucose, and LDL

cholesterol and an increase in insulin sensitivity were observed when flavone (25 mg/kg, 50 mg/kg, and 100 mg/kg) was fed to rats with non-insulin dependent diabetes mellitus for 2 weeks (Zhao 2007).

***Diacylated Anthocyanin from Sweet Potato Root***

A diacylated anthocyanin isolated from Ayamurasaki sweet potato root was shown to inhibit maltase in Sprague-Dawley rats. Blood glucose levels were significantly lower ( $P < 0.05$ ) 30 minutes after rats were fed 400 mg/kg diacylated anthocyanin with 2 g/kg maltose ( $143.8 \pm 4.2$  mg/dl) compared to the control group that was only fed 2 g/kg maltose ( $170.3 \pm 4.6$  mg/dl). Serum insulin was significantly reduced ( $P < 0.01$ ) in the group fed diacylated anthocyanin after 30 minutes ( $1.15 \pm 0.34$  ng/mL) and 60 minutes ( $0.80 \pm 0.22$  ng/mL) compared to the control group at 30 minutes ( $2.85 \pm 0.13$  ng/mL) and 60 minutes ( $2.48 \pm 0.34$  ng/mL) (Matsui and others 2002).

## **GUT ENZYME INHIBITORS**

### **Amylase Inhibitors**

Amylase inhibitors are naturally present in many plants and protect the plant from pests by not allowing the insect to break down starch and gain energy from it. Plants may contain separate protease inhibitors as well or amylase inhibitors may play a dual role and also inhibit proteases. Protein amylase inhibitors as well as non-protein amylase inhibitors exist. Amylase inhibitors may be active against a wide variety of amylases or may be specific to certain insect amylases or mammalian amylases (Franco and others 2002).

### **Structure of Proteinaceous Amylase Inhibitors**

The determination of the structure of a complex between porcine pancreatic amylase and a protein amylase inhibitor isolated from bean (*Phaseolus vulgaris*) showed interaction between the pancreatic amylase active site and the inhibitor. Conformational changes were observed in the pancreatic amylase upon the binding of the inhibitor. The inhibitor was found to be a dimer with a disaccharide attached to one of the amino acid residues. (Bompard-Gilles and others 1996).

### **Amylase Inhibitors in Sweet Potatoes**

Shivaraj and others (1979) reported that sweet potatoes do not contain amylase inhibitors while Rekha and others (1999) reported the presence of amylase inhibitors in 79 of the 100 varieties tested. Cultivar differences as well as isolation procedure could account for these differing results. Before performing amylase inhibitor assays, Shivaraj and others homogenized sweet potato with water, allowed the samples to sit for 1 hour, centrifuged the samples, collected the supernatant, and then subjected the supernatant to heat treatment

(80°C for 10 minutes) to destroy native amylases. Rekha and others homogenized sweet potatoes in a sodium phosphate buffer containing polyvinyl pyrrolidone and sodium chloride, stored the samples in the refrigerator, centrifuged the samples, and then performed trichloroacetic acid precipitation to remove native amylases. Rekha and others chose to use TCA precipitation rather than the heat treatment Shivaraj and others used because Rekha and others found heat treatment to be ineffective at destroying all native amylase activity.

### **Amylase Inhibitors in Plants Used to Treat Diabetes**

Amylase inhibitors have been identified in several plants that are used to treat diabetes. In vitro assays suggest the presence of  $\alpha$ -amylase inhibitors in *Phyllanthus amarus*, a plant used to treat diabetes in Malaysia. Various components were isolated and oleanolic acid and ursolic acid were deemed the major components responsible for  $\alpha$ -amylase inhibition (Ali and others 2006). Inhibition of  $\alpha$ -amylase has also been demonstrated in vitro by extracts of rosmarinic acid from lemon balm and oregano. Various herbs containing rosmarinic acid are used to treat diabetes in Southern Europe, Japan, and India (McCue and Shetty 2004).

### **Alpha-Glucosidase Inhibitors in Plants Used to Treat Diabetes**

A number of plants have been shown to possess  $\alpha$ -glucosidase inhibitors that may be useful in the treatment of diabetes. Inhibition of  $\alpha$ -glucosidase slows the degradation of disaccharides and thus reduces the amount of monosaccharides available for absorption. Kwon and others (2005) found  $\alpha$ -glucosidase inhibitors in oregano, chocolate mint, lemon balm, sage, and rosemary. The concentration of phenolic compounds was linked to the

degree of  $\alpha$ -glucosidase inhibition. Chebulagic acid isolated from *Terminalia chebula* Retz has also been shown to inhibit  $\alpha$ -glucosidase in vitro (Gao and others 2008).

## **CONCLUSION**

Sweet potato cultivars in the United States such as the Beauregard variety may contain components that lower blood glucose and could be useful in the management of diabetes. Possible mechanisms include inhibition of pancreatic amylase and inhibition of  $\alpha$ -glucosidases. Testing for pancreatic amylase inhibition can be done in vitro by using starch as a substrate, pancreatic amylase as the enzyme, sugar reducing ends as the product, and dinitrosalicylic acid as the detection medium. Similarly, testing for inhibition of  $\alpha$ -glucosidases in vitro can be done using sucrose, maltose, isomaltose, or lactose as the substrate, sucrase, maltase, isomaltase, or lactase respectively as the enzyme, sugar reducing ends as the product, and dinitrosalicylic acid as the detection medium. The focus of this paper is on testing for amylase inhibition.

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## **CHAPTER 2: ISOLATION OF SWEET POTATO PROTEIN**

## **ABSTRACT**

The goal of this experiment was to determine the optimum buffer for extraction of protein from Beauregard sweet potatoes. The ideal buffer would utilize the least amount of chemical while extracting the greatest amount of protein. Buffers containing varied amounts of sodium phosphate (0.025 M and 0.05 M) and sodium chloride (0.1 M, 0.2 M, and 0.3 M) were compared to determine the most efficient buffer for sweet potato protein extraction. Sweet potato flesh was homogenized with each buffer and then protein was precipitated with calcium chloride and resuspended in 10 mM EDTA/2% SDS. Bicinchoninic acid (BCA) assay was used to determine protein concentration in solution after homogenization and after precipitation and resuspension.

The most efficient buffer for protein extraction from sweet potato was distilled water. The concentration of protein in solution after homogenization and the concentration of protein in solution after precipitation and resuspension was statistically the same for all buffers. Since the most efficient buffer not only allows for the greatest protein yield but also utilizes the least amount of chemical, distilled water was determined to be the most efficient buffer.

## **INTRODUCTION**

The ideal method of protein extraction would produce a high protein yield with minimal chemical usage. Peters (2007) compared sweet potato protein extraction with a distilled water buffer to extraction with a 0.05 M sodium phosphate and 0.3 M NaCl buffer and protein precipitation with ammonium sulfate to precipitation with calcium chloride. In this study sweet potato homogenized with 0.05 M sodium phosphate and 0.3 M NaCl buffer followed by precipitation with calcium chloride produced the highest protein yield ( $36.6 \pm 8.66$  mg/100 mL homogenate) while sweet potato homogenized with distilled water and precipitated with calcium chloride produced the lowest yield ( $24.6 \pm 3.07$  mg/100 mL homogenate).

There was no statistical difference between homogenization with 0.05 M sodium phosphate and 0.3 M NaCl buffer followed by precipitation with calcium chloride ( $36.6 \pm 8.66$  mg/100 mL homogenate) and homogenization with 0.05 M sodium phosphate and 0.3 M NaCl buffer followed by precipitation with ammonium sulfate ( $34.2 \pm 8.66$  mg/100 mL homogenate). Given the lack of difference in yield between the two methods, precipitation with calcium chloride was chosen to yield a product safe for human consumption with minimal further processing.

Peters (2007) determined that homogenization with 0.05 M sodium phosphate and 0.3 M NaCl buffer yielded a greater amount of protein than homogenization with distilled water, however, the amount of sodium phosphate and sodium chloride required to maintain a high protein yield was not determined. A lesser amount of sodium phosphate or sodium chloride could still yield a high amount of protein and thus be more efficient by utilizing fewer

chemicals. In this experiment, buffers utilizing varied amounts of sodium phosphate (0.05 M and 0.025 M) and sodium chloride (0.3 M, 0.2 M, and 0.1 M) were compared to determine the most efficient method of protein extraction.

## **MATERIALS AND METHODS**

### **Acquisition and Storage of Sweet Potatoes**

Beauregard sweet potatoes were obtained from the Department of Horticulture at North Carolina State University. Sweet potatoes were peeled and stored in a freezer at -20°C. The frozen sweet potatoes were transferred to a refrigerator at 4°C to thaw for 48 hours before use.

### **Preparation of Buffers**

Sodium phosphate monobasic monohydrate, ACS reagent, 98.0-102.0% (Sigma-Aldrich; St. Louis, MO) and sodium chloride certified A.C.S. (Fisher; Waltham, MA) were used to make the buffers. The five buffers tested were (1) 0.05 M sodium phosphate and 0.3 M sodium chloride, (2) 0.05 M sodium phosphate and 0.2 M sodium chloride, (3) 0.05 M sodium phosphate and 0.1 M sodium chloride, (4) 0.025 M sodium phosphate and 0.3 M sodium chloride, and (5) distilled water. All buffers were adjusted to pH=7.

### **Homogenization of Sweet Potato and Buffer**

Sweet potato flesh was shredded in a Cuisinart food processor (Conair Corporation; East Windsor, NJ) and then mixed with a plastic spoon. Sweet potato flesh (50 g) was added to 250 mL of buffer. The mixture was then homogenized in a Waring blender (Conair Corporation; East Windsor, NJ) for 2 minutes.

### **Analysis of Protein in Solution**

The samples were allowed to sit for 1 hour at 4°C. A portion of each sample (12 mL) was removed and centrifuged for 20 minutes at a G-force of 1077 g units (3363 rpm). A portion (0.1 mL) of the supernatant was removed for protein analysis by BCA assay.

### **Analysis of Precipitated Protein**

A portion (10 mL) of the supernatant was removed for protein precipitation with calcium chloride. A 0.05 mL portion of saturated (35%) calcium chloride solution was added to each 10 mL sample. Samples were allowed to sit for 24 hours at 4°C. Samples were centrifuged for 20 minutes at a G-force of 1077 g units (3363 rpm) and the supernatant was discarded. The pellet was resuspended in 10 mL of 10 mM EDTA/2% SDS for protein analysis by BCA assay. The experiment was repeated 3 times.

### **BCA Assay**

A BCA Assay Kit (Pierce Biotechnology; Rockford, IL) was used to determine protein concentration. A 50 mL portion of reagent A (500 mL containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) was mixed with 1 mL reagent B (25 mL containing 4% cupric sulfate) to make the working reagent. A 100 µL portion of each sample and each standard was added to a test tube. Bovine serum albumin (BSA) of concentration 2.0 mg/mL was diluted to make the standards. The standards were of BSA concentration 0.0 mg/mL, 0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, and 2.0 mg/mL. Working reagent (2 mL) was added to each test tube. The tubes were mixed and incubated at 37°C for 30 minutes in a water bath. The samples were cooled in an ice bath and the absorbance at 562 nm of each of the samples was measured.

### **Calculations**

The total protein extracted from 50 g of sweet potato was calculated by multiplying the protein concentration as determined by BCA assay by the total volume of the homogenized solution (250 mL). The total protein recovered was calculated by dividing the

protein concentration after precipitation and resuspension by the protein concentration after homogenization and then multiplying by the total protein extracted.

### **Statistical Analysis**

The data were analyzed with the GLM procedure of SAS software. When there was a significant main effect, individual treatments were compared with Least Squares Means analysis (SAS Inc., Cary, NC).

## **RESULTS**

The protein concentration of each of the supernatant samples after homogenization of 50 g of sweet potato flesh with 250 mL of the various buffers is shown in table 2.1. While none of the samples contained statistically different concentrations of protein, homogenization with 0.05 M sodium phosphate and 0.1 M NaCl produced the highest average concentration ( $1.086 \pm 0.174$  mg/mL) followed by homogenization with 0.05 M sodium phosphate and 0.2 M NaCl ( $1.017 \pm 0.012$  mg/mL) and then homogenization with distilled water ( $1.008 \pm 0.141$  mg/mL). Homogenization with 0.025 M sodium phosphate and 0.3 M NaCl produced a slightly higher average concentration ( $0.999 \pm 0.063$  mg/mL) than homogenization with 0.05 M sodium phosphate and 0.3 M NaCl ( $0.953 \pm 0.144$  mg/mL).

The protein concentration of each of the samples after precipitation with calcium chloride and resuspension is shown in table 2.1 as well. The sample homogenized with 0.05 M sodium phosphate and 0.1 M sodium chloride exhibited the highest protein concentration ( $0.620 \pm 0.097$  mg/mL) followed by the sample homogenized with 0.05 M sodium phosphate and 0.2 M sodium chloride ( $0.581 \pm 0.255$  mg/mL) and then the sample homogenized with 0.05 M sodium phosphate and 0.3 M sodium chloride ( $0.524 \pm 0.160$  mg/mL). The sample homogenized with distilled water produced a slightly higher protein concentration ( $0.475 \pm 0.030$  mg/mL) than the sample homogenized with 0.025 M sodium phosphate and 0.3 M sodium chloride ( $0.470 \pm 0.164$  mg/mL).

The amount of protein extracted from 50 g sweet potato flesh and the amount of protein recovered are shown in table 2.2. Since total protein extracted was calculated by

multiplying the protein concentration after homogenization by the total volume of homogenate and protein recovered was calculated by multiplying the protein concentration after precipitation and resuspension by the total volume of homogenate, the results follow the same trend as the protein concentration data.

The average percent protein in the homogenate recovered by precipitation is shown in table 2.3. The greatest average percent protein recovered was achieved with the 0.05 M sodium phosphate and 0.2 M NaCl buffer (57.2%) followed by the 0.05 M sodium phosphate and 0.1 M NaCl buffer (57.1%) and then the 0.05 M sodium phosphate buffer and 0.3 M NaCl buffer (55.0%). The average percent protein recovered with distilled water was 47.1% and the average percent protein recovered with 0.025 M sodium phosphate and 0.3 M NaCl buffer was 47.1%. None of these values were significantly different from each other.

## **DISCUSSION**

The concentration of salt in a solution can affect the solubility of proteins through the mechanisms of salting in and salting out. Salting in refers to the increase in protein solubility as salt concentration increases due to salt ions interacting with the charged portions of protein. Salting out refers to the decrease in protein solubility once salt concentration reaches a certain level at which the interactions between salt and protein decrease the interactions of the protein with the solvent (Voet and Voet 1990).

The mechanisms of salting in and salting out do not appear to play a role in the solubility of protein extracted from sweet potato flesh. The amount of protein in solution after homogenization in the various buffers was not statistically different. The addition of sodium phosphate and sodium chloride did not increase the solubility of the protein as would be expected through salting in or decrease the solubility of the protein as would be expected with salting out when compared to distilled water.

The amount of protein in solution after precipitation with calcium chloride and resuspension showed greater differences, although none of the differences were statistically significant. The highest average protein concentration after precipitation and resuspension was reached using the 0.05 M sodium phosphate and 0.1 M sodium chloride buffer.

## **CONCLUSIONS**

The most efficient solvent system for extraction of protein from sweet potato was found to be distilled water. There were no statistical differences in protein extracted and protein recovered between homogenization of sweet potato with 0.05 M sodium phosphate and 0.3 M sodium chloride buffer, 0.05 M sodium phosphate and 0.2 M sodium chloride buffer, 0.05 M sodium phosphate and 0.1 M sodium chloride buffer, 0.025 M sodium phosphate and 0.3 M sodium chloride, and distilled water.

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Table 2.1 Effect of Buffer on Protein Solubility after Homogenization and Protein Solubility after Precipitation and Resuspension

Buffer	Protein Concentration after Homogenization (mg/mL)		Protein Concentration after Precipitation and Resuspension (mg/mL)	
	Average	Standard Deviation	Average	Standard Deviation
0.05 M sodium phosphate and 0.3 M NaCl	0.953 <sup>a</sup>	0.144	0.524 <sup>a</sup>	0.160
0.05 M sodium phosphate and 0.2 M NaCl	1.02 <sup>a</sup>	0.012	0.581 <sup>a</sup>	0.255
0.05 M sodium phosphate and 0.1 M NaCl	1.09 <sup>a</sup>	0.174	0.620 <sup>a</sup>	0.097
0.025 M sodium phosphate and 0.3 M NaCl	0.999 <sup>a</sup>	0.063	0.470 <sup>a</sup>	0.164
Distilled water	1.01 <sup>a</sup>	0.141	0.475 <sup>a</sup>	0.030

\*Values with the same letter superscript are not statistically different.

Table 2.2 Total Protein Extracted and Total Protein Recovered from 50 g Sweet Potato Flesh

Buffer	Total Protein Extracted (mg)		Total Protein Recovered (mg)	
	Average	Standard Deviation	Average	Standard Deviation
0.05 M sodium phosphate and 0.3 M NaCl	238 <sup>a</sup>	36.1	131 <sup>a</sup>	40.0
0.05 M sodium phosphate and 0.2 M NaCl	254 <sup>a</sup>	2.89	145 <sup>a</sup>	63.8
0.05 M sodium phosphate and 0.1 M NaCl	272 <sup>a</sup>	43.5	155 <sup>a</sup>	24.4
0.025 M sodium phosphate and 0.3 M NaCl	250 <sup>a</sup>	15.8	118 <sup>a</sup>	41.1
Distilled water	252 <sup>a</sup>	35.1	119 <sup>a</sup>	7.49

\*Values with the same letter superscript are not statistically different.

Table 2.3 Percent Protein in Homogenate Recovered by Precipitation

Buffer	Percent Protein Recovered
0.05 M sodium phosphate and 0.3 M NaCl	55.0 <sup>a</sup>
0.05 M sodium phosphate and 0.2 M NaCl	57.2 <sup>a</sup>
0.05 M sodium phosphate and 0.1 M NaCl	57.1 <sup>a</sup>
0.025 M sodium phosphate and 0.3 M NaCl	47.1 <sup>a</sup>
Distilled water	47.1 <sup>a</sup>

\*Values with the same letter superscript are not statistically different.

## **CHAPTER 3: DIGESTION OF SWEET POTATO PROTEIN WITH PEPSIN**

## **ABSTRACT**

The goal of the experiment was to determine the effect of pepsin on sweet potato protein. Pancreatic amylase is active in the small intestine so in order to inhibit pancreatic amylase, a protein inhibitor must not be inactivated by pepsin digestion in the stomach. Protein that is resistant to pepsin digestion could pass through the stomach and into the small intestine unaltered.

SDS-PAGE was used to determine the extent to which pepsin digests Beauregard sweet potato protein. Protein isolated from the flesh, peel, and leaf were treated with pepsin and then run on a gel next to samples not treated with pepsin.

Proteins resistant to pepsin digestion as well as proteins susceptible to pepsin digestion were found. A protein of the same molecular weight (22 kDa) as the protein reportedly isolated from Caiapo and deemed the active component was found in the flesh, peel, and leaf of Beauregard sweet potato and remained present after pepsin digestion.

## **INTRODUCTION**

Pancreatic amylase is responsible for the breakdown of starches to maltose, isomaltose, and maltotriose. Brush border enzymes then further break down these products to glucose for absorption and blood glucose levels rise (Gropper and others 2005). Reducing hyperglycemia is of great importance to the diabetic patient as hyperglycemia is associated with increased risk of complications (Turkoski 2006). Caiapo, an extract of Japanese white-skinned sweet potato, has been shown to reduce the glycemic response to white potato (Corbitt 2007) but not the glycemic response to glucose (Zakir and others 2006). Inhibition of pancreatic amylase was proposed as a mechanism of action because inhibition of pancreatic amylase would slow the digestion of starches but would have no effect on glucose since it does not need further digestion before absorption. Slowing the digestion of starch would decrease the absorption of glucose and the corresponding rise in blood glucose.

A 22 kDa glycoprotein was identified as the antidiabetic component in Caiapo (Kusano and others 2001). Pancreatic amylase is active in the small intestine, however, protein digestion begins in the stomach with the action of hydrochloric acid and pepsin. Hydrochloric acid can denature proteins and pepsin can hydrolyze peptide bonds (Gropper and others 2005). In order for a dietary protein to inhibit an enzyme in the small intestine the protein must pass through the stomach without losing its inhibitory activity. It was hypothesized that if the low glycemic index of sweet potato is due to the protein fraction, proteins resistant to pepsin digestion would be present.

## **MATERIALS AND METHODS**

### **Acquisition and Storage of Sweet Potatoes**

Beauregard sweet potato roots and leaves were obtained from the Department of Horticulture at North Carolina State University. Sweet potato roots were washed and peeled, leaves were washed, and all components were freeze dried. The freeze dried components were stored in a refrigerator at 4°C.

### **Isolation of Sweet Potato Protein**

A 10 g portion of freeze dried sweet potato flesh, peel, or leaf was blended with 250 mL of 0.05 M phosphate buffer containing 0.1 M sodium chloride for 2 minutes. The mixture was allowed to sit for 1 hour and then centrifuged for 20 minutes at a G-force of 1077 g units (3363 rpm). The supernatant was collected and 35% calcium chloride was added so that the final concentration was 0.5% calcium chloride. The mixture was allowed to sit for 24 hours and then the precipitate was removed by filtration through a Whatman 4 filter. The precipitate was resuspended in 10 mM EDTA/2% SDS buffer, pH=7.

### **Digestion with Pepsin**

A 0.025 g portion of pepsin from porcine gastric mucosa (Sigma-Aldrich; St. Louis, MO) was added to 10 mL of sweet potato protein solution. The pH was adjusted to 2 by the addition of 1 N hydrochloric acid. The solution was agitated for 45 minutes at 22°C. The pH was then adjusted to 7 by the addition of 1 N sodium hydroxide.

### **Gel Electrophoresis**

The running buffer was prepared by adding 270 mL of water to 30 mL of 10x Tris/Glycine/SDS buffer (Bio-Rad; Hercules, CA). The sample buffer was prepared by

adding 50  $\mu\text{L}$  of 2-mercaptoethanol to 950  $\mu\text{L}$  of Laemmli sample buffer (Bio-Rad; Hercules, CA). One part sweet potato protein sample was mixed with one part sample buffer and heated in boiling water for 5 minutes. A 15% Tris-HCl, 10 well, 30  $\mu\text{L}$  gel (Bio-Rad, Hercules, CA) was loaded in to a Mini Protean II Electrophoresis Cell (Bio-Rad, Hercules, CA). The running buffer was added to the cell and the comb was removed from the gel. A 10  $\mu\text{L}$  portion of SeeBlue Plus2 Pre-Stained Standard (Invitrogen; Carlsbad, CA) was added to the first well. Undigested sweet potato flesh protein (20  $\mu\text{L}$ ) was added to the second well. Pepsin digested sweet potato flesh protein (20  $\mu\text{L}$ ) was added to the third well. Undigested sweet potato peel protein (20  $\mu\text{L}$ ) was added to the fourth well. Pepsin digested sweet potato peel protein (20  $\mu\text{L}$ ) was added to the fifth well. Undigested sweet potato leaf protein (20  $\mu\text{L}$ ) was added to the sixth well. Pepsin digested sweet potato leaf protein (20  $\mu\text{L}$ ) was added to the seventh well. The chamber lid was attached and the power supply was turned on. The voltage was set to 200 V and the initial current was 60 A. When the tracking buffer reached the bottom of the gel the power supply was turned off and the gel was removed for staining.

### **Silver Staining**

A mixture of 160 mL methanol and 40 mL acetic acid was added to 200 mL water to make fixative. A 20 mL portion of oxidizer concentrate (Bio-Rad; Hercules, CA) was added to 180 mL water to make oxidizer. A 20 mL portion of silver reagent concentrate (Bio-Rad; Hercules, CA) was added to 180 mL water to make silver reagent. One bottle of silver stain developer (Bio-Rad; Hercules, CA) was dissolved in 3.6 L water to make developer. The gel was placed in 400 mL fixative for 30 minutes. Then the gel was placed in 200 mL oxidizer for 5 minutes. The gel was placed in 400 mL water for 5 minutes repeating until yellow

color was gone but no longer than 15 minutes. Then the gel was placed in 200 mL silver reagent for 20 minutes followed by 400 mL water for 30 seconds. The gel was placed in 200 mL developer for 30 seconds and then the developer was replaced with fresh developer. The developer was replaced with fresh developer every 5 minutes until the desired band intensity was obtained. Finally, the gel was placed in 400 mL 5% acetic acid for 15 minutes.

## **RESULTS**

Figure 3.1 shows the digestion of sweet potato protein isolated from the peel, flesh, and leaf by pepsin. From left to right the lanes are marker (A), undigested sweet potato flesh protein (B), pepsin digested sweet potato flesh protein (C), undigested sweet potato peel protein (D), pepsin digested sweet potato peel protein (E), undigested sweet potato leaf protein (F), and pepsin digested sweet potato leaf protein (G). A 22 kDa protein was present in all fractions. A greater number of low molecular weight proteins were present in the samples digested with pepsin compared to samples not digested with pepsin.

## **DISCUSSION**

Zakir and others (2006) used gel electrophoresis and Coomassie Brilliant Blue stain to compare protein band patterns of peel and flesh extracts from Beauregard as well as White Star sweet potatoes to Caiapo. Protein band patterns from Beauregard, White Star, and Caiapo were essentially identical. Silver stain was used for this experiment rather than Coomassie blue stain to achieve a greater amount of protein band detail with a minimal concentration of protein.

In order to inhibit pancreatic amylase, protein must survive pepsin digestion in the stomach and make it to the small intestine. Proteins that are resistant to pepsin digestion could inhibit pancreatic amylase in the small intestine. Proteins that are resistant to pepsin digestion are present in each of the extracts, flesh, peel, and leaf.

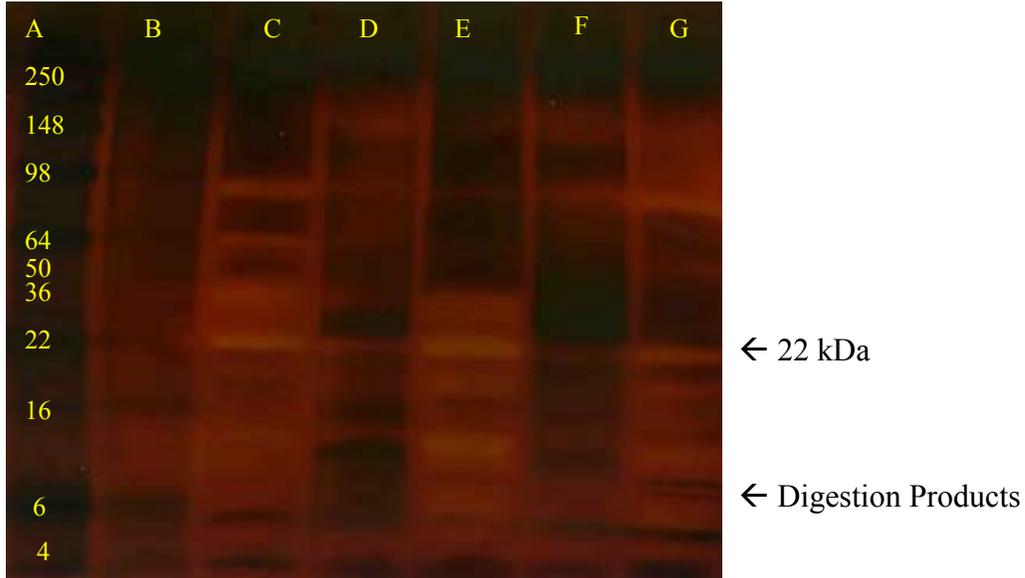
Kusano and others (2001) isolated the active component of Caiapo and estimated it to be an acidic glycoprotein with a molecular weight of 22 kDa. A 22 kDa protein is present in the flesh, peel, and leaf protein extracts of Beauregard sweet potato and remains present after pepsin digestion. The 22 kDa protein band becomes more pronounced after pepsin digestion indicating that larger proteins are cleaved by pepsin in to smaller, possibly more active proteins. The protein responsible for Caiapo's antidiabetic properties may be present in the Beauregard variety of sweet potato.

## **CONCLUSIONS**

Proteins resistant to pepsin digestion are present in Beauregard sweet potato flesh, peel, and leaf. The 22 kDa protein isolated from Caiapo and deemed the active antidiabetic component may be present in Beauregard sweet potato flesh, peel, and leaf.

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- A- marker
- B- undigested sweet potato flesh protein
- C- pepsin digested sweet potato flesh protein
- D- undigested sweet potato peel protein
- E- pepsin digested sweet potato peel protein
- F- undigested sweet potato leaf protein
- G- pepsin digested sweet potato leaf protein

Figure 3.1 Digestion of Sweet Potato Protein with Pepsin

**CHAPTER 4: AMYLASE ACTIVITY AND AMYLASE INHIBITORY ACTIVITY  
OF SWEET POTATO PROTEIN**

## **ABSTRACT**

Sweet potatoes have been shown to have a low glycemic index. One proposed mechanism of action is the inhibition of amylase by sweet potato protein. Testing this hypothesis is complicated by the presence of native amylase in sweet potato. The objectives of this experiment were to measure amylase activity and amylase inhibitory activity of sweet potato protein isolated from the peel, flesh, and leaf and to determine the effect of pepsin on these enzymes. Enzymatic activity was measured using soluble starch as the substrate and reducing sugars as the product. Dinitrosalicylic acid solution reacts with the sugar reducing ends to form a compound that absorbs at 540 nm.

The amylase activity of pancreatic amylase, sweet potato protein with and without incubation in the presence of pepsin, and a combination of pancreatic amylase and sweet potato protein with and without incubation in the presence of pepsin was determined using the above method. The expected amylase activity was calculated by adding the pancreatic amylase activity and the native sweet potato amylase activity. Inhibition was calculated by subtracting the experimental amylase activity of the combination of pancreatic amylase and sweet potato protein from the expected amylase activity.

The sweet potato peel and flesh protein extracts exhibited amylase activity while the leaf protein extract did not exhibit amylase activity. The amylase activity of the flesh protein extract was much higher ( $32.7 \pm 5.93$  units/mg protein) than the amylase activity of the peel protein extract ( $13.0 \pm 1.12$  units/mg protein). Incubation with pepsin eliminated the amylase activity of the flesh and peel extracts.

Amylase inhibitors were not apparent in any of the protein extracts before or after digestion with pepsin. The experimental amylase activity was higher than the expected amylase activity in all cases. The low glycemic index of sweet potatoes does not appear to be due to amylase inhibition by sweet potato protein.

## **INTRODUCTION**

Over 14 million people in the United States have been diagnosed with diabetes. Diabetes increases risk of heart attack, stroke, kidney failure, nerve damage, and blindness. Risk of complications can be reduced with proper disease management, especially control of blood glucose levels (Turkoski 2006). The glycemic index can be used to differentiate carbohydrates based on glycemic response. A meta-analysis found that low glycemic index diets reduced H<sub>b</sub>A<sub>1c</sub> levels and glycated proteins compared to high glycemic index diets (Brand-Miller and others 2003). Sweet potatoes have a low glycemic index and certain cultivars have been shown to have antidiabetic properties. One proposed mechanism for the low glycemic response to sweet potatoes is inhibition of pancreatic amylase.

Rekha and others (1999) found amylase inhibitors in sweet potatoes while Shivaraj and others (1979) did not find amylase inhibitors in sweet potatoes. Rekha and others (1999) homogenized sweet potatoes in a sodium phosphate buffer containing polyvinyl pyrrolidone and sodium chloride while Shivaraj and others (1979) homogenized sweet potatoes with water. Shivaraj and others (1979) heated the sweet potato samples for 10 minutes at 80°C to destroy native amylase activity. Rekha and others (1999) found that this procedure was not effective in destroying all native amylase activity and thus precipitated the native amylases with trichloroacetic acid.

Amylase inhibitors could be inactivated by heat treatment and protein amylase inhibitors could be precipitated with trichloroacetic acid. Protein amylase inhibitors could be present in sweet potatoes but not apparent following the above procedures. In this experiment, native amylase activity was adjusted for rather than removed.

Cultivar differences could also lead to differences in amylase inhibitor activity as suggested by Rekha and others (1999) finding amylase inhibitor activity in only 79 of the 100 sweet potato varieties tested. The Beauregard variety of sweet potato is one of the top cultivars grown in the United States (Bovell-Benjamin 2007) and thus was the cultivar of interest for this experiment.

Digestion of protein can affect activity. Cleavage of protein can decrease activity as well as create an active fragment. In vivo, sweet potato protein would be subjected to pepsin digestion before entering the small intestine, therefore, it is important to test the amylase inhibitory activity of sweet potato protein after digestion with pepsin.

## **MATERIALS AND METHODS**

### **Acquisition and Storage of Sweet Potatoes**

Beauregard sweet potato roots and leaves were obtained from the Department of Horticulture at North Carolina State University. Sweet potato roots were washed, peeled and stored in a freezer at -20°C. Sweet potato leaves were washed and stored in a freezer at -20°C.

### **Isolation of Sweet Potato Protein**

A 25 g portion of each sweet potato component (peel, flesh, and leaf) was blended with 125 mL of 0.05 M phosphate buffer containing 0.1 M sodium chloride for 2 minutes. The mixture was allowed to sit for 1 hour and then centrifuged for 20 minutes at 3363 rpm. The supernatant was collected and 35% calcium chloride was added so that the final concentration was 0.5% calcium chloride. The mixture was allowed to sit for 24 hours and then the precipitate was removed by filtration through a Whatman 4 filter. The precipitate was resuspended in 20 mM sodium phosphate buffer. Samples not undergoing pepsin digestion were stored frozen in 5 mL volumes until 12 hours before needed upon which they were transferred to the refrigerator.

### **Digestion of Sweet Potato Protein by Pepsin**

A 250 mL portion of immobilized pepsin (Thresher 1989) was added to 10 mL of each sweet potato protein solution. The pH was adjusted to 2 by addition of 1 N hydrochloric acid. The samples were shaken for 4 hours at 22°C. The pH was adjusted to 7 by addition of 1 N sodium hydroxide. The immobilized pepsin was removed by centrifugation at a G-force of 1077 g units (3363 rpm) for 15 minutes. Samples were stored

frozen in 5 mL volumes until 12 hours before needed upon which they were transferred to the refrigerator.

### **BCA Assay**

A BCA Assay Kit (Pierce Biotechnology; Rockford, IL) was used to determine the protein concentration in each sample. A 50 mL portion of reagent A (500 mL containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1 M sodium hydroxide) was mixed with 1 mL reagent B (25 mL containing 4% cupric sulfate) to make the working reagent. A 100  $\mu$ L portion of each sample and each standard was added to a test tube. Bovine serum albumin (BSA) of concentration 2.0 mg/mL was diluted to make the standards. The standards were of BSA concentration 0.0 mg/mL, 0.5 mg/mL, 1.0 mg/mL, 1.5 mg/mL, and 2.0 mg/mL. Working reagent (2 mL) was added to each test tube. The tubes were mixed and incubated at 37°C for 30 minutes in a water bath. The samples were cooled in an ice bath and the absorbance at 562 nm of each of the samples was measured. Based on the results of the BCA assay, each sample was diluted with 20 mM sodium phosphate buffer to a protein concentration of 0.309 mg/mL to aid in comparability of results. The concentration before adjustment of each of the samples is shown in table 4.1.

### **Determination of the Substrate Concentration**

Various concentrations of starch solution were subjected to digestion by amylase under the conditions of the assay to ensure adequate substrate availability. The percentage starch versus the absorbance at 540 nm is shown in figure 4.1. The 2% starch solution was chosen because amylase activity did not increase with the addition of substrate beyond this concentration.

### **Preparation of Reagents for Assay**

A 2% soluble starch solution was prepared by dissolving Starch Soluble Certified A.C.S. (Fisher Scientific; Waltham, MA) in warm distilled water. The pancreatic amylase solution was prepared by adding 5  $\mu$ L  $\alpha$ -Amylase, Type I-A: PMSF treated, From Porcine Pancreas (Sigma-Aldrich; St. Louis, MO) to 10 mL of 20 mM sodium phosphate buffer. The dinitrosalicylic acid (DNS) solution was prepared by adding 0.735 g DNS to 73.25 mL of water and 21.25 g potassium sodium tartrate to a solution of 1.125 g NaOH in 25 mL of water.

### **Assay for Amylase Activity and Amylase Inhibitor Activity**

This procedure is a modified version of the procedures of Ali and others (2006) and Shivaraj and others (1979). A 0.25 mL portion of each protein solution was added to 0.25 mL amylase solution in duplicate (experimental activity). A 0.25 mL portion of DI water was added to 0.25 mL amylase solution in duplicate (pancreatic amylase activity). A 0.25 mL portion of each protein solution was added to 0.25 mL DI water in duplicate (sweet potato amylase activity). Samples were incubated at 37°C for 15 minutes. Starch solution (0.5 mL) was added to each sample. DNS solution (2 mL) was immediately added to one set of samples. The other set of samples was allowed to sit for 3 minutes before addition of 2 mL DNS solution. Samples were heated in boiling water for 10 minutes. Samples were then cooled in a 27°C water bath for 3 minutes. Absorbance at 540 nm was measured. The final absorbance value was calculated by subtracting absorbance at 0 minutes from absorbance at 3 minutes. The experiment was repeated 3 times.

### **Calculation of Inhibition**

Expected activity was calculated by adding pancreatic amylase activity and sweet potato amylase activity. Experimental activity was the amylase activity measured from the solution containing both pancreatic amylase and sweet potato protein. Inhibition was calculated by subtracting experimental activity from expected activity.

### **Calculation of Specific Activity**

The relationship between maltose concentration and absorbance at 540 nm was determined by reacting various concentrations of maltose with DNS reagent under the conditions of the assay and recording the absorbance at 540 nm. The maltose standard curve shown in figure 4.2 was used to calculate amylase activities based on observed absorbance. A unit of amylase activity is defined as the amount that liberated 1  $\mu\text{mol}$  of reducing sugar per minute under the conditions of the assay. A unit of amylase inhibitory activity is defined as the amount that prevented the liberation of 1  $\mu\text{mol}$  of reducing sugar per minute under the conditions of the assay. Specific activity was calculated by dividing the units of activity by the amount of protein (mg) in the sample.

### **Statistical Analysis**

The data were analyzed with the GLM procedure of SAS software. When there was a significant main effect, individual treatments were compared with Least Squares Means analysis (SAS Inc., Cary, NC).

## **RESULTS**

The amylase activity of sweet potato protein isolated from the flesh, peel, and leaf is shown in table 4.2. The amylase activity of the flesh protein before pepsin digestion ( $32.7 \pm 5.93$  units/mg protein) was significantly higher than the amylase activity of the peel protein before pepsin digestion ( $13.0 \pm 1.12$  units/mg protein). Pepsin digestion eliminated the amylase activity of the flesh protein ( $0.0130 \pm 0.812$  units/mg protein) and the peel protein ( $0.609 \pm 0.795$  units/mg protein). No amylase activity was exhibited by the leaf protein before pepsin digestion ( $-1.40 \pm 0.469$  units/mg protein) or after pepsin digestion ( $-0.625 \pm 5.93$  units/mg protein).

The expected amylase activity and the experimental amylase activity of the combination of sweet potato protein and porcine pancreatic amylase (PPA) are shown in table 4.3. The experimental amylase activity was higher than the expected amylase activity in all cases. The expected amylase activity of the flesh extract with PPA was  $42.0 \pm 4.84$  units/mg protein while the experimental amylase activity was  $53.0 \pm 5.93$  units/mg protein. The expected amylase activity of the pepsin treated flesh extract with PPA was  $10.7 \pm 1.84$  units/mg protein while the experimental amylase activity was  $28.7 \pm 1.60$  units/mg protein. The expected amylase activity of the peel extract with PPA was  $23.1 \pm 1.92$  units/mg protein while the experimental amylase activity was  $36.1 \pm 2.36$  units/mg protein. The expected amylase activity of the pepsin treated peel extract with PPA was  $11.2 \pm 0.499$  units/mg protein while the experimental amylase activity was  $28.7 \pm 1.27$  units/mg protein. The expected amylase activity of the leaf extract with PPA was  $9.33 \pm 1.23$  units/mg protein while the experimental amylase activity was  $26.0 \pm 2.79$  units/mg protein. The expected

amylase activity of the pepsin treated leaf extract with PPA was  $10.1 \pm 1.16$  units/mg protein while the experimental amylase activity was  $29.4 \pm 2.33$  units/mg protein.

## **DISCUSSION**

The extracts were resuspended in 20 mM sodium phosphate buffer for this experiment because native amylase activity of the sweet potato flesh and peel extracts and pancreatic amylase activity were eliminated when 10 mM EDTA/2% SDS was used.

There was variability in the amount of protein extracted from the flesh, peel, and leaf. To aid in comparability of amylase and amylase inhibitory activity in the different components the protein concentration was adjusted to 0.309 mg/mL by the addition of 20 mM phosphate buffer. Table 4.2 shows the original concentration of protein in each of the samples.

The elimination of the native amylase activity in the flesh and peel by pepsin digestion indicate that in vivo sweet potato amylase would likely not be active in the small intestine and thus not aggravate the diabetic condition by increasing starch digestion.

Amylase inhibition by sweet potato protein does not appear to be the mechanism for the low glycemic response to sweet potatoes. The experimental amylase activity of all samples was statistically higher than the expected amylase activity indicating a lack of amylase inhibitors and possibly the presence of amylase activators. The experimental amylase activities of the flesh and peel extracts after pepsin treatment were significantly lower than the experimental amylase activities of the flesh and peel extracts prior to pepsin treatment. This was expected since pepsin digestion eliminated native amylase activity in the flesh and peel extracts.

Non-protein components present in Beauregard sweet potatoes may inhibit pancreatic amylase because the amylase inhibitors Rekha and others (1999) observed were not

characterized as protein. It is also possible that the Beauregard cultivar may not contain amylase inhibitors as was the case for 21 of the 100 varieties of sweet potato tested by Rekha and others (1999).

Inhibition of maltase by sweet potato protein could also explain the decreased glycemic response to white potato with the addition of Caiapo (Corbitt 2007) while the addition of Caiapo to glucose did not exhibit a decreased glycemic response (Zakir and others 2006). Before being absorbed, starch must be broken down to maltose by pancreatic amylase and then to glucose by maltase (Gropper and others 2005). Inhibition of this second step could account for the lower than expected blood glucose levels exhibited by sweet potato and white potato in combination with Caiapo.

## **CONCLUSIONS**

Amylase inhibition by sweet potato protein does not appear to be the mechanism for the low glyceimic response to Beauregard sweet potatoes. Gastric digestion is likely to eliminate native amylase activity in the sweet potato thereby requiring pancreatic amylase to digest carbohydrate in vivo. Non-protein components or protein components using a different pathway may provide a mechanism for the low glyceimic response to sweet potatoes.

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Table 4.1 Protein Concentration after Precipitation and Resuspension (Prior to Adjustment)

Component	Protein Concentration (mg/mL)
Flesh Extract 1	0.473
Flesh Extract 2	1.045
Flesh Extract 3	0.561
Peel Extract 1	0.318
Peel Extract 2	0.309
Peel Extract 3	0.611
Leaf Extract 1	0.576
Leaf Extract 2	0.903
Leaf Extract 3	1.253

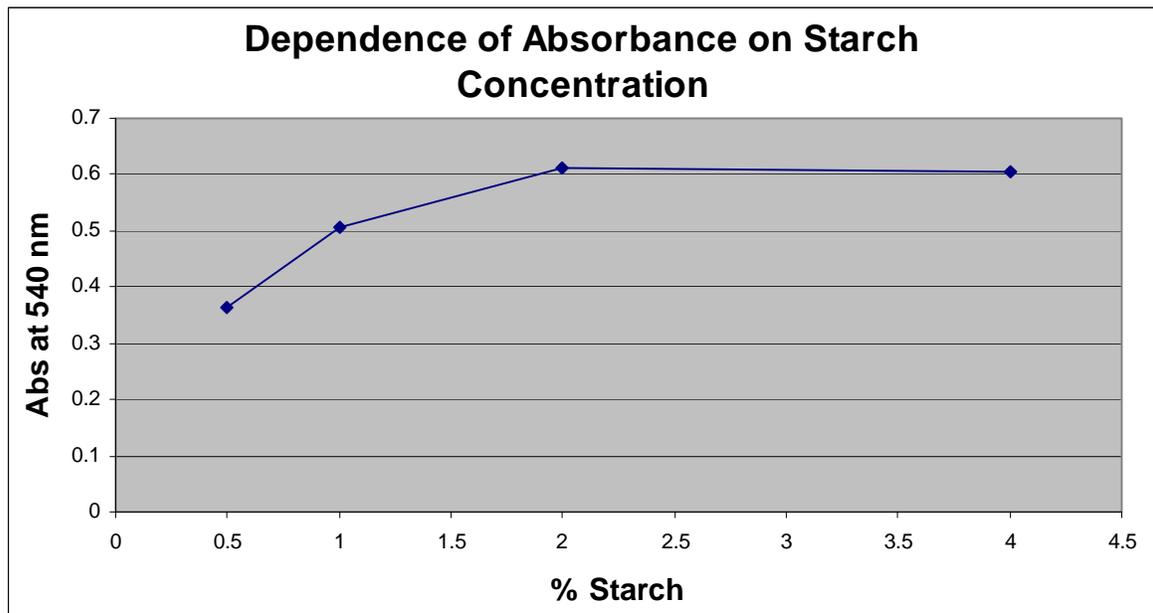


Figure 4.1 Dependence of Absorbance on Starch Concentration in the Amylase Assay

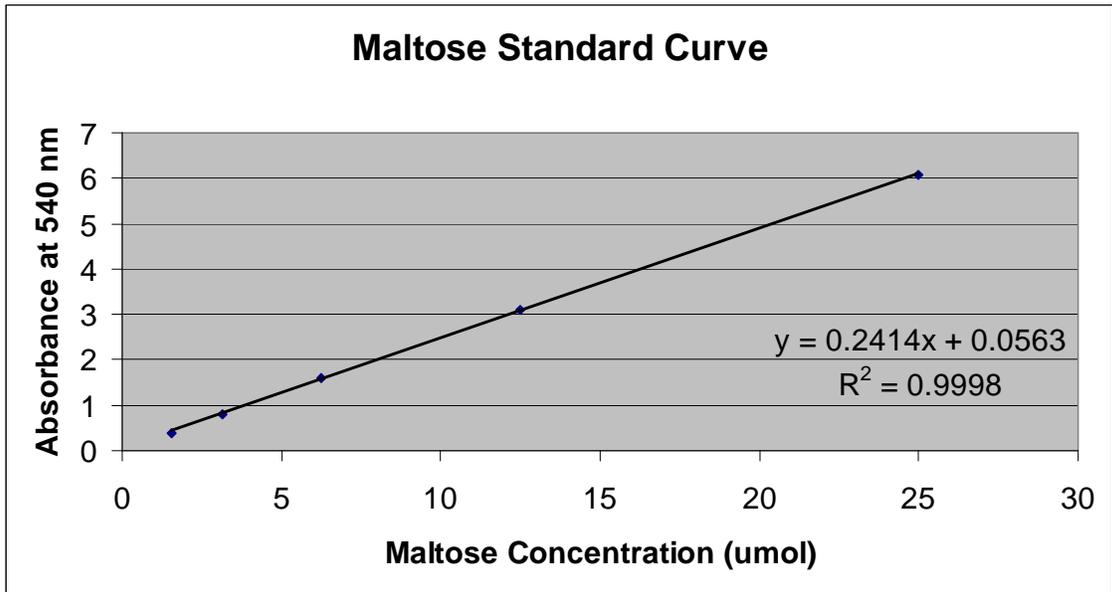


Figure 4.2 Maltose Standard Curve

Table 4.2 Amylase Activity of Sweet Potato Components

Sweet Potato Component	Amylase Activity (units/mg protein)	
	Average	Standard Deviation
Flesh	32.7 <sup>a</sup>	5.93
Flesh (Pepsin Treated)	0.0130 <sup>c</sup>	0.812
Peel	13.0 <sup>b</sup>	1.12
Peel (Pepsin Treated)	0.609 <sup>c</sup>	0.795
Leaf	-1.40 <sup>c</sup>	0.469
Leaf (Pepsin Treated)	-0.625 <sup>c</sup>	0.179

\*Values with the same letter superscript are not statistically different.

\*Negative values are statistically the same as zero.

Table 4.3 Amylase Inhibitory Activity of Sweet Potato Components

Components	Expected Amylase Activity (units/mg protein)		Experimental Amylase Activity (units/mg protein)		Amylase Inhibitory Activity (units/mg protein)	
	Average	Standard Deviation	Average	Standard Deviation	Average	Standard Deviation
Flesh + PPA <sup>1</sup>	42.0 <sup>b</sup>	4.84	53.0 <sup>a</sup>	5.14	-11.0	0.386
Flesh (Pepsin Treated) + PPA <sup>1</sup>	10.7 <sup>f</sup>	1.84	28.7 <sup>d</sup>	1.60	-18.0	1.19
Peel + PPA <sup>1</sup>	23.1 <sup>e</sup>	1.92	36.1 <sup>c</sup>	2.36	-13.0	2.02
Peel (Pepsin Treated) + PPA <sup>1</sup>	11.2 <sup>f</sup>	0.499	28.7 <sup>d</sup>	1.27	-17.5	0.775
Leaf + PPA <sup>1</sup>	9.33 <sup>f</sup>	1.23	26.0 <sup>de</sup>	2.79	-16.7	3.36
Leaf (Pepsin Treated) + PPA <sup>1</sup>	10.1 <sup>f</sup>	1.16	29.4 <sup>d</sup>	2.33	-19.4	3.45

\*Values with the same letter superscript are not statistically different.

<sup>1</sup>PPA=porcine pancreatic amylase

## **CHAPTER 5: CONCLUSION**

## **CONCLUSION**

The native amylase activity of sweet potato flesh and peel appear to be eliminated by pepsin digestion. The diabetic condition should not be aggravated by native sweet potato amylases because amylase activity will be destroyed during digestion in the stomach. An animal model should be used to confirm the elimination of the native amylase activity of sweet potato in vivo.

Inhibition of pancreatic amylase by sweet potato protein does not appear to be the mechanism for the low glycemic response to sweet potatoes. Inhibition of maltase by sweet potato protein could account for the decreased glycemic response to white potato with the addition of Caiapo (Corbitt 2007) and the lack of decreased glycemic response to glucose with the addition of Caiapo (Zakir and others 2006). Starch must be broken down to maltose by pancreatic amylase and then maltose must be broken down to glucose by maltase before absorption can occur (Gropper and others 2006). Decreased maltose breakdown to glucose due to inhibition of maltase could explain the reduced glycemic response to white potato with the addition of Caiapo (Corbitt 2007). Likewise, maltase inhibition would have no effect on glucose absorption and thus the glycemic response to glucose would not be changed by the addition of Caiapo. Further research is needed to determine if maltase inhibitors are present in Beauregard sweet potatoes.

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