

## ABSTRACT

PETERS, TIFFANY BETH. Isolation of *Ipomoea batatas* Protein and Characterization of Amylase Inhibitory Activity (Under the direction of Dr. Jonathan C. Allen).

Caiapo, a protein extract of sweet potato (*Ipomoea batatas*), has been shown to decrease fasting plasma glucose levels, low-density lipoprotein cholesterol, and total cholesterol in type 2 diabetic patients. As an additive in foods, Caiapo has also been shown to modify the glycemic index of foods, suggesting that Caiapo inhibits pancreatic amylase. A number of studies have been completed linking Caiapo and  $\alpha$ -amylase inhibitor properties. However, previous work in the extraction of these compounds has been completed with chemicals unsafe for human consumption, including trichloroacetic acid. The present study aimed to determine a food grade method of extraction of sweet potato protein, and determine  $\alpha$ -amylase inhibitory effects of the sweet potato protein fractions.

A 2x2 treatment was used to determine the most efficient method of protein extraction. Proteins were homogenized into distilled water or 50 mM phosphate buffer with 0.3 M NaCl and precipitated with either 60% ammonium sulfate or 0.5% calcium chloride. The water-soluble and the insoluble fractions were assayed for total and soluble protein concentration using the EZQ Protein Assay and the BCA Protein Assay, respectively. Of the four different extraction methods, homogenizing with 50 mM phosphate buffer and 0.3 M NaCl followed by a 0.5% calcium chloride precipitation was the most efficient technique.

For the determination of amylase activity and amylase inhibition, proteins were precipitated from the water-soluble fraction of sweet potato flesh with ammonium sulfate.

When the precipitated protein pellet was dialyzed against water, a portion of the protein pellet redissolved and an insoluble pellet remained. Both fractions were subsequently heated at 95 °C for 30 minutes. All four fractions were assayed for total protein concentration, soluble protein concentration, amylase and  $\alpha$ -amylase inhibitor activity. The samples were allowed to incubate with or without  $\alpha$ -amylase before the addition of starch. Amylase activity was calculated from absorbance of the starch solution after iodine was added. Native sweet potato amylases, found in both insoluble and water-soluble protein fractions, were reduced by heat treatment, and calculation of expected vs. observed amylase suggested the presence of heat-stable amylase inhibitor in the material isolated. Our data show amylase activity in the protein extract when combined with porcine  $\alpha$ -amylase to be less than the amylase activity we expected to find. Thus, amylase inhibition may be responsible for the hypoglycemic effect of sweet potato.

**ISOLATION OF *IPOMOEA BATATAS* PROTEIN AND  
CHARACTERIZATION OF AMYLASE INHIBITORY ACTIVITY**

By

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North Carolina State University  
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## **BIOGRAPHY**

Tiffany Beth Peters was born on July 25, 1983, in Pueblo, Colorado to Beth and Joseph Peters. Due to her father's profession, the family moved from Colorado to Arkansas to Tennessee and finally settled in Cornelius, North Carolina, just north of Charlotte. Tiffany pursued two bachelor's degrees in Textile Chemistry and Chemistry at NC State University and graduated in 2005 with high distinction completing both the University Scholars Program and the University Honors Program. After successful completion of this Master's Thesis she hopes to attend medical school to become a Doctor of Emergency Medicine.

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## **CHAPTER 1: LITERATURE REVIEW**

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## 1.1 THE SWEET POTATO

The sweet potato, *Ipomoea batatas* L. (Lam), is grown as a food plant in Malasia, China, and many other countries. The family Convolvulaceae, to which the sweet potato belongs, contains approximately 50 genera and over 1000 species. Only *Ipomoea batatas* is considered to have a major economic importance as a food. Because of human intervention by domestication selection, as well as spontaneous mutations and natural hybridization of the sweet potato there are currently a large number of sweet potato cultivars in existence, differing in the color of the root skin and flesh, depth of rooting, resistance to disease, size and shape of roots and leaves, time to maturity, and texture of the cooked roots (Woolfe, 1992).

Ranking seventh in total crop production, the sweet potato is cultivated in over 100 countries, and serves as an important monetary source in many parts of the world. Due to the *Ipomoea batatas*' ability to grow in tropical areas where a high proportion of the world's poorest population live, there is great interest in further developing its production and consumption, for both raw and processed foods, as well as providing a raw material for industrial processes (Woolfe, 1992).

### **Soluble Proteins in Sweet Potato**

Ipomoein is the soluble protein with the greatest concentration in the storage roots of the sweet potato plant. Ipomoein comprises between 82.9% and 99.7% of the extracted soluble protein at harvest, 63.5% to 84.1% at five months after harvest, and 72.3% to 88.8% after presprouting. Ipomoein is a 25 kDa protein strand and includes a protein formerly known as sporamin (Varon, 1987).

### **Distribution of Protein in Sweet Potato**

There is a significant difference in sweet potato protein distribution between the flesh and the skin. Total protein has been reported to be in higher concentrations close to the skin in the outer layer of flesh, which is removed through peeling (Makki et al., 1986). The peel removed by scraping (2.5% of the total weight) or by deep peeling (8.5% of the total weight) resulted in an increase of 87% and 47% more protein per unit weight, respectively, than that contained the remaining peeled material. Scraping results in a 4% loss of total protein, while deep peeling results in a loss of 12% protein in the roots (Bradbury et al. 1984). Another study indicated that removing 1-2 mm of the flesh only reduces the total protein content by 0.1-0.2% (Kimber, 1976). While the skin of the sweet potato is protein-rich, it is present in small quantities. Protein is unevenly distributed throughout the root, having a small, however significant, end-to-end gradient. A higher concentration of proteins can be found at the stem (proximal) end than at the distal (root) end (Purcell et al., 1976a). The previous results indicate that sampling for sweet potato proteins should utilize longitudinal sections of sweet potato tissue.

### **Factors Influencing Protein Concentration**

Sweet potato protein concentration is highly dependent on environmental conditions and cultural management practices. In over 300 lines of sweet potatoes grown in Taiwan under similar conditions within a single season, total protein varied from 1.27% to 10.07% on fresh weight basis, with the majority falling between 4% and 5% (Li, 1974). One hundred seedlings from seven parental clones grown in America in a single location for a season ranged from 4.38% to 8.98% (mean of 6.29%) (Dickey et al., 1984). Enhanced protein

content can be achieved by selective breeding, as evidenced by a Peruvian cultivar having a protein content that ranges between 8.9% and 14.9% (Carpio Burga, 1985). Six American cultivars grown under identical conditions contained between 1.36% and 2.13% protein (Picha, 1985), while ten cultivars from Papua New Guinea ranged from 1.29% to 1.81% protein (Bradbury et al., 1985).

Within cultivars, there is significant variation among roots from the same plant as well as variation between plants (Bradbury et al., 1985). Location within a field and field-to-field variability also causes significant differences in protein concentration within cultivars (Purcell et al., 1978).

Environmental conditions, including climate, soil, incidence of pests and diseases, which vary with location, season, and year, have a greater effect on some cultivars more than others. Total protein variation between and within cultivars showed significant effects of environment, genotype, and interaction between genotype and environment (Collins and Walter, 1982).

A Taiwanese study demonstrated that significant protein differences could be found between years (4.3-5.8%), seasons (3.4-6.1%), locations (2.8-6.5%), and cultivars (3.2-8.3%). Cultivar location interactions for protein concentrations were significant, indicating that it is important to test for interactions. The cultivar by year interaction for protein concentrations, however, was not significant, indicating that cultivar differences from different years should be comparable (Li, 1975).

Cultural management techniques, including plant spacing, irrigation, fertilization, variations in planting and harvest time, also affect total protein concentration. A direct correlation between the application of nitrogenous fertilizer to total sweet potato protein

content has been documented (Constantin et al., 1974; Yeh et al. 1981). On the other hand, after concentrations reach an optimum level, the efficiency of nitrogen utilization by the root decreases (Kimber, 1976), as well as dry matter content (Li, 1975) and decreased root production (Yeh et al., 1981).

While nitrogen fertilization has been shown to increase the concentration of protein in a sweet potato, potassium fertilization has not been shown to have a significant effect on increasing protein concentration; with potassium application levels of 140/kg/ha (Constantin et al., 1977) and 240 kg/ha (Caraballo Llosas, 1974), total protein concentration significantly drops. However, potassium application to sweet potato plants results in a greater yield of roots, producing a greater quantity of protein per hectare (Purcell et al., 1982).

Similar to potassium, phosphorus application decreases protein concentration (Hammett et al., 1982), while sulfur application has no significant effect (Purcell et al., 1982). Root total protein concentration increased with the application of waterhyacinth in combination with bonemeal as protein fertilizers from 3.8% to 5.45% in Indian sweet potato (Maurya and Dhar, 1976).

Protein concentration has an inverse relationship with moisture content of the soil, indicating that supplemental irrigation decreases protein concentration (Constantin et al., 1974). No significant difference was found in protein concentration was noted when planting density varied between 35,000 to 50,000 plants/ha (Li, 1975).

The length of growing season on American cultivars demonstrated that protein percentage dropped when the plants were harvested at 165 days after planting in comparison to 102 days after planting (Purcell et al., 1976b). However, these results conflict with a

Brazilian study that showed higher protein concentrations at eight months compared to four and six months (240 vs. 120-180 days) (Menezes et al., 1976).

The percentage of soluble protein, as well as total protein concentration, tends to increase with storage in most genotypes. The rate of dry matter loss during storage is two times higher than the rate of protein loss. While some proteins are hydrolyzed, there is not significant loss of major proteins after seven months of storage (Varon, 1987).

## **1.2 DIABETES**

Diabetes, a health problem reaching epidemic proportions, is a disease where the body either produces little or no insulin, or where the body becomes progressively resistant to the action of insulin. This disease affects more than 200 million people worldwide (Zimmet and McCarty 1995), including more than 16 million people in the United States (Harris et al., 1998). There are two types of Diabetes – Type 1, formerly designated as Juvenile Diabetes; and Type 2, formerly designated as Adult Onset Diabetes. Type 1 diabetes accounts for 10% of all diabetes patients and results from a decreased ability to produce insulin due to a poorly understood autoimmune disease of the pancreas. Type 2 diabetes, accounting for 90% of diabetes patients is a disease of insulin resistance and abnormal carbohydrate metabolism (Chakrabarti and Rajagopalan, 2002). Chronic complications include neuropathy, accelerated atherosclerosis, and retinopathy, nephropathy resulting in end-stage renal disease, premature cardiovascular mortality, blindness, and amputation (DeFronzo and Ferannini, 1991; Gerich, 1977). The incidence of the disease in the world's population is driven by type 2 diabetes, currently with 5% of the world

population having diabetes. The global cost of treating diabetes and its complications could reach one trillion US dollars annually by the year 2025 (Chakrabarti and Rajagopalan, 2002).

Type 2, diabetes mellitus (DM), has both environmental and genetic causes. The primary pathogenic factors contributing to type 2 diabetes mellitus are decreased insulin secretion and insulin resistance resulting from defects within the pancreatic beta-cells, skeletal muscle, and the liver (Clark, 1998). These two abnormalities vary between different type 2 diabetic patients. Impaired insulin secretion is primarily found in lean diabetic patients and is due to insufficiencies of the pancreatic beta-cells. Impaired insulin sensitivity is predominantly found in obese diabetics and occurs at the liver and skeletal muscle tissue level (DeFronzo, 1988). The pathophysiology of type 2 DM is as follows:

1. The pancreatic beta cells are unable to respond to excess glucose with appropriate insulin secretion.
2. An increased demand for insulin due to induced insulin resistance factors (e.g., obesity) begins.
3. A compensation by the beta cells to increase insulin secretion results in maintenance of normal glucose levels.
4. A gradual increase in insulin resistance and a gradual decrease of insulin secretion results in suppression of hepatic glucose output and impaired glucose tolerance appear.
5. As insulin resistance increases, there is a simultaneous increase in hepatic glucose synthesis leading to fasting hyperglycemia.
6. Concurrently, the pancreas fails to compensate for the increased demand of insulin and further contributes to hyperglycemia (Gerich, 1996).

Most DM patients suffer from visceral obesity and have high circulating levels of cholesterol, triglycerides, and low levels of high density cholesterol, which are factors known to contribute to the development of cardiovascular disease (Donahue, 1994). Separate from the above listed complications, changes in the electrical and mechanical properties of the heart can contribute to diabetic cardiopathy (Kannel and McGee, 1979). The most devastating consequences of complications include lower-limb amputation, loss of vision, myocardial infarction, end stage renal failure, and death (Chakrabarti and Rajagopalan, 2002).

### **Oral Therapies for Type 2 Diabetics**

The three abnormalities contributing to type 2 diabetes mellitus are insulin resistance, insulin deficiency, and increased hepatic glucose output (DeFronzo et al., 1992). Therapies used to treat type 2 diabetic patients are aimed at correcting or alleviating one or more of the abnormalities. The most common pharmacological treatment for type 2 diabetics is insulin treatment even though studies have demonstrated that metabolic control and reduced risk of complications can be achieved through strict lifestyle management and intensive drug therapy. There are six different classes of hypoglycemic agents in use today – sulfonylureas, insulin, alpha-glucosidase inhibitors, meglitinides, biguanides, and thiazolidinediones (Chakrabarti and Rajagopalan, 2002).

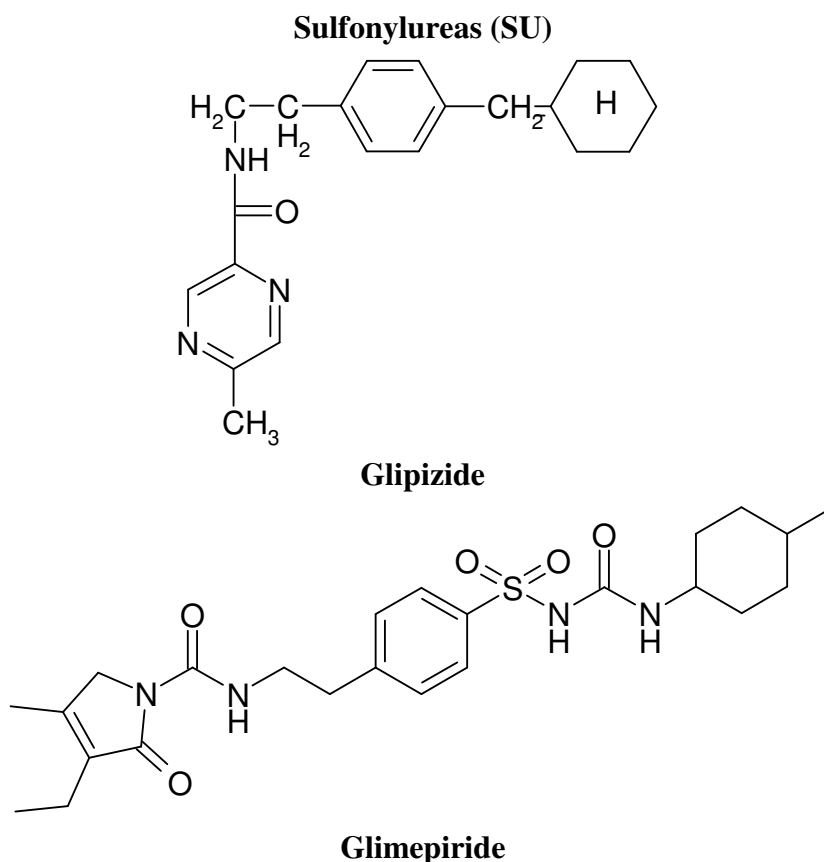
There are several classes of oral pharmacologic agents on the market that are used for correcting or alleviating one or more of the abnormalities associated with diabetes mellitus (Table 1.1). There are agents that act by reducing insulin resistance of adipose tissue, liver, and skeletal muscle (thiazolidinediones or glitazones); increasing insulin output (sulfonylureas and meglitinides); suppression of excessive hepatic glucose output

(metformin); and delaying gastrointestinal glucose absorption ( $\alpha$ -glucosidase inhibitors) (Mudaliar et al., 2001). The first line of treatment, recommended by the American Diabetes Association (ADA), includes a modified diet and exercise. If glycemic control cannot be achieved and controlled by diet and exercise within a specified period, pharmacological intervention is recommended (American Diabetes Assn., 1995). A strong correlation has been shown between insulin resistance and the development of hypertension, vasculopathy, dyslipidemia, hypercoagulation, and finally atherosclerotic cardiovascular disease (Fagan and Deedwania, 1998).



**Table 1.1 Pharmacological Differences Among Oral Treatments (Clark, 1998)**

Characteristic	Sulfonylureas	Repaglinide (Meglitinides)	Metformin (Biguanides)	Troglitazone (Glitazone)	Acarbose ( $\alpha$ -glucosidase inhibitor)
<b>Mechanism of Action</b>	↑pancreatic insulin secretion	↑pancreatic insulin secretion	↓gluconeogenesis  ↓hepatic glucose production  ↑peripheral glucose utilization  ↓intestinal glucose absorption	↓gluconeogenesis  ↓hepatic glucose production	↓digestion of complex carbohydrates  ↓disaccharides to absorbable monosaccharides
<b>Blood Glucose Levels</b>	↓in hyperglycemic state  ↓in normoglycemic state	↓in hyperglycemic state  ↓in normoglycemic state	↓in hyperglycemic state	↓in hyperglycemic state	↓in hyperglycemic state
<b>Plasma insulin levels</b>	↑	↑	↓ or unchanged	↓	Unchanged
<b>Body weight</b>	↑	Unknown	↓ or unchanged	Unchanged	↓ or unchanged
<b>Plasma lipids</b>	No significant effects	No significant effects	↓total cholesterol, LDL, triglycerides  ↓or no effects on HDL	↑LDL, total cholesterol  ↓triglycerides  ↑ or no effects on HDL cholesterol	No consistent effects



**Figure 1.1 The Structure of Sulfonylureas (Melander, 2004)**

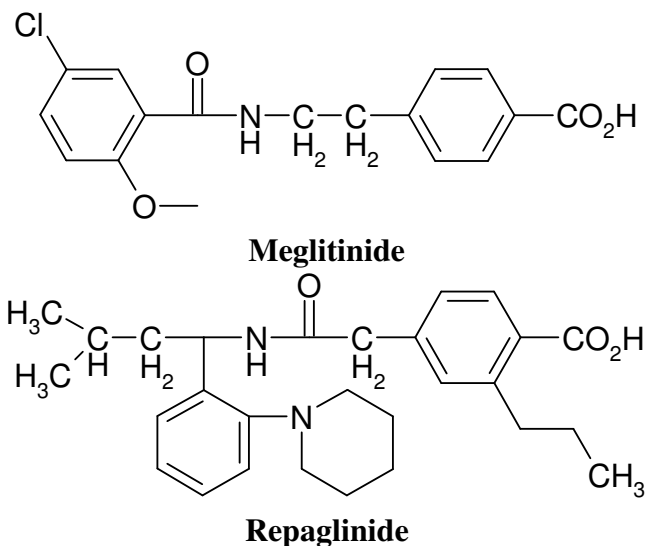
Sulfonylureas (SU; Figure 1.1) have been a popular pharmaceutical antidiabetic therapy for over thirty years. The first generation of sulfonylureas, acetohexamide, chlorpropamide, tolbutamide, and tolazamide, were no longer administered after a University Group Diabetes Program study linked increased mortality secondary to cardiovascular events (Feinglos and Bethel, 1999). The second generation of sulfonylureas, including glyburide, glimepiride, and glipizide, have fewer detrimental side effects and are currently in widespread use (Chakrabarti and Rajagopalan, 2002).

Sulfonylureas inhibit  $K_{ATP}$  channels which initiate insulin secretion (Lebovitz, 1990), which limits the use of this drug class to treating patients with type 2 diabetes mellitus who still have functional beta cells for insulin production. It has also been suggested that SUs

alter protein kinase C, increase calcium ionophore-like activity, and increase cAMP levels (Chakrabarti and Rajagopalan, 2002).

The greatest problem associated with the use of sulfonylureas is overt hypoglycemia (Krentz et al., 1994), especially with agents, such as chlorpropamide and glyburide, which are metabolized to active forms with significant renal excretion (Lebovitz, 1990). Elderly patients should avoid these pharmaceuticals, especially those with impaired renal function (Berger, 1985). Of the second generation SUs, glipizide and glimepiride have been associated with lower rates of hypoglycemia. Independent of the specific type, all sulfonylureas are associated with weight gain, hence, they are not an optimal choice for obese diabetic patients (Chakrabarti and Rajagopalan, 2002).

### Meglitinides



**Figure 1.2 The Structure of Meglitinides (Melander, 2004)**

Repaglinide (Figure 1.2), a member of the glinides, a carbomyl methyl benzoic acid family, is structurally different from traditional sulfonylureas (Figure 1.1) (Melander, 2004). However, structurally it resembles a nonsulfonylurea analog of the glibenclamide molecule.

Recently, a new meglitinide, nateglinide, has become available. A third drug within this class, mitiglinide, is in Phase III clinical trials. One of the advantages of this class is a decreased risk of hypoglycemia and a greater decrease in post-prandial glucose levels (Clark, 1998).

The meglitinides act as insulin secretagogues, stimulating insulin release from beta cells in the pancreas. The action takes place via binding the sulfonylurea receptor on the beta cell (Fuhendorff et al., 1998). This class of drugs, however, have different characteristics in comparison to the sulfonylureas. Meglitinides have not been shown to stimulate calcium dependent exocytosis, in comparison to glibenclamide, a sulfonylurea. Only glibenclamide can stimulate *in vitro* insulin secretion in the complete absence of glucose; however, in the presence of 5 to 10 mM of glucose, meglitinides are five times more potent than glibenclamide in stimulating insulin secretion (Chakrabarti and Rajagopalan, 2002).

Because meglitinides have a short half-life and a rapid onset of action, it is advised that patients are administered a dose immediately prior to eating (Clark, 1998). If a diabetic patient makes the choice to forgo a meal during the day, the drug should also be omitted to prevent hypoglycemia. Similarly, if an extra meal is added, the patient should add a dose of the meglitinides to compensate for the meal. This flexible dosing regimen is more convenient for patients who do not have a consistent eating schedule (Chakrabarti and Rajagopalan, 2002).

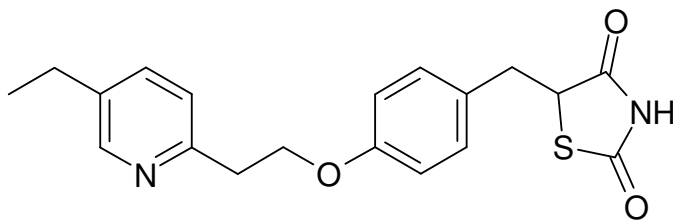
### **Biguanides**

Biguanides, recently introduced into the United States, have been utilized for many years in other countries. The only agent available from this class in the US, metformin, is the largest selling orally administered hypoglycemic agent. The target of metformin is the

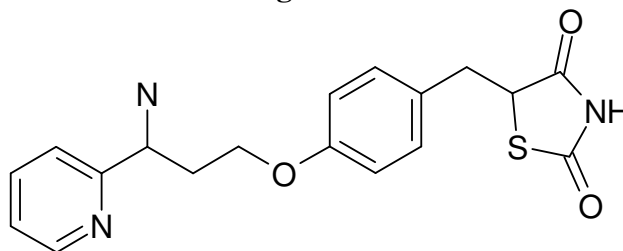
inhibition of gluconeogenesis, thereby reducing hepatic glucose production (Jackson et al., 1987). Metformin also acts to further enhance insulin sensitivity in both hepatic and peripheral tissues (Marena et al., 1994). Unlike other oral antidiabetic drugs, metformin is considered to be an anti-hyperglycemic agent due to the fact that if used as monotherapy in diabetic patients, hypoglycemia will not occur as commonly as in other oral antidiabetic drugs (Hermann et al., 1994).

Beneficial side effects of metformin include a reduction in low-density lipoprotein (LDL) and plasma triglyceride levels. Unlike other oral antidiabetic drugs, metformin is unique in that its use is not associated with weight gain (Clarke and Duncan, 1968). In some instances, there has been weight loss in obese patients (Campbell et al., 1994). Additional side effects are generally favorable, and tend to be associated with the initiation of treatment. A very rare adverse effect in some patients is lactic acidosis, which was the reason for withdrawal of phenformin, another drug in the biguanides class, in the US in the 1970s (Bailey, 1992). Metformin therapy is contraindicated with liver disease, pulmonary insufficiency with hypoxemia, congestive heart failure, cardiogenic or septic shock, and severe tissue hypoperfusion (Chakrabarti and Rajagopalan, 2002).

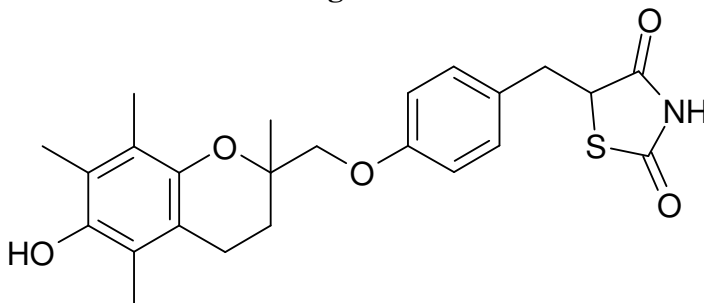
### Thiazolidinediones and Glitazones



**Pioglitazone**



**Rosiglitazone**



**Troglitazone**

**Figure 1.3 The Structure of Thiazolidinedione Agents**

Ciglitazone, a thiazolidinedione-based compound (Figure 1.3), is derived by fibrate lipid-lowering agents and has been reported to be a novel oral hypoglycemic drug that maximizes the effects of insulin. GlaxoSmithKline, Inc., (North Carolina, USA) has invested in synthesizing new analogues of ciglitazone and in the determination of action and molecular targets of this class of compounds. Thiazolidinediones activate a peroxisome proliferator activated receptor,  $\gamma$ (PPAR $\gamma$ ), a member of a nuclear receptor family that regulates insulin sensitivity through regulation of gene expression regulation and mediates adipocyte differentiation (Olefsky, 2000). Triglyceride-lowering fibrates, the class of

compounds from which thiazolidinedione compounds are derived, have been shown to be agonistic to PPAR $\alpha$ , a different isoform of the PPAR family (Lemberger et al., 1996).

Troglitazone by Daiichi Sankyo (Munich, Germany) was the first thiazolidinedione compound to be approved for use in the US and Japan. However, following reports of severe liver toxicity in patients, the product was removed from the market. GlaxoSmithKline's (North Carolina, USA) rosiglitazone and Takeda/Pfizer's (New York, USA) pioglitazone are the two available thiazolidinedione drug analogues currently on the market (Mudaliar and Henry, 2001). These compounds work to improve hyperglycemia through increased insulin sensitivity in muscle and fat tissues (Clark, 1998). These compounds also cause small decreases in hepatic glucose production. Thiazolidinediones have been shown to modify lipid profiles in diabetes mellitus patients including decreased triglyceride levels, and increased LDL-cholesterol and total cholesterol levels (Garg, 1998).

Hypoglycemia is not of concern in use with thiazolidinediones. All thiazolidinediones have been associated with significant weight gain – a great concern as most type 2 diabetic patients are already obese (Actos, 1999; and Avandia, 2000). This class of drugs is safe for patients with impaired renal function, however it is unsafe for patients with impaired hepatic function (Actos, 1999; and Avandia, 2000). Some studies have reported decreases in liver function after treatment with rosiglitazone. Another significant side effect is mild to moderate edema, which is reported in 5% to 7% of patients treated with pioglitazone or rosiglitazone. This side effect is significant and of concern for patients with congestive heart failure (Actos, 1999; and Avandia, 2000). Besides their effects on lipid metabolism, hepatic glucose metabolism, and insulin sensitivity, the glitazones have been

shown to have beneficial effects on fibrinolysis, cancer cell apoptosis, endothelial function, ovarian steroidogenesis, and atherogenesis (Saleh et al., 1999; Takahashi et al., 1999).

### **Alpha-glucosidase inhibitors**

Alpha-glucosidase inhibitors prevent the cleavage of oligosaccharides, trisaccharides, and disaccharides into sugars by reversibly inhibiting the alpha-glucosidase enzyme found in brush border cells that line the small intestine. The antidiabetic effect is a result of the agents' ability to delay the digestion of disaccharides and carbohydrates to monosaccharides (Balfour and McTavish, 1993). This results in a decrease in postprandial hyperglycemia (Rodger et al., 1995). The two drugs currently available within this class are acarbose and miglitol (Chakrabarti and Rajagopalan, 2002).

Abdominal discomfort, flatulence, diarrhea, and bloating are the most common side effects of this class of compounds (Clissold and Edwards, 1988). Acarbose therapy has been shown to increase serum transaminase levels and should not be used in patients with liver cirrhosis. The levels of alpha-glucosidase inhibitors directly increase in proportion to the degree of renal dysfunction. Patients with a history of bowel obstruction or patients with inflammatory bowel disease should not be treated with this drug (Precose, 1995). Because the agent's target is the enzymes of the small intestine, it does not appear to have direct effects on insulin-stimulated glucose uptake or insulin resistance (Balfour and McTavish, 1993).



## **Future Work**

Based on the previous discussion, the current pharmacological treatments are not adequate in their ability to optimally control of blood glucose levels. Furthermore their use is associated with undesirable side effects and in many patients their use is contraindicated. There is a need for more research and development in the area of oral agents with unique physiological mechanism of actions. The areas of active investigation currently include insulin sensitizers, and PPAR agonists (Chakrabarti and Rajagopalan, 2002).

## **1.3 CAIAPPO**

Due to undesirable side effects of the previously mentioned oral antidiabetic drugs, there has been an increased interest in the use of nutraceuticals or herbal products for the treatment of type 2 diabetes. The safety and efficacy of these natural products have not been investigated, and it is possible that these substances might harm rather than help individuals. However, Caiapo, isolated from the white-skinned sweet potato (WSSP) has recently been shown to reduce fasting plasma glucose levels, low-density lipoprotein cholesterol, and total cholesterol in type 2 diabetic patients. At high doses Caiapo has been shown to reduce insulin resistance (Ludvik et al., 2003).

The sweet potato, *Ipomoea batatas*, has been used to treat symptoms of diabetes, such as weight loss and thirst, by Native Americans. Similarly, in Japan an extract of the root and skin have been utilized in treatment of type 2 diabetes. In an uncontrolled study of 145 type 2 diabetic patients in Japan, a decrease in blood glucose levels exceeding 10% of the basal values was observed in 77% of the subjects. Furthermore, uncontrolled studies in non-diabetic patients have shown decreases in blood glucose levels. An acidic glycoprotein,

isolated from sweet potato homogenate, has been proposed to act as the active antidiabetic component of Caiapo (Ludvik et al., 2003).

### **Ludvik et al. Human Male Long-Term Caiapo Trial**

Parameters related to glucose disappearance, glucose tolerance, and insulin secretion were monitored in a trial of 18 male type 2 diabetic patients. Patients were divided into a control group, a low dose Caiapo group, and a high dose Caiapo dose group (Ludvik et al., 2003). With high doses of Caiapo, insulin sensitivity increased as indicated by both an oral glucose tolerance test (OGTT) and a frequently sampled intravenous glucose tolerance test (FSIGT). With low doses only the FSIGT showed an increase in insulin sensitivity. The high-dose groups also demonstrated a marked reduction in basal glucose levels from  $150 \pm 10$  mg/dL to  $130 \pm 7$  mg/dL. Insulin clearance, distribution, basal rates, and hepatic extraction did not change through the trial, indicating that insulin dynamics did not change. In this trial there were no negative side effects noted, including no change in body weight, blood pressure, insulin dynamics, or glucose tolerance, indicating that Caiapo could be a valuable agent for lowering fasting glucose and glucose response in type 2 diabetics.

Reduced intestinal glucose absorption, improved insulin sensitivity, and increased insulin secretion are responsible for improved glycemic control. As a result of the data from the OGTT and FSIGT dynamic tests, Ludvik et al., (2003) concluded that Caiapo benefits type 2 diabetic patients by reducing insulin resistance. This study has also shown that there is an increase in insulin sensitivity regardless of body weight. In the low-dose Caiapo group, the beneficial effects of Caiapo were observed even in patients that had a lower BMI than in the high-dose group, and therefore more moderate insulin resistance. After six weeks of

treatment there was no change in hematology, urinalysis, or blood chemistry, including hepatic enzymes, with the exception of cholesterol and glucose (Ludvik et al., 2003).

After the administration of the high-dose Caiapo there was a congruent lowering of LDL and total cholesterol. This effect, independent of the improved insulin sensitivity, supports the idea that Caiapo could contain more than one metabolically active agent. No changes were noted in overall triglycerides, which may be explained in part by the short period of the study (Ludvik et al., 2003).

Of note for this study is the difference in the physical makeup of the low- and high-dose Caiapo groups. Although the groups were randomized, the low-dose individuals tended to be leaner, while the high-dose individuals demonstrated higher baseline BMI levels. The effects of the high-dose Caiapo could be skewed due to the predisposition of the individuals in the group to treatment. With the low-dose, moderately insulin-resistant individuals, Caiapo still exerted an insulin sensitizing effect, supporting its use as an antidiabetic agent (Ludvik et al., 2003).

The results of this study suggested the beneficial effects of Caiapo treatment on type 2 diabetic plasma glucose levels and LDL cholesterol levels as a result of a decrease in insulin resistance. There was not a significant change in body weight, nor were there any negative side effects. The results of this study indicate that type 2 diabetic patients could benefit from Caiapo treatment (Ludvik et al., 2003, Ludvik et al., 2002).

### **Kusano et al., db/db Mice and Zucker Fatty Rats Trial**

In an initial trial utilizing streptozotocin (STZ)-induced insulin-deficient Zucker fatty rats, both troglitazone and white skinned sweet potato (WSSP; *Ipomoea batatas* L.) extracts were compared. Hyperinsulinemia in the rats at three, four, six, and eight weeks after

initiation of the WSSP treatment, decreased by 23%, 26%, 60% and 50%, respectively. These results were very similar to the results obtained with troglitazone, a commercial pharmaceutical. In addition to decreases in hyperinsulinemia, there were improvements in glucose tolerance, decreases in circulating free fatty acid lactate levels and triglyceride concentrations. Troglitazone administration also resulted in similar effects on glucose tolerance, circulating free fatty acid, lactate, and triglyceride concentrations. Unlike troglitazone, WSSP did not result in weight gain, as compared to the control group. Also notable is the reggranulation of pancreatic beta-cells in both the WSSP group and the troglitazone group after the eight weeks of treatment. This study concluded that WSSP reduces insulin resistance, thereby improving both glucose and lipid metabolism. In normal rats it was also shown that administration of WSSP decreases insulin resistance, and suppresses the increase in blood glucose concentration after glucose loading (Kusano et al., 2001).

A second study by Kusano et al. (2001) focused on the isolation and purification of the antidiabetic component of the white skinned sweet potato. Mice (Db/db) were administered water extracts of the whole, pith, and cortex for three weeks. While all treatments exhibited a marked decrease in insulin and glucose levels, the group fed the water extract of the cortex had the greatest decrease. Similarly, normal mice who were fed the WSSP water extracts had an increase in blood insulin activity, with the largest change occurring in the group administered the water extract from the cortex. These two trials indicate that the highest concentration of antidiabetic compounds are found in the cortex of the white skinned sweet potato (Kusano et al., 2001).

Glucose tolerance tests were conducted using a purified sample, designated CAF, of the water extract from the cortex of the white skinned sweet potato. Insulin and blood glucose levels were monitored in Wistar rats after glucose loading. Blood glucose levels decreased after 60 minutes in the groups given the purified extract (CAF). The control group had lower blood insulin levels than the CAF group for up to 120 minutes after glucose loading (Kusano et al., 2001).

In longer term studies, there was a significant decrease in blood glucose and insulin levels after fourteen days of repeated administration of CAF to db/db mice as compared to the control group. The results from the glucose tolerance tests and the fourteen-day study utilizing the CAF extract suggests that the active antidiabetic component that increased insulin activity in normal rats and was responsible for the hypoglycemic activity in db/db mice is the same compound (Kusano et al., 2001).

The molecular weight of the CAF compound was approximately 22,000, as determined by gel filtration chromatography (GFC) analysis. Because the active fraction contained both protein and a sugar and bound to a QA ion exchange column (strong anion exchanger). It was presumed to be an acidic glycoprotein (Kusano et al., 2001).

The extract from the white skinned sweet potato lowered blood glucose levels and reduced the high insulin levels in KK Ay db/db mice and Zucker fatty rats, and it increased blood insulin levels in STZ-diabetic rats. Based on the previous results, Kusano et al., (2001) concluded that the WSSP extract works via different mechanisms than that of tolbutamide and thiazolidine derivatives.

While troglitazone and tolbutamide have been shown to have effects after a single dose, it takes two to three weeks to see the effects of WSSP extract if administered orally.

However, if dosed intravenously, WSSP extract can increase blood insulin levels. Because of its high molecular weight, it has not been determined if this compound is digested prior to absorption or if it is absorbed intact. Because it benefits both insulin-resistant animals and insulin-deficient animals, this agent could become a popular therapeutic agent. It has a unique mechanism of action and no known undesirable side effects (Kusano et al., 2001).

### **Caiapo effects on KK-Ay Mice**

Sakuramata et al. (2004) studied a combination therapy of Caiapo with mulberry leaf powder, a known  $\alpha$ -glucosidase inhibitor, and loquat leaf extract, which has an insulin-like effect. The combination therapy was hypothesized to enhance Caiapo's antidiabetic properties, and further decrease the time necessary for decreasing blood glucose levels. Male KK-Ay mice were orally administered mixtures of pulverized tuber of Caiapo and mulberry leaf powder, or pulverized skin of Caiapo and powdered loquat leaf extract for 28 days with a glucose loading test being conducted every 7 days. After one week of feeding, there was a reduction in blood glucose 60 minutes after the glucose loading test in the groups that received both mixtures. It took 2-3 weeks, however, for the group of mice only receiving Caiapo to reach the same glucose levels (Sakuramata et al. 2004), suggesting that these substances did not work by the same mechanism.

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

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## 2.1 ABSTRACT

The sweet potato, *Ipomoea batatas*, has been used to treat symptoms of diabetes, such as weight loss and thirst, by Native Americans. Similarly, in Japan the extract of the root and skin have been utilized in the treatment of type 2 diabetes. Sweet potato protein has been shown to decrease fasting plasma glucose levels, low-density lipoprotein cholesterol, and total cholesterol in type 2 diabetic patients. A dietary supplement from sweet potato, Caiapo, has also been shown to modify the glycemic response. A number of studies have demonstrated  $\alpha$ -amylase inhibitor properties in sweet potato protein extract. However, previous work on the extraction of this compound used contains chemicals unsafe for consumption. The objectives of this study were to determine a safe and effective protein extraction method, and determine its repeatability within sweet potatoes.

Protein content was determined for four different extraction methods performed on the same sweet potato and for four different fractions derived from three different sweet potatoes, through the EZQ Protein Assay and the BCA Protein Assay. Of the four different extraction methods, homogenizing sweet potato flesh with 50 mM phosphate buffer and 0.3 M NaCl followed by a 0.5% calcium chloride precipitation produced the highest protein mass.

Two fractions from the sweet potato protein precipitation, the supernatant and the protein pellet, were subjected to heat treatment. The amount of protein produced varied between sweet potatoes and treatments. The highest amount of protein could be found in the heat-treated protein pellet, with slightly higher recovery of proteins treated at the higher

temperatures. Although protein content varies in different sweet potatoes, the protein can efficiently be extracted, through homogenization into a buffer, followed by calcium chloride.

## 2.2 INTRODUCTION

Sweet potato crude protein content has been reported to range from 1.3 to over 10% on a dry matter basis. There is great genetic variability between cultivars; however, there is also high variability within cultivars depending on growing conditions. Root nitrogen content is dependent on water availability and nitrogen fertilization (Walter et al. 1984). It is important that comparisons between extraction methods should use homogeneous samples from the same sweet potatoes. In contrast, when determining repeatability between experiments, it is important to obtain samples from multiple sweet potatoes to estimate variation.

Several methods have been used to extract protein from sweet potatoes. The most commonly used methods are time and resource intensive. Furthermore, these methods do not utilize food grade chemicals that are safe for human consumption. The extracted material cannot be used as a nutritional supplement.

Kusano et al. (2001) utilized a juice extractor and a homogenizer to obtain extracts of white skinned sweet potato (WSSP) which was then lyophilized. The WSSP-cortex was dialyzed and then was extracted using 85% ethanol. The resultant supernatant was concentrated and lyophilized. A distilled water/30% trichloroacetic acid (TCA) solution (v/v) was used to dissolve the powder. The supernatant obtained after centrifugation was dialyzed against distilled water to remove the TCA and then lyophilized. The resultant powder was dissolved in 50 mM phosphate buffer (pH 7.0) containing 2 M ammonium sulfate. This solution was applied to a column of butyl-Toyopearl 650 M, which had been equilibrated with 50 mM phosphate buffer (pH 7.0) and 1.5 M ammonium sulfate. Fractions

were eluted with a stepwise gradient of 1.5 M, 0 M ammonium sulfate and 50% ethanol and the same buffer, respectively. Each of the active fractions were collected, concentrated, and dialyzed against distilled water, followed by the addition of solid ammonium sulfate to a concentration of 1.5 M. This solution was centrifuged and then placed on a column of phenyl-Toyopearl 650M and equilibrated with 50 mM phosphate buffer (pH 7.0) and 1.5 M ammonium sulfate. The resultant fractions underwent ultrafiltration using Ultracent 30 (Tosoh Company; Tokyo, Japan).

Rehka et al. (1999) extracted  $\alpha$ -amylase inhibitor from *Colocasia esculenta* tubers, taro, using a sodium phosphate buffer (0.02 M; pH 6.9) containing 1.3 M NaCl, sodium phosphate buffer (0.02 M; pH 6.9) with 0.1% Triton X-100, Tris-HCl buffer (0.02 M; pH 7.6), and Tris-HCl buffer (0.02 M; pH 7.6) with 0.1% Triton X-100. The highest amount of amylase inhibitor activity extracted from taro roots was achieved through the use of the sodium phosphate buffer (0.02 M; pH 6.9) that contained 0.3 M NaCl. Since both native  $\alpha$ -amylases and the  $\alpha$ -amylase inhibitor was extracted through this solution, the extracts were subjected to heating at 80 °C for 10, 20, or 30 minutes to determine the time needed to deactivate native  $\alpha$ -amylases. Heating for 10 minutes at 80 °C was sufficient to inactivate the native  $\alpha$ -amylases.

Steed et al. (2005) compared different methods of protein precipitation on both the flesh and the peel of North Carolina Sweet Potatoes. Raw sweet potato was homogenized in 50 mM sodium phosphate buffer (pH 7.0) and precipitated with ammonium sulfate at 30%, 60%, and 90% concentrations. The solutions were then dialyzed against a dialysis buffer. A higher protein concentration was found in sweet potato peel than in sweet potato flesh. A 60% ammonium sulfate solution was found to be sufficient to precipitate most of the proteins



in solution. The objective of this study was to determine food-grade, simple methods of extracting sweet potato protein and to test the repeatability and efficiency of the methods.

## **2.3 MATERIALS AND METHODS**

### **2.3.1 Sweet Potatoes**

Beauregard Sweet Potatoes were obtained from the North Carolina State University Department of Horticulture. Samples were harvested in 2006 and stored at  $12.8\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and at 85-90% relative humidity.

### **2.3.2 Preparation of Sweet Potato Homogenate**

One-hundred grams of sweet potato flesh was initially homogenized in a KitchenAid® Quisinaart food processor (KitchenAid; Michigan, USA) with either 500 ml of either distilled water or 50.0 mM phosphate buffer (pH 6.9) containing 0.3 M NaCl. A more homogeneous homogenate was created by further blending in a Waring Blender (Waring; Connecticut, USA). The water-insoluble fractions were initially separated from the water-soluble fraction by gravity settling for approximately 10 minutes. The water-soluble fraction was centrifuged for 20 minutes ( $22\text{ }^{\circ}\text{C}$ ) at 3363 rpm ( $1,000 \times g$ ) to further remove insoluble material. Proteins were precipitated from the resulting supernatant with ammonium sulfate or calcium chloride.

### **2.3.3 Sweet Potato Protein Precipitation with Ammonium Sulfate**

A saturated solution of ammonium sulfate was added to 100 ml of the water-soluble fraction of the sweet potato homogenate to a final concentration of 60% ammonium sulfate

(w/v) and left for 24 hours at 22 °C. The precipitated suspension was centrifuged for 20 minutes (22 °C) at 3363 rpm (1000 x g). The protein suspension was dialyzed against distilled water for at least 24 hours (22 °C), with a change of water bath at least once during dialysis. The dialyzed suspension was centrifuged for 20 minutes (22 °C) at 3363 rpm (1000 x g) resulting in a protein pellet and solubilized protein fractions.

#### **2.3.4 Sweet Potato Protein Precipitation with Calcium Chloride**

A saturated solution of calcium chloride was added to 100 ml of the water-soluble fraction of sweet potato homogenate to yield a final concentration of 0.5% (w/v) calcium chloride. After 24 hours, the precipitated suspension was centrifuged for 20 minutes (22 °C) at 3363 rpm (1000 x g) resulting in a protein pellet and solubilized protein fractions.

#### **2.3.5 Heat Treatment**

The water-soluble fraction and the protein pellet were suspended in water or phosphate buffer and heated at 95 °C for 30 minutes. The water-soluble fraction was then centrifuged for 20 minutes (22 °C) at 3363 rpm (1000 x g) and the precipitated proteins removed. The protein pellet was resuspended for protein and enzyme activity measurements.

#### **2.3.6 EZQ Protein Quantitation Assay**

The EZQ® Protein Quantitation Kit (R33200) purchased from Molecular Probes™ (Invitrogen; California, USA) was used to determine total protein concentration in the different samples. Ovalbumin supplied with the kit was diluted to a 10 mg/ml stock solution. The control samples were serially diluted to concentrations ranging from 0.375 to 5 mg/ml. Each of the protein samples were diluted not to exceed concentrations of 5 mg/ml and then diluted two additional times to be sure that at least one dilution was within the range of the

standard curve. The samples were spotted onto assay paper in triplicate using 1 µl of sample per well. The paper was dried and fixed with methanol to remove contaminating substances. The assay paper was then spotted with EZQ® protein quantitation reagent for 30 minutes. The paper was washed a total of three times with a 10% methanol/7% acetic acid (v/v) solution. The fluorescence is read at ~485/590 nm (Cytoflour II, Perceptive Biosystems; Minnesota, USA).

### **2.3.7 BCA Assay**

The BCA Assay Kit purchased from Pierce (Pierce Biotechnology; Illinois, USA) was used to determine the soluble protein concentration in the different samples. Bovine serum albumin supplied with the kit was serially diluted in buffer to concentrations ranging from 2.0 mg/ml to 0.25 mg/ml. Each of the protein samples were diluted to provide concentrations within that range and diluted two additional times. Twenty µl of each standard and sample was placed in triplicate onto a 96-well microplate. Two-hundred µl of the working reagent was added to each well prior to incubating at 37 °C for 30 minutes. After returning to room temperature, the absorbance was read at 540 nm on a plate reader (Multiskan MMC, Fisher Scientific Corp).

## **2.4 EXPERIMENTAL DESIGN**

### **2.4.1 Experiment 1: Mass Production Experiment**

One hundred g of sweet potato was homogenized with 400 ml distilled water and further divided into two fractions, 100 ml which underwent ammonium sulfate precipitation

(DA) and 100 ml of the same solution which underwent calcium chloride precipitation (DC) as described above.

One hundred g from the same sweet potato was homogenized with 400 ml 50 mM phosphate buffer with 0.3 M NaCl (pH 6.9); 100 ml of this solution was precipitated with ammonium sulfate (PBA) and 100 ml of the same solution with calcium chloride (PBC). After precipitation all samples were centrifuged to obtain a protein pellet.

### **Experiment 1: Protein Concentration and Solid Mass Study**

Sample DA and Sample DC protein pellets were resuspended into 6 ml of distilled H<sub>2</sub>O while Samples PBA and PBC were resuspended into 6 ml of 50 mM phosphate buffer 0.3 M CaCl<sub>2</sub> (pH 6.9). The samples were serially diluted using equal parts sample and distilled water for preparation for both the EZQ Protein Assay and the BCA Protein Assay. The samples used in the BCA Assay were taken from the same preparations as for the EZQ Protein Assay. Statistical analysis completed using ANOVA and test of significance among the means using Statistix 8 (Analytical Software; Florida, USA).

All four samples were placed separately in aluminum weigh dishes and placed in a drying oven at 60 °C for 72 hours or until a constant weight was reached. Each sample was weighed to determine the comparative yields of protein and dry matter.

### **2.4.2 Experiment 2: Protein Assays**

One hundred g of three different sweet potatoes was homogenized with 400 ml of distilled water. After gravity settling and centrifugation, proteins were precipitated from 100 ml of the supernatant with calcium chloride. After precipitation, all samples were centrifuged to obtain a protein pellet (3) and the remaining supernatant (2). Replicates are

designated R, S, and T. Samples of both the supernatant (2HT) and protein pellet (3HT) underwent heat treatment.

The protein pellets from each of the samples were resuspended into 6 ml of 50 mM phosphate buffer with 0.3 M  $\text{CaCl}_2$  (pH 6.9), while the supernatant was not diluted. The samples were serially diluted using equal amounts of sample and distilled water for preparation for the EZQ protein assay and the BCA protein assays. The samples used in the BCA assay were taken from the same preparations as for the EZQ protein assay.

## 2.5 RESULTS

### 2.5.1 EZQ Concentration Assay Study

Data and the standard curve for the EZQ protein assay are shown in Table 2.1. Each of the four samples from the mass production samples were serially diluted to ensure fluorescence values within the range of the standard curve. Sample 1, sweet potato, homogenized in distilled water followed by an ammonium sulfate precipitation, resulted in an average of  $33.8 \pm 6.65$  mg of protein produced for the 100 ml sweet potato homogenate (Table 2.2). Sample 2, sweet potato homogenized into distilled water, followed by calcium chloride precipitation, resulted in an average of  $24.6 \pm 3.07$  mg of protein produced for 100 ml of sweet potato homogenate (Table 2.2). Sample 3, sweet potato homogenized into phosphate buffer and NaCl water followed by an ammonium sulfate precipitation, resulted an average of  $34.2 \pm 6.05$  mg of protein produced for the 100 ml of sweet potato homogenate (Table 2.2). The three dilutions for sample 4, sweet potato homogenized into phosphate buffer and NaCl, followed by calcium chloride precipitation, averaged  $36.6 \pm 8.66$  mg of protein per 100 ml of sweet potato homogenate (Table 2.2).

Table 2.3 shows the EZQ assay results for the triplicate sweet potatoes, by treatment, and dilution. The quantity of protein given is the total mass in milligrams resulting from 100 ml of sweet potato homogenate. Missing values are due to fluorescence values beyond the range of the standard curve (Table 2.1).

Table 2.4 describes the analysis of variance between each of the three controlled variables – sweet potato, treatment, and dilution. Variation between sweet potatoes (R, S, T) showed a F-value of 4.37 and a P-value of 0.0375 (DF = 2), treatment variation (2, 2HT, 3, 3HT) obtained a F-value of 52.6 and a P-value < 0.0001 (DF = 3), and dilution variation

resulted in a F-value of 2.35 and a P-value of 0.138 (DF = 2). In models of sweet potato x treatment, sweet potato x dilution, and treatment x dilution, the F-values and P-values were 2.98 and 0.0510, 1.02 and 0.435, and 3.39 and 0.372, respectively. Because P value of the Treatment x Dilution interaction was less than 0.05, the variance for treatment was compared with the MSE for the interaction term, and still showed a significant treatment effect.

### **2.5.2 BCA Assay Concentration Study**

The standard curve for the BCA protein assay is shown in Table 2.5. Each of the fractions from the extraction study were serially diluted to ensure that some of the absorbance values were within the range of the standard curve. Sample 1, sweet potato homogenized into distilled water followed by an ammonium sulfate precipitation, resulted in an average of  $22.0 \pm 5.2$  mg of protein per 100 ml of sweet potato homogenate (Table 2.6). Sample 2, sweet potato homogenized into distilled water, followed by calcium chloride precipitation, had a calculated quantity of protein for 100 ml of sweet potato homogenate of 15.38 mg, 7.877 mg, and 26.30 mg, respectively, with an average of  $16.5 \pm 5.35$  mg (Table 2.6). Sample 3, sweet potato homogenized into phosphate buffer and NaCl water followed by an ammonium sulfate precipitation, resulted in an average of  $17.6 \pm 5.94$  mg of protein per 100 ml sweet potato homogenate (Table 2.6). Sample 4, sweet potato homogenized into phosphate buffer and NaCl, followed by calcium chloride precipitation, had a calculated quantity of protein for 100 ml of sweet potato homogenate of 20.9 mg, 36.0 mg, and 37.5 mg, respectively, with an average of  $31.5 \pm 5.29$  mg (Table 2.6).

Each of the triplicate sweet potato protein samples were diluted as described above. Table 2.7 shows the BCA assay results for the experiment by sweet potato, treatment, and dilution. The quantity of protein given is the total mass in milligrams resulting from 100 ml

of sweet potato homogenate. The supernatant (2) from the three sweet potatoes contained  $0.658 \pm 0.903$  mg protein for sample R,  $0.745 \pm 0.746$  mg protein for Sample S, and  $1.36 \pm 0.133$  mg protein for Sample T. Heat treatment (HT) of the supernatant (2) resulted in values for Sample R(2HT) of  $1.50 \pm 0.0544$  mg, Sample S(2HT)  $1.32 \pm 0.0426$  mg, and Sample T(2HT)  $1.27 \pm 0.0216$  mg.

The protein content of pellet (3) of Sample R(3) was  $10.7 \pm 0.118$  mg, Sample S(3)  $13.2 \pm 0.153$  mg, and Sample T(3)  $12.0 \pm 0.192$  mg. Heat treatment (HT) of the protein pellet (3) resulted in values for Sample R(3HT) of  $11.8 \pm 0.313$  mg, Sample S(3HT)  $13.4 \pm 0.0161$  mg, and Sample T(3HT)  $18.1 \pm 0.413$  mg.

Table 2.8 describes the analysis of variance between each of the three controlled variables – sweet potato, treatment, and dilution. Variation between sweet potatoes (R, S, T) showed a F-value of 136 and a P-value  $< 0.0001$  (DF = 2), treatment variation (2, 2HT, 3, 3HT) obtained a F-value of 4920 and a P-value  $< 0.0001$  (DF = 3), and dilution variation resulted in a F-value of 1.25 and a P-value of 0.321 (DF = 2). In models of sweet potato x treatment, sweet potato x dilution, and treatment x dilution, the F-values and P-values were 91.2 and  $<0.0001$ , 1.74 and 0.205, and 0.630 and 0.706, respectively. There was no significant variability between sweet potatoes, treatments, and across both sweet potatoes and treatments.

### **2.5.3 EZQ and BCA Comparative Concentration Assay Study**

Table 2.10 displays side by side comparisons of the quantity, averages, standard error, and P-values from the EZQ Protein Assay and the BCA Protein Assay for each of the mass production samples and their three dilutions. Sample 1 (DA), Sample 2 (DC), Sample 3 (PBA), and Sample 4 (PBC) had two-tailed P-values of 0.0246, 0.374, 0.00540, and 0.350,



respectively. The P-values were determined by comparing the quantity of matter produced as measured by the EZQ Protein Assay and the BCA Protein Assay for the total dilutions (DF = 4). The P-value for the overall comparison between the two protein assays is 0.000725 (DF = 11).

Table 2.10 displays side by side comparisons of the produced quantity, averages, standard error, and P-values from the EZQ Protein Assay and the BCA Protein Assay for soluble and insoluble fractions for three separate sweet potatoes measured at three dilutions. The P-values were determined by comparing the quantity of matter produced as measured by the EZQ Protein Assay and the BCA Protein Assay for the total dilutions (DF = 4). The P-value for the overall comparison between the two protein assays is 0.00228 (DF = 29).

#### **2.5.4 Experiment 1: Mass Production Study**

Table 2.11 shows the dried precipitate recovered in g from the mass production experiment. Sample 1 (DA) had a 11.9% yield, Sample 2 (DC) had a 1.2% yield, Sample 3 (PBA) had a 13.9% yield, and Sample 4 (PBC) had a 14.2% yield of dry matter as measured after drying as described in Section 2.4.1.

### **2.6 DISCUSSION**

#### **2.6.1 Experiment 1: Mass Production Samples**

The best extraction procedure would yield the highest amount of total protein from the precipitation while utilizing smaller amounts of chemicals. The method using the least amount of chemicals, sweet potato homogenized into distilled water and precipitated with calcium chloride, was the least effective at extracting protein as measured by both the EZQ protein assay and the BCA protein assay (Table 2.9). The highest yield of protein came from the procedure involving homogenization of the sweet potato into phosphate buffer and NaCl followed by a calcium chloride precipitation (Table 2.9). The remaining two methods, homogenization into distilled water with ammonium chloride precipitation and homogenization into phosphate buffer and NaCl with ammonium chloride precipitation, produced similar averages with respect to each analytical method. The EZQ protein assay and BCA protein assay are statistically different, with the EZQ method detecting almost twice as much protein as the BCA method. Since the EZQ method measures both soluble and insoluble proteins whilst the BCA method only measures soluble proteins. The results might be expected since the sweet potato was only partly soluble.

Crude protein content in sweet potatoes has been reported to range from 1.3% to over 10% on a dry matter basis (Li, 1974). Our most efficient extraction resulted in 37.5 mg of protein from 100 ml of sweet potato homogenate based on wet weight. One hundred grams of sweet potato would therefore produce 225 mg of protein from this extraction procedure, resulting in a 0.225% protein precipitation wet weight and 12% precipitation dry mass basis. This value could be different than published data which could be due to protein remaining in the initial insoluble protein fraction. Kusano et al. (2001) reported a 0.011% yield of crude protein after precipitation, purification on a column, and fractionation.

### **2.6.2 Experiment 2: Variability Among Potatoes**

While each sweet potato used in Experiment 2 underwent the same extraction procedure at the same time, there was marked difference among the sweet potatoes when comparing the data by treatment (Table 2.5 and Table 2.8). Each treatment also yielded a statistically significant difference in protein precipitation. Each dilution, when calculated back to the initial concentration, was statistically dissimilar, indicating that the dilutions did not change the amount of calculated protein. Data did not vary when looking at total protein in regards to sweet potato x treatment and sweet potato x dilution. There were significant interactions for treatment x dilution in protein quantity analysis of variance.

The highest amount of protein was found in the heat-treated protein pellet (3HT) followed closely by the protein pellet (Table 2.11). There was significantly less protein found in the supernatant, indicating that the calcium chloride precipitation was sufficient to precipitate most of the protein, as the resulting material remained poorly soluble. It is possible that the heat treatment of the protein pellet may have denatured and aggregated the

soluble protein to yield a larger pellet, resulting in higher values for the heat-treated fraction as compared to its non-heat-treated counterpart.

## **2.7 CONCLUSIONS**

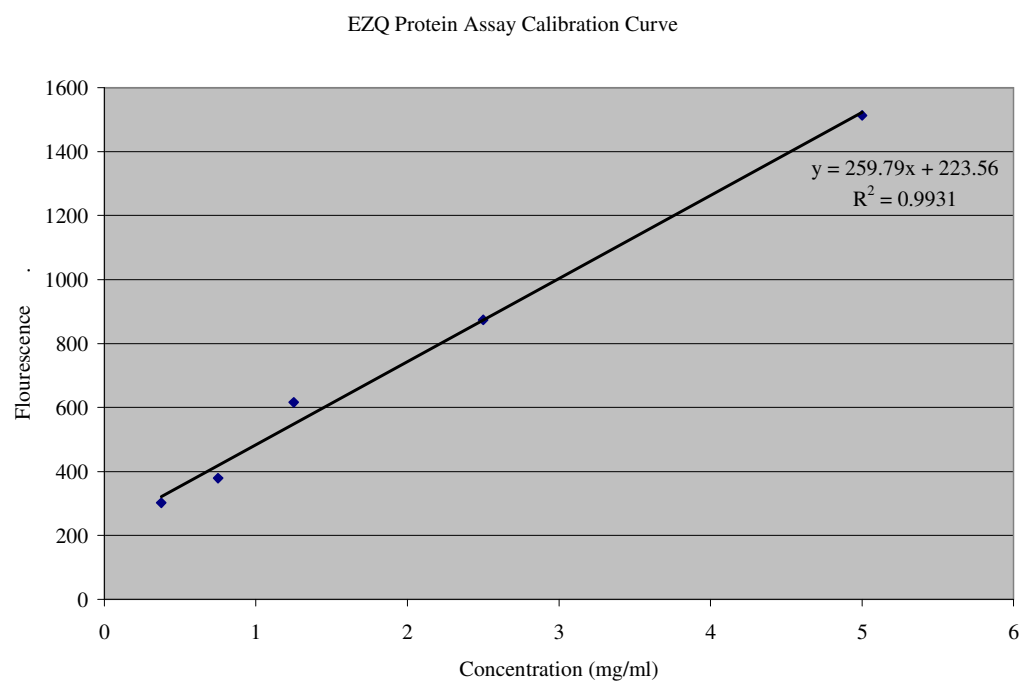
Four methods for sweet potato protein precipitation were described and compared. While statistically the results from the EZQ Protein Assay and the BCA Protein Assay are different, the values are comparable with respect to the types of protein being measured – total proteins, and soluble proteins, respectively. When comparing protein values derived from the same method, there is great variability between sweet potatoes used and the treatment they undergo. Variability between sweet potato protein content is well documented and dependent on field-to-field, location, environment, cultural management, and irrigation. There is also noted variability in protein content within the same sweet potato. Calcium chloride precipitation is sufficient to precipitate most of the protein out of the supernatant. Heat treatment of either the supernatant or the protein pellet causes a greater amount of protein to denature and go into solution, causing higher values for protein produced.

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**Table 2.1 EZQ Protein Assay Calibration Curve Data**

Concentration	Fluorescence	Calculated Concentration
5 mg/ml	1510	4.96
2.5 mg/ml	874	2.50
1.25 mg/ml	616	1.51
0.75 mg/ml	379	0.598
0.375 mg/ml	302	0.302
Line Equation	$y=260x + 224$	$R^2=0.993$



**Figure 2.1 EZQ Protein Assay Calibration Curve**

**Table 2.2 Experiment 1: EZQ Protein Assay Data**

Sample	Dilution Factor	Quantity (mg/100 ml SP homogenate)	Average	Standard Error
<b>1 (DA)</b>	1	22.7	33.8	6.65
	2	33.0		
	4	45.7		
<b>2 (DC)</b>	1	19.3	24.6	3.07
	2	30.0		
	4	24.7		
<b>3 (PBA)</b>	1	24.2	34.2	6.05
	2	33.3		
	4	45.1		
<b>4 (PBC)</b>	1	21.0	36.6	8.66
	2	37.7		
	4	50.9		

\*Quantity of protein was calculated using a dilution factor.



**Table 2.3 Experiment 2: Triplicate Data Samples EZQ Protein Assay Data**

Sample	Treat- ment	Dilution Factor	Quantity (mg/100 ml SP homogenate)	Average	Standard Error
<b>R</b>	2	1	0.705	0.377	0.331
		2	(0.0461)		
		4	(-)		
	2HT	1	4.35	4.01	0.689
		2	5.00		
		4	2.68		
	3	1	25.8	20.9	6.18
		2	28.3		
		4	(8.63)		
	3HT	1	25.2	31.0	4.08
		2	29.1		
		4	38.9		
<b>S</b>	2	1	1.36	1.36	0.954
		2	(-)		
		4	(-)		
	2HT	1	2.59	2.44	0.148
		2	2.58		
		4	2.14		
	3	1	17.3	14.4	7.41
		2	25.6		
		4	(0.379)		
	3HT	1	24.0	26.7	1.36
		2	27.7		
		4	28.4		
<b>T</b>	2	1	4.04	2.44	1.56
		2	(0.837)		
		4	(-)		
	2HT	1	3.42	2.81	0.620
		2	3.44		
		4	(1.57)		
	3	1	24.3	20.6	2.51
		2	21.3		
		4	15.8		
	3HT	1	20.8	14.7	3.57
		2	15.0		
		4	(8.43)		

\*Values in parenthesis are below the range of the standard and were not used when calculating the average concentrations.

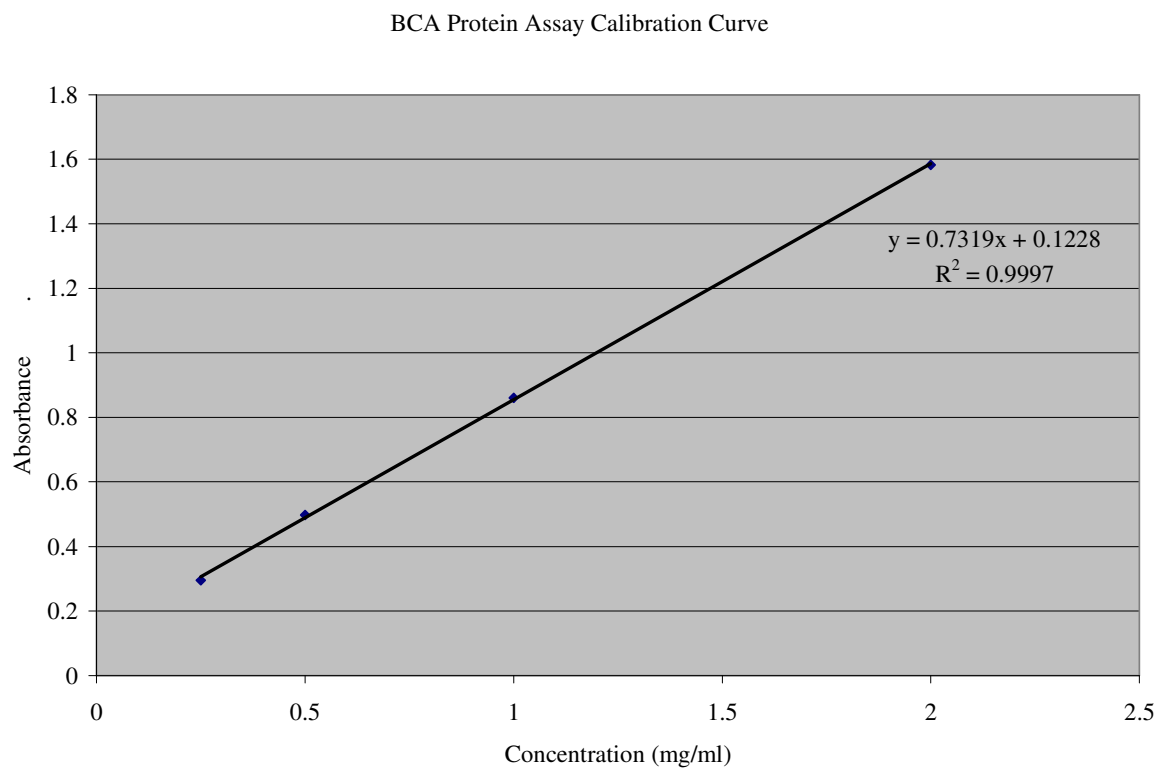
\*\*Quantity of protein was calculated using a dilution factor.

**Table 2.4 Experiment 2: EZQ Protein Assay Analysis of Variance Table**

<b>Source</b>	<b>DF</b>	<b>F</b>	<b>P</b>
Sweet Potato (SP)	2	4.37	0.0375
Treatment	3	52.55	0.0000
Dilution	2	2.35	0.138
SP x Treatment	6	2.98	0.0510
SP x Dilution	4	1.02	0.435
Treatment x Dilution	6	3.39	0.0342

**Table 2.5 BCA Protein Assay Calibration Curve Data**

Concentration	Absorbance	Calculated Concentration (mg/ml)
2 mg/ml	1.583	2.00
1 mg/ml	0.8605	1.01
0.5 mg/ml	0.4974	0.512
0.25 mg/ml	0.2949	0.235
Line Equation	$Y=0.732x + 0.123$	$R^2=0.999$



**Figure 2.2** BCA Protein Assay Calibration Curve

**Table 2.6 Experiment 1: BCA Protein Assay of Samples from Extraction Study**

Sample	Dilution Factor	Quantity (mg/100 ml SP homogenate)	Average	Standard Error
<b>1 (DA)</b>	1	(14.7)	22.0	5.16
	2	19.3		
	4	32.0		
<b>2 (DC)</b>	1	(15.4)	16.5	5.35
	2	7.88		
	4	26.3		
<b>3 (PBA)</b>	1	9.27	17.6	5.94
	2	14.3		
	4	29.1		
<b>4 (PBC)</b>	1	(20.9)	31.5	5.29
	2	(36.0)		
	4	37.5		

\*Values in parentheses are outside the range of the standard and were excluded.

\*\*Quantity of protein was calculated using a dilution factor.

**Table 2.7 Experiment 2: BCA Protein Assay**

Sample	Treat- ment	Dilution Factor	Quantity (mg/100 ml SP homogenate)	Average	Standard Error
<b>R</b>	2	1	0.743	0.658	0.903
		2	0.754		
		4	(0.478)		
	2HT	1	1.44	1.50	0.0544
		2	1.44		
		4	1.60		
	3	1	10.5	10.7	0.118
		2	10.6		
		4	10.9		
	3HT	1	11.6	11.8	0.313
		2	11.6		
		4	12.4		
<b>S</b>	2	1	0.782	0.745	0.0746
		2	0.852		
		4	(0.602)		
	2HT	1	1.36	1.32	0.0426
		2	1.37		
		4	1.24		
	3	1	(13.0)	13.2	0.153
		2	13.1		
		4	13.5		
	3HT	1	(13.5)	13.4	0.0161
		2	13.4		
		4	13.4		
<b>T</b>	2	1	1.13	1.36	0.133
		2	1.585		
		4	1.376		
	2HT	1	1.316	1.27	0.0216
		2	1.264		
		4	1.243		
	3	1	11.61	12.0	0.192
		2	12.22		
		4	12.14		
	3HT	1	(17.74)	18.1	0.413
		2	18.87		
		4	17.55		

\*Values in parentheses are outside the range of the standard.

\*\*Quantity of protein was calculated using a dilution factor.

**Table 2.8 Experiment 2: Triplicate Data BCA Protein Assay Analysis of Variance Table**

<b>Source</b>	<b>DF</b>	<b>F</b>	<b>P</b>
Sweet Potato (SP)	2	136	0.000
Treatment	3	4920	0.000
Dilution	2	1.25	0.321
SP x Treatment	6	91.2	0.000
SP x Dilution	4	1.74	0.205
Treatment x Dilution	6	0.630	0.706

**Table 2.9 Experiment 1: Mass Production Samples EZQ and BCA Comparative Concentration Assay**

Sample	Dilution Factor	Averages		Standard Error	
		EZQ Assay (mg/ml)	BCA Assay (mg/ml)	EZQ Assay (mg/ml)	BCA Assay (mg/ml)
<b>1</b> <b>(DA)</b>	1	33.8	22.0	6.65	5.16
	2				
	4				
<b>2</b> <b>(DC)</b>	1	24.6	16.5	3.07	5.35
	2				
	4				
<b>3</b> <b>(PBA)</b>	1	34.2	17.6	6.05	5.94
	2				
	4				
<b>4</b> <b>(PBC)</b>	1	36.6	31.5	8.66	5.29
	2				
	4				

\*Quantity of protein was calculated using a dilution factor.



**Table 2.10 Experiment 2: Triplicate Data Samples EZQ and BCA Comparative Concentration Assay**

Sample	Treatment	Dilution Factor	Averages		Standard Error	
			EZQ Assay	BCA Assay	EZQ Assay	BCA Assay
R	2	1	0.377	0.658	0.331	0.903
		2				
		4				
	2HT	1	4.01	1.50	0.689	0.0544
		2				
		4				
	3	1	20.9	10.7	6.18	0.118
		2				
		4				
	3HT	1	31.0	11.8	4.07	0.313
		2				
		4				
S	2	1	1.36	0.745	0.954	0.0746
		2				
		4				
	2HT	1	2.44	1.32	0.148	0.0426
		2				
		4				
	3	1	14.4	13.2	7.41	0.153
		2				
		4				
	3HT	1	26.7	13.4	1.36	0.0161
		2				
		4				
T	2	1	2.44	1.36	1.56	0.133
		2				
		4				
	2HT	1	2.81	1.27	0.620	0.0216
		2				
		4				
	3	1	20.6	12.0	2.51	0.1917
		2				
		4				
	3HT	1	14.7	18.1	3.57	0.413
		2				
		4				

\*Quantity of protein was calculated using a dilution factor.

**Table 2.11 Experiment 1: Mass Production Study Dry Mass Data**

<b>Sample</b>	<b>% Yield</b>
<b>Sample 1 (DA)</b>	11.9%
<b>Sample 2 (DC)</b>	1.2%
<b>Sample 3 (PBA)</b>	13.9%
<b>Sample 4 (PBC)</b>	14.2%

### **CHAPTER 3: AMYLASE ACTIVITY OF SWEET POTATO PROTEIN**

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### 3.1 ABSTRACT

Sweet potato protein has been shown to decrease fasting plasma glucose levels, low-density lipoprotein cholesterol, and total cholesterol in type 2 diabetic patients. A dietary supplement from sweet potato, Caiapo, has also been shown to modify the glycemic response. Based on this short term response, one hypothesis for the function of Caiapo is as an  $\alpha$ -amylase inhibitor. The objective of this study was to characterize the amylase and amylase inhibitory properties of Caiapo.

Protein was extracted based on the method outlined in Chapter 2 of this document. Native amylase activity and amylase inhibition activity were determined through the use of an iodine-starch assay. Amylase activity was characterized from a calculated amylase activity unit.

Sweet potato protein has native amylase activity. In combination with porcine pancreatic amylase, sweet potato protein demonstrates inhibitory properties. On a weight basis, the water-soluble fraction and the insoluble protein pellet had similar inhibitory properties. However, upon heat treating, the water-soluble fraction lost both native amylase activity and amylase inhibitory properties. Heat treatment of the insoluble protein pellet resulted in a small decrease in native amylase activity and an even smaller decrease in inhibitory activity.

### 3.2 INTRODUCTION

Sweet potato protein, Caiapo, has been used in traditional Japanese medicine as a method of controlling diabetes. Studies have shown that Caiapo decreases HbA1c, glucose levels, and cholesterol when consumed over a long term, and lowers the glycemic response of foods in the short term (Ludvik et al., 2004; Ludvik et al., 2003)). Caiapo has been shown to have the same effects of tolbutamide, a pharmaceutical used in the treatment of type 2 diabetes, through increasing blood insulin levels and increasing insulin activity. However, unlike tolbutamide, Caiapo showed hypoglycemic activity, improved glucose tolerance, and reduced high insulin concentrations (Kusano et al., 2001). The short term effect of Caiapo when taken with a meal is a change in the glycemic index of that food. It is hypothesized that Caiapo serves as an amylase inhibitor, reducing the activity of the body's ability to break down longer starches into sugars to be absorbed.

Rekha et al. (2002) studied the inhibitor potential of the  $\alpha$ -amylase inhibitors of sweet potato on the digestive enzymes of root crop storage pests. Sweet potato  $\alpha$ -amylase inhibitors showed great variation between both the inhibitor found in the sweet potato, and the  $\alpha$ -amylase it was inhibiting. Inhibition ranged from 0.80% to 94% of amylase activity.

$\alpha$ -Amylase inhibitors have been known to play a role in pest and disease resistance mechanism in plants, however, the biological significance of these inhibitors is not fully understood. Raw sweet potatoes are known to have high amounts of  $\alpha$ -amylase inhibitors, however, it is not known how processing affects the inhibitor. It has been shown that the  $\alpha$ -amylase inhibitors of sweet potato retained between 0.8-10% activity when processed at 90 °C and 100 °C for 24 hours. Boiling the sweet potato pieces in water allowed for a retention

of 29-59 % activity of the  $\alpha$ -amylase inhibitors. Baking in the microwave resulted in complete inactivation of the amylase (Rekha et al., 2002).

A method of measurement of inhibitor activity, as defined by Rekha et al. (2002), utilizes a soluble starch solution which interacts with a porcine pancreatic  $\alpha$ -amylase and the sample containing  $\alpha$ -amylase inhibitors. After the reaction is stopped, the residual starch concentration is measured through the absorbance of the starch and an iodine solution (Rekha et al., 1997). A similar method for assaying amylase activity measures the decrease in iodine staining power throughout the reaction using human serum albumin and pancreatic  $\alpha$ -amylase (Marshall et al., 1975). The objective of this study was to characterize the native amylase activity of sweet potato protein and its amylase inhibitory properties.

### **3.3 MATERIALS AND METHODS**

#### **3.3.1 Sweet Potato Protein Samples**

Sweet potato protein was extracted using calcium chloride following extraction into distilled water as explained in Chapter 2.

#### **3.3.2 Amylase Activity by Iodine Assay**

The amylase activity of each of the samples was quantified by the method of Rekha et al. (1997). A 0.5 ml portion of each sample was placed in duplicate test tubes. To one of the duplicate test tubes, 0.25 ml of a solution of porcine pancreatic  $\alpha$ -amylase was added (100  $\mu$ g porcine pancreatic  $\alpha$ -amylase/ml, Sigma A-3176 Type VI-B (Sigma; Wisconsin, USA). Phosphate buffer, 1.5 ml of a 50 mM  $\text{Na}_2\text{PO}_4$  with 0.3 M NaCl, pH = 6.9, was added to each of the test tubes. This mixture was incubated for approximately 30 minutes. After the

incubation, 0.5 ml of a 0.5% soluble starch solution was added to each test tube. To stop the reaction, 1.0 ml of 1.0 N HCl was added approximately 10 minutes after the initiation of the reaction. A 0.5 ml portion of an iodine reagent (0.2 g iodine crystals and 2.0 g potassium iodide in 100 ml distilled water) was added to each of the test tubes and the solution was measured at 620 nm in a spectrophotometer.

The amylase activity unit (AAU) is a value relative to the difference in absorbance between the control without pancreatic amylase and the control with pancreatic amylase, which then set to 1.000 AAU. Table 3.2 describes the coefficients of activity derived from the iodine assay. The activity of the standard was set to have an amylase coefficient of 1.000. Native activity was determined by the following equation.

$$amylase.coefficientSample2 = \frac{absorbance(tube2) - absorbance(tube4)}{absorbance(tube2) - absorbance(tube1)}$$

Activity of the sample which was incubated with  $\alpha$ -amylase was determined by the following equation.

$$amylase.coefficientSample2 = \frac{absorbance(tube2) - absorbance(tube3)}{absorbance(tube2) - absorbance(tube1)}$$

Expected activity was based on the native amylase activity and the activity of the standard, as determined by the following equation.

$$Sample2 = absorbance(tube2) - (absorbance(tube4) - absorbance(tube3)) - absorbance(tube1)$$

Differences in activity were based on the activity with enzyme and the expected activity, as determined by the following equation.

$$Difference = (activity.with.enzyme) - (expected.activity)$$

### **3.3.3 EnzChek® Ultra Amylase Assay Kit (E33651)**

The EnzChek® Ultra Amylase Assay Kit (E33651) (Molecular Probes™, Invitrogen; California, USA) was used to determine native amylase activity in the different samples. *Bacillus sp.*  $\alpha$ -amylase was used as the control. The control samples were prepared through a dilution scheme ranging from 20 mU/ml to 2.5 mU/ml, in triplicate. A 200  $\mu$ g/ml working solution of the provided DQ™ substrate was created. Fifty microliters of the samples and control were added to wells in a 96-well plate in duplicate or triplicate. Fifty microliters of the diluted DQ solution was added to each well as delivered by a multichannel pipette. The samples were incubated at room temperature and protected from light. The fluorescence intensity was measured in a fluorescence microplate reader at excitation 485 nm and emission at 530 nm, every two minutes for 30 minutes starting 10 minutes after initiation of reaction.

## **3.4 EXPERIMENTAL DESIGN**

Samples R, S, T, derived from the methods described in Chapter 2, were tested to determine their native amylase activity and amylase inhibitor properties. Each test tube was laid out according to Table 3.1.

## **3.5 RESULTS**

The controls, without sample, are found in tubes 1 and 2. Each sample was measured for native amylase activity (even numbered tubes) and native inhibitor activity (odd numbered tubes).



The native amylase activity for the soluble protein fraction (2), heat-treated soluble protein fraction (2HT), insoluble protein fraction (3), and heat-treated insoluble protein fraction (3HT), were  $1.72 \pm 0.0473$  amylase activity units (AAU),  $-0.178 \pm 0.139$  AAU,  $1.80 \pm 0.02$  AAU, and  $1.61 \pm 0.12$  AAU units, respectively. The expected activity, the combination of the native amylase and the standard porcine amylase, was  $2.72 \pm 0.047$  AAU (2),  $0.822 \pm 0.139$  AAU (2HT),  $2.80 \pm 0.0196$  AAU (3), and  $2.61 \pm 0.124$  AAU (3HT). The actual values of the native amylase and the standard porcine amylase were  $1.89 \pm 0.0370$  AAU (2),  $1.40 \pm 0.0798$  AAU (2HT),  $1.99 \pm 0.0707$  AAU (3), and  $2.04 \pm 0.0428$  AAU (3HT), indicating that the inhibition for each fraction was  $0.830 \pm 0.0193$  AAU (2),  $-0.573 \pm 0.142$  AAU (2HT),  $0.813 \pm 0.0512$  AAU (3), and  $0.576 \pm 0.0838$  AAU (3HT).

Table 3.3 Describes the coefficients of activity after the concentration of the protein, as determined by the BCA Protein Assay, was taken into account.

The native amylase for the soluble protein fraction (2), heat treated soluble protein fraction (2HT), insoluble protein fraction (3), and heat treated insoluble protein fraction (3HT), are  $2.06 \pm 0.403$  AAU,  $-0.131 \pm 0.106$  AAU,  $0.152 \pm 0.00786$  AAU, and  $0.114 \pm 0.0102$  AAU, respectively. The expected activity, the combination of the native amylase and the standard porcine amylase, is  $3.26 \pm 0.638$  AAU (2),  $0.606 \pm 0.112$  AAU (2HT),  $0.237 \pm 0.0131$  AAU (3), and  $0.186 \pm 0.0174$  AAU (3HT). It was found that the actual values were  $2.26 \pm 0.429$  AAU (2),  $1.04 \pm 0.105$  AAU (2HT),  $0.167 \pm 0.00555$  AAU (3), and  $0.146 \pm 0.0162$  AAU (3HT), indicating that the inhibition for each fraction was  $1.00 \pm 0.209$  AAU (2),  $-0.427 \pm 0.114$  AAU (2HT),  $0.0691 \pm 0.00856$  AAU (3), and  $0.0400 \pm 0.00415$  AAU (3HT).

Table 3.4 shows the results from the EnzChek Ultra Amylase Assay Kit for each of the three sweet potato samples and their four treatments. The average describes the mean of each of the wells containing the sample, since the samples were done in quadruplicate.

Table 3.5 shows the native amylase coefficients as determined by the iodine assay for each of the three sweet potato samples and their four treatments.

Table 3.6 shows the amylase coefficients as determined by the EnzChek for each of the three sweet potato samples and their four treatments. Since a standard was not used, the “2HT” fraction was set equal to zero because of the lack of activity as demonstrated by the iodine assay.

Figure 3.1 graphically shows the amylase activity per mg protein for each of the four fractions. Expected activity is the addition of the pancreatic amylase activity and the native sweet potato amylase. Inhibition is calculated from the difference between the expected activity and the actual activity (S.P. + Panc.).

### **3.6 DISCUSSION**

The characterization of amylase and amylase inhibitory properties was completed through a starch-iodine assay. All fractions, with the exception of the heat treated soluble protein fraction (2HT), demonstrated native amylase activity on a per weight basis, with the soluble protein fraction (2) having the highest value of native amylase activity. Likewise, the soluble fraction had the highest amylase activity in combination with the porcine pancreatic amylase, with the remaining three fractions having similar activity. The inhibition properties were similar for all of the fractions with the exception of the heat treated soluble protein fraction (2HT), which did not exhibit any inhibitory properties.

Rekha et al. (2004) studied the inhibitor potential of  $\alpha$ -amylase inhibitors of sweet potato on the digestive enzymes of root crop storage pests. They found that, depending on the digestive enzyme of the pests, there was between 25-58% inhibition. Inhibition for the pancreatic amylase in the present study was approximately in this range.

The data show that there are inhibitory properties when sweet potato protein extract is combined with pancreatic amylase. Based on the current methods there is not a way to determine which amylase, the porcine pancreatic amylase or the native sweet potato amylase, is responsible for the remaining amylase activity seen in the presence of the inhibitor. Further research is needed to chemically characterize the molecular structure of the amylase inhibitor.

### **3.7 CONCLUSIONS**

The amylase activity and inhibitory activity of sweet potatoes was characterized. All fractions, with the exception of the heat treated soluble protein fraction, had native amylase activity, as well as inhibitory properties. Heat treatment of the insoluble protein pellet decreased native amylase activity and slightly decreased inhibitory properties. This indicates that even if sweet potatoes are cooked, the protein maintains its integrity and can still act as a pancreatic amylase inhibitor.

### 3.8 REFERENCES

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**Table 3.1 Iodine Assay Experiment Design**

	Phosphate Buffer	Sample	$\alpha$ -Amylase	Starch	Sample R	Sample S	Sample T
<b>1</b>	2 ml	0.0 ml	0.25 ml	0.5 ml	-	-	-
<b>2</b>	2 ml	0.0 ml	0.0 ml	0.5 ml	-	-	-
<b>3</b>	1.5 ml	0.5 “2” ml	0.25 ml	0.5 ml	-	-	-
<b>4</b>	1.5 ml	0.5 “2” ml	0.0 ml	0.5 ml	-	-	-
<b>5</b>	1.5 ml	0.5 “2HT” ml	0.25 ml	0.5 ml	-	-	-
<b>6</b>	1.5 ml	0.5 “2HT” ml	0.0 ml	0.5 ml	-	-	-
<b>7</b>	1.5 ml	0.5 “3” ml	0.25 ml	0.5 ml	-	-	-
<b>8</b>	1.5 ml	0.5 “3” ml	0.0 ml	0.5 ml	-	-	-
<b>9</b>	1.5 ml	0.5 “3HT” ml	0.25 ml	0.5 ml	-	-	-
<b>10</b>	1.5 ml	0.5 “3HT” ml	0.0 ml	0.5 ml	-	-	-

**Table 3.2 Iodine Assay Coefficients of Activity**

	<b>Average</b>	<b>Standard Error</b>
<b>Native Activity</b>		
Amylase Coefficient	1.00	0.00
"2" Activity	1.72	0.0473
"2 H.T." Activity	-0.178	0.139
"3" Activity	1.80	0.0196
"3 H.T." Activity	1.61	0.124
<b>Expected Activity</b>		
"2"	2.72	0.0473
"2 H.T."	0.822	0.139
"3"	2.80	0.0196
"3 H.T."	2.61	0.124
<b>Activity with Enzyme</b>		
"2"	1.89	0.0370
"2 H.T."	1.40	0.0798
"3"	1.99	0.0707
"3 H.T."	2.04	0.0428
<b>Differences in Activity (Inhibition)</b>		
"2"	0.830	0.0193
"2 H.T."	-0.573	0.142
"3"	0.813	0.0512
"3 H.T."	0.576	0.0838

**Table 3.3 Iodine Assay Coefficients of Activity by Weight**

	Average	Standard Error
<b>Native Amylase</b>		
"2" Activity	2.06	0.403
"2 H.T." Activity	-0.131	0.106
"3" Activity	0.152	0.00786
"3 H.T." Activity	0.114	0.0102
<b>Expected Activity</b>		
"2"	3.26	0.638
"2 H.T."	0.606	0.112
"3"	0.237	0.0131
"3 H.T"	0.186	0.0174
<b>Activity with Enzyme</b>		
"2"	2.26	0.429
"2 H.T."	1.04	0.105
"3"	0.167	0.00555
"3 H.T"	0.146	0.0162
<b>Differences in Activity (Inhibition)</b>		
"2"	1.00	0.209
"2 H.T."	-0.427	0.114
"3"	0.0691	0.00856
"3 H.T"	0.0400	0.00415

**Table 3.4 EnzChek Ultra Amylase Assay Kit**

<b>Treatment</b>	<b>R</b>		<b>S</b>		<b>T</b>	
	Average (nm)	Standard Error	Average (nm)	Standard Error	Average (nm)	Standard Error
<b>2</b>	1815	23.1	1672	46.56	3023	304
<b>2HT</b>	1838	52.7	1791	42.9	1801	36.8
<b>3</b>	2758	32.5	2794	84.6	2390	173
<b>3HT</b>	2280	371	1931.5	30.9	3251	406

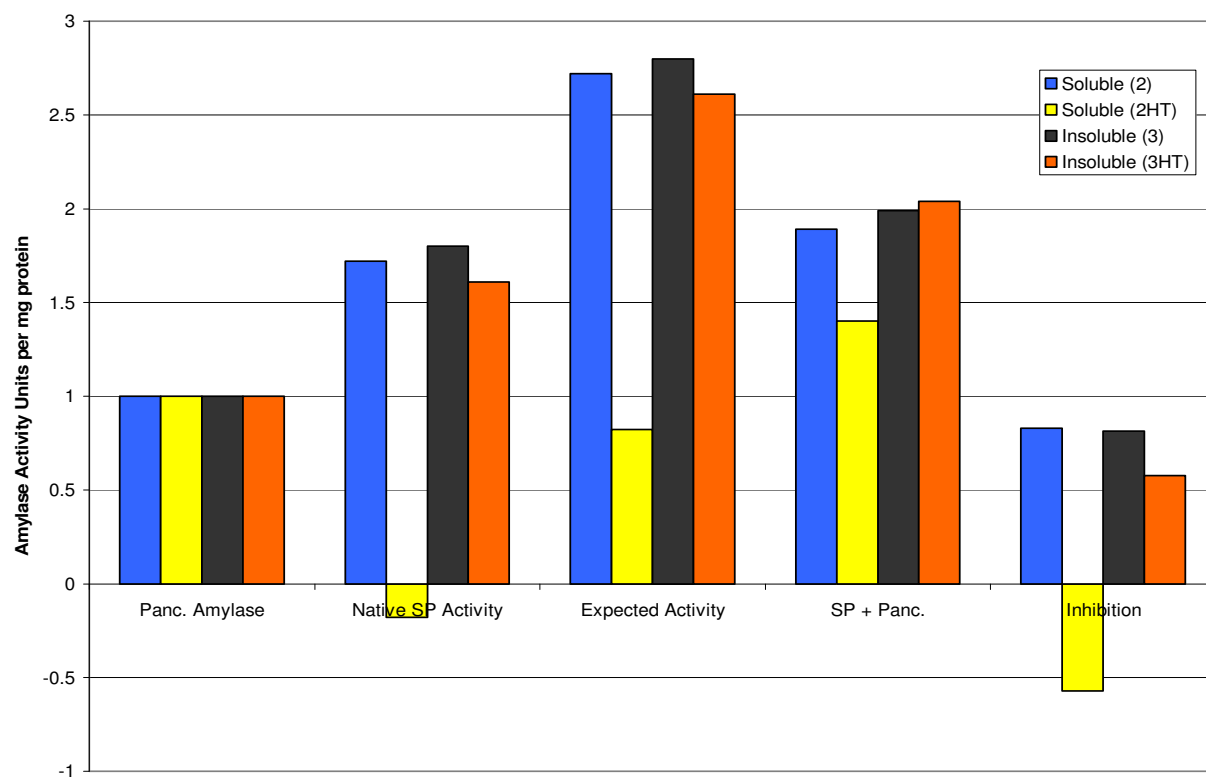


**Table 3.5 Iodine Assay Native Amylase Activity**

	<b>R</b>	<b>S</b>	<b>T</b>	<b>Average</b>	<b>Standard Error</b>
<b>"2"</b>	0.365	0.378	0.363	0.368	0.00470
<b>"2HT"</b>	0.000	0.000	0.000	0.000	0.000
<b>"3"</b>	0.391	0.382	0.388	0.387	0.00265
<b>"3HT"</b>	0.306	0.367	0.366	0.346	0.0202

**Table 3.6 EnzChek Ultra Amylase Assay Kit Native Amylase Activity**

	<b>R</b>	<b>S</b>	<b>T</b>	<b>Average</b>	<b>Standard Error</b>
<b>"2"</b>	-22.5	-119	1222	360.	432
<b>"2HT"</b>	0.000	0.000	0.000	0.000	0.000
<b>"3"</b>	920.	1000	589.	837	126.
<b>"3HT"</b>	442.	141	1450	677	396



**Figure 3.1 Characterization of Sweet Potato Protein**

**CHAPTER 4: SUMMARY**

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In summary, sweet potato protein was precipitated using either an ammonium sulfate precipitation or calcium chloride precipitation following homogenization with distilled water or phosphate buffer. It was shown that homogenization with phosphate buffer followed by a calcium chloride precipitation produced the greatest amount of precipitate. There is variability between sweet potatoes in the quantity of protein which is produced through precipitation.

Both the water-soluble and insoluble protein fractions have native amylase activity, which is destroyed with heat treating in the case of the water-soluble fraction and partially destroyed in the case of the insoluble protein fraction. When incubated with porcine pancreatic amylase the heat treated and non-heat treated insoluble fractions and non-heat treated soluble protein fraction exhibited amylase inhibitory properties.

Future work could include simulation of the human digestive system, changes in protein and characterization of amylase activity following different sweet potato processing methods, a large scale (pilot-plant) production of protein, and using the precipitated protein in human trials.