FUNCTIONAL BIOACTIVE COMPOUNDS FROM SWEET POTATOES FOR HUMAN HEALTH BENEFITS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Global food and nutritional insecurities, public health challenges of diet-linked non-communicable chronic diseases (NCDs), and rapid climate change-linked agricultural production challenges are interconnected and require urgent attention. Therefore, to address these complex and interconnected challenges, it is essential to advance robust and resilient strategies based on sustainable agricultural production practices, wider integration of nutritionally-balanced plant-based foods in the diet, improvement of human health-targeted nutritional qualities, post-harvest preservation qualities and food processing optimization. Therefore, food plants that are climate resilient and rich source of human health protective nutritional bioactives, such as sweet potato are ideal dietary targets for advancing global food and nutritional security solutions, while also addressing emerging NCD-linked health challenges. Sweet potatoes are rich source of stress protective phenolic bioactives with dual functional benefits relevant for resilience to climate change and countering diet-linked NCD challenges. However, the phenolic bioactive compounds and associated health protective functionalities of sweet potatoes vary widely between different flesh color and cultivars, due to different pre-harvest production practices, post-harvest storage conditions, and with different food processing strategies. Therefore, the aim of this dissertation was to screen sweet potato cultivars of different flesh color (off-white, orange, purple) and optimizing different food processing strategies based on optimum phenolic bioactive-linked antioxidant, anti-diabetic and anti-hypertensive properties using metabolically-targeted *in vitro* assay models. Overall, high soluble phenolic-linked antioxidant activity was observed in purple-fleshed cultivar, while high type 2 diabetes relevant anti-hyperglycemic and anti-hypertensive properties were observed in orange and white-fleshed sweet potatoes. Additionally, improvement in stability and retention of phenolic bioactives and associated functionalities were present in

bio-transformed sweet potatoes after fermentation with beneficial lactic acid bacteria (LAB). Furthermore, food processing (deep-frying, baking, steaming, and boiling) optimization studies revealed optimum food processing conditions (cooking temperature, cooking time, and sweet potato sample size) based on higher retention of phenolics and associated antioxidant and anti-hyperglycemic functionalities. We also advanced metabolically-driven elicitation strategy based on the conceptual foundation of dual functional benefits of phenolic compounds to improve wound-healing in bruised potato tubers through stimulation of redox-linked pathway (pentose phosphate pathway) regulation associated with stress-protective phenolic biosynthesis and antioxidant enzyme responses.

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DEDICATION

I would like to dedicate this project to my family, friends and teachers who supported me throughout my life.

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CHAPTER 1. INTRODUCTION

1.1. Human Health Benefits of Whole Plant-Based Diet Related to Emerging Non-Communicable Chronic Disease (NCDs) Challenges

A whole plant-based diet is characterized as minimally processed, nutritionally balanced, and ecologically sustainable with a higher proportion of fresh vegetables, whole grains, legumes, fruits, and nuts. Such plant-based diet with a rich nutritional profile are gaining wider interest as health-targeted food solutions because of provision of dietary protection against rapidly emerging diet and lifestyle-linked non communicable chronic diseases (NCDs). Additionally, in the context of rapid global climate change, the agricultural production of plant-based food also needs to be ecologically sustainable by supporting both macro- and microenvironments of food plants. The current global food production systems are emphasized by industrialized agricultural production, mono-cropping, hyper-processing of food, and intensive animal farming, which provide global macronutrient-based calorie needs, but have several disadvantages such as higher utilization of natural resources, greater reliance on chemical fertilizers and synthetic pesticides, reduction in food diversity and related nutritional imbalances, and higher carbon footprints contributing to the global climate change with an estimated 14.5% of total greenhouse gas emissions (Sustainable Agriculture Network, 2020). To better balance these diverse challenges, advancing nutritionally balanced and ecologically sustainable agricultural food production systems promoting a whole plant-based diet can help to reduce the agricultural carbon footprints while also supporting global food and nutritional security solutions.

The presence of essential nutrients beyond just calorie-linked macronutrients such as bioactive compounds, vitamins, minerals, proteins, and dietary fibers of whole plant-based foods are also needed for its diverse dietary and health benefits but are often overlooked (Willett et al.,

2019). These needed nutritional benefits are particularly found to be rich in a plant-based diet comprised of phytonutrient-dense foods such as vegetables and fruits. Although the nutritional profile of these foods varies widely based on different ecologies and growing environments, overall a diet with portions and diversity of whole plant-based food such as whole grains, legume seeds, vegetables, fruits, and nuts along with supplement meat and dairy products have been considered as nutritionally balanced and promoting good human health . A popular and prominent example for this kind of diet is the Mediterranean diet which is largely considered as a healthy diet due to its wider diversity of plant-based foods and their health protective functions (U.S News & World Report Annual Rankings, 2020). Such a whole plant-based and nutritionally balanced diet not only just provides essential nutrients, but also supports and protects human health by reducing the risk of cardiovascular diseases and other NCDs (Morin et al., 2019).

Due to such wider health benefits, consumers across the globe are also making informed choices and integrating whole plant-based foods in their daily diet. Over the past few years, there has been an increasing interest in choosing the whole plant-based dietary pattern in the United States as about 5 % of American adults identified themselves as vegetarians in 61 different online Foods surveys (Gallup poll, 2018). The selective choice of plant-based diet has shown an increasing trend from the year 2013 (only 1.3 %) to 2018 (5.3 %) in the United States (Simcikas, 2018). In order to attain nutritional adequacy, individual dietary choices are critical, dietary choices are largely driven by several factors such as ecological conditions, race/ethnicity, socio-economic status of a person, availability, and affordability of food. A study explained importance of socio-economic status as a contributing factor for dietary choices, body mass index, and obesity issues compared to health-related psychosocial factors such as self-efficacy, beliefs and nutrition knowledge about positive effects of healthy diet (Wang et al., 2012).

Additionally, ensuring the bioavailability of certain nutrients are critical for meeting the basic nutritional needs of an individual. For instance, higher intake of dietary fiber, calcium, potassium, vitamins, and phytonutrients provides nutritional adequacy, while also reducing mortality rate associated with NCDs such as hypertension, type 2 diabetes, obesity and cardiovascular diseases (CVDS). In this context, an optimal diet is one that contains more of vegetables and fruits, which are considered as well-balanced diet due to their rich sources of minerals, fibers, and phytochemicals with potential antioxidant properties and other human health protective functionalities. Therefore, whole plant-based dietary approaches are found to be effective in providing all essential nutrients required for all stages of life like childhood, adulthood, pregnancy, lactation, late adulthood (Farmer et al., 2011). On the contrary, lower consumption of complex carbohydrates and dietary fiber can lead to potential health risks (WHO, 2017). A positive impact of consuming a whole plant-based diet on reduced risk of ischemic heart disease was observed in a cohort study conducted in nine European countries over twelve years of time period (Timothy et al., 2019). This study reported a positive correlation between excess intake of red and processed meat with higher cardiometabolic risks and related cardiovascular disease, especially in middle aged people (Key et al., 2019). Similarly, Satija et al. (2017) reported reduction of coronary heart disease with whole plant-based diet in a prospective cohort study conducted on adults over several years. This study also provided improvement in cardiometabolic health in association with higher intake of whole plant-based foods and its benefits over intake of hyper processed foods. Therefore, all these studies indicated a potential health protective role of whole plant-based diet that are rich in human health relevant bioactives, especially for prevention and management of major NCDs, such as type 2 diabetes, obesity and cardiovascular diseases (CVDs).

1.2. Type 2 Diabetes

Imbalance of glucose homeostasis causes a serious chronic metabolic disorder in humans and other animals, which is commonly referred as Diabetes Mellitus (DM). Diabetes, especially type 2 diabetes has been a leading cause for increased risk of heart diseases, kidney failure, and blindness. The health impacts of type 2 diabetes on individuals are severe irrespective of age, gender, geographic and demographic factors. This chronic disease is the fastest growing health challenge of the 21st century affecting 79% of individuals living in low- and medium-income economies with wider impacts on individuals, societies, countries, and nations. The economic cost of type 2 diabetes is estimated as $760 billion, which accounts 10 % health expenditure for treating various complications (e.g. diabetic ketoacidosis) and it is expected to reach $845 billion by 2045 globally (IDF Diabetes Atlas, 2019).

According to the American Diabetes Association (ADA), diabetes has been categorized into type 1, type 2, and gestational diabetes. Complete absence of insulin secretion defines Type 1 which is described as an autoimmune disorder related to destruction of beta cells of the pancreas and constitutes 5 % to 10 % of total diabetes. Whereas, type 2 diabetes is described as a metabolic disorder arising as a result of a prolonged hyperglycemic condition and accounts for 90 % to 95 % of overall diabetes population. Gestational diabetes observed in 2 % to 5 % of cases during pregnancy which has significant impact on health of both mother and pre-born child (National Diabetes Statistics Report, 2017). Type 2 diabetes, which has serious social and economic impacts causing a severe effect on the overall health care system, life expectancy, and quality of life. The wider health effects of type 2 diabetes on life expectancy is through increased risks of several health-related complications such as cardiovascular disease, stroke, Alzheimer's disease, foot wound problems, eye problems, dental problems, kidney disease, and nerve

damage. In addition to life altering complications, type 2 diabetes has been described as a leading cause for early death, which is estimated to be one death every eight seconds comprising nearly 4.2 million deaths in 2019 (IDF Diabetes Atlas, 2019). Global prevalence of type 2 diabetes has tripled during past few years and in the year 2019, it was estimated at 463 individuals. According to IDF, an exponential rise of diabetes is expected to increase to 700 million individuals by 2040 (IDF Diabetes Atlas, 2019). This exponential upsurge in the global diabetic prevalence is due to rise in type 2 diabetes with an associated increase in contributing risk factors such as increasing obesity, sedentary behaviour, poor nutrition, and prolonged psychological stress.

1.2.1. Type 2 Diabetes and Associated Risks

Type 2 diabetes is a complex and heterogeneous chronic disease condition that develops from effects of multiple and interrelated physiological, metabolic and social factors such as lifestyle, behavioral, genetic, and socioeconomical conditions. The pathophysiology and associated health risks of type 2 diabetes include insulin resistance, impaired glucose tolerance, β cell dysfunction, central adiposity, hypertension, retinopathy, and nephropathy. These diverse type 2 diabetes related disease risks are characterized as ‘cardiometabolic syndrome’ or “metabolic syndrome X”, which is widely accepted by World Health Organization and the American Society of Endocrinology as a disease entity (Castro et al., 2003). Therefore, type 2 diabetes and associated metabolic breakdowns can lead to several disease-related complications such as cardiovascular disease includingstroke, Alzheimer’s disease, foot wound problems, eye problems, dental problems, kidney disease, and acute nerve damage. The American Heart Association considers type 2 diabetes as major contributing factor for cardiovascular disease (CVD), which is the leading cause of death globally. Additionally, poorly managed diabetes can

lead to mortality, which is estimated at approximately 4.2 million deaths, comprising 11.3 % of global deaths in 2019 (IDF Diabetes Atlas, 2019).

Therefore, prevention and management of type 2 diabetes by advancing robust health care strategies are essential to reduce the morbidity and mortality associated with type 2 diabetes, CVD and other NCDs. Solely relying on pharmaceutical drug-based treatment approaches are ineffective to prevent and counter type 2 diabetes and associated risks. Such health care strategies also have huge economic costs and add up burden to the overall economy of developing and developed countries worldwide. Therefore, multifaceted and dynamic strategies focusing on balanced diet and healthy lifestyle along with formal health care measures are required to address the complexity of type 2 diabetes and associated disease challenges. Like type 2 diabetes, gestational diabetes of pregnant women is also becoming a major global health risk that needs an urgent attention.

1.2.2. Maternal and Child Health

Preconception health care protects the mother during pregnancy and childbirth and supports pre- and post-natal health of the baby, which is important to avoid any serious disease complication before and after the birth. This preconception health care varies from one individual to another depending on their health status, diet, socio-economic status, and access to good medical care and supporting environment. Overall, many factors contribute to maternal health such as accessibility to balanced nutrition, daily regime of physical activity before and during pregnancy, healthy body mass index to avoid premature childbirth, maternal mental health during pregnancy and after childbirth, and socio-economic condition of the household. According to WHO estimate, approximately 810 women die from preventable causes related to pregnancy and childbirth annually, which accounts for 94 % of maternal deaths in developing

countries (WHO, 2019). The major causes of such high maternal mortality in developing countries are mostly due to persistent poverty, lack of quality health care services, poor nutrition, and lack of skilled health care personnel (WHO, 2019). Among different health complications, high blood pressure (preeclampsia) and high blood glucose level (gestational diabetes) during pregnancy, high risks of infections during and after childbirth due to poor sanitation and lack of hygiene, and severe bleeding during childbirth are major causes for maternal death. Gestational diabetes is a condition with high blood glucose levels during pregnancy, which could be fatal to both mother and baby if it is not diagnosed and remains untreated. So, it is very important to have early diagnosis and treatment to avoid any serious complications associated with gestational diabetes. There are several symptoms that emerge during early stages of the disease development such as frequent urination, excessive thirst, nausea, tiredness or lack of energy, blurred vision, and urinary tract infections. Routine health monitoring can help early diagnosis and prevent future complications to arise from gestational diabetes. However, the majority of women worldwide, especially in developing countries do not have physical and economic access to regular health check-ups and therefore the disease remains undiagnosed and untreated. The factors causing gestational diabetes are complex, which includes poor nutrition, obesity, prediabetic conditions such as impaired glucose tolerance, and genetic predisposition. According to the WHO pregnant women with diabetes are at higher risk of having frequent health complications during pregnancy when compared to non-diabetic pregnant women. Additionally, gestational diabetes also increases the risk of developing type 2 diabetes later in life both for mother and baby, increase the risk of chronic obesity (fetal macrosomia), premature birth of a baby with respiratory diseases, hypoglycemia (low blood glucose) at pre-natal stage, and stillbirth. Therefore, the magnitude and health impact of gestational diabetes on pregnancy

outcomes is severe and needs sound public health policy measures to address this challenge. An adequate and balanced supply of nutrition to pregnant women is one of the key strategies to prevent and manage gestational diabetes. Therefore, dietary strategies based on whole and diverse plant-based diet with health protective functions can be advanced to address type 2 diabetes, gestational diabetes, and associated health challenges.

1.3. Dietary Strategies to Prevent and Manage Type 2 Diabetes and Associated Risks

According to the American Diabetes Association (2007), dietary interventions can effectively prevent or delay the onset of type 2 diabetes and associated health risks. Furthermore, WHO (2019) specified the importance of higher intake of fruits and vegetables that are rich in dietary fiber and protective bioactive compounds to mitigate the risks of type 2 diabetes and other NCDs. Dietary interventions that include a whole plant-based diet are highly recommended as an effective strategy to prevent and slow down type 2 diabetes associated health risks. In this context, previous studies found positive impact of higher consumption of fruits and vegetables that are rich in dietary fiber and bioactive compounds on prevention and management of type 2 diabetes (Bauer et al., 2013; Eshak et al., 2013). Lindstrom et al. (2016) reported higher chances (approximately 90 %) of reduction in development of type 2 diabetes in high-risk individuals by following dietary strategies with increased intake of whole grains, fruits, legumes, and vegetables, combining with lifestyle interventions such as regular physical activity. Whole grains, fruits and vegetables are especially rich in health protective bioactive compounds and these bioactives provide diverse health benefits including protection against type 2 diabetes, CVD, obesity, dyslipidemia and other NCD related risks.

1.4. Plant Bioactives

Plant bioactives are secondary metabolites primarily involved in the endogenous protective defense mechanism against biotic and abiotic stresses. Overall, in plants 20 % of total carbon fixation are diverted to secondary metabolic pathways such as the phenylpropanoid pathway for the biosynthesis of secondary metabolic compounds such as phenolic bioactives. These secondary metabolites are classified based on origin (natural and artificial), and mode of action (primary antioxidants and secondary antioxidants) as phenolic acids, flavonoids, alkaloids, stilbenes, tannins, coumarins, and lignans. Apart from their important role in endogenous defense responses, bioactives of food and medicinal plants also provide human health relevant benefits when integrated in dietary and therapeutic interventions. Among many human health protective functions, plant bioactive compounds are reported to influence gut microbiota, which directly influence nutrient absorption and metabolism. Previous studies reported a strong correlation between higher intake of plant-based diet and improvement of gut microbiota, and associated health protective role in reducing the risks of obesity, type 2 diabetes, CVD, and a few types of cancer (Backhed et al., 2004; Sarkar and Shetty, 2014; Turnbaugh et al., 2006). As mentioned previously whole plant-based foods such as fruits, vegetables, nuts, legume seeds, and whole grains, are known to be rich sources of various bioactive compounds/phytochemicals. Overall, protective plant bioactives play a critical metabolic role in maintaining redox homeostasis by countering chronic oxidative stress –induced damages. Chronic oxidative stress and over inflammation of tissues are linked to pathophysiology of many NCDs such as type 2 diabetes, CVD, respiratory diseases, age-related eye diseases, and cancer. When consumed, bioactive compounds of plant-based foods can act as natural antioxidants in neutralizing or stabilizing the most reactive free radicals. Furthermore, natural antioxidants from plant-based foods can act as

hydrogen donors, electron donors, singlet oxygen quenchers, enzyme inhibitors, and metal-chelating agents (Goiris et al. 2012). Health protective roles of these bioactive compounds largely depends on several factors such as their bio-accessibility and bioavailability during intestinal digestion which is related to food matrix, food preparation and processing methods, intestinal absorption, metabolic rate of an individual, and composition and population of host gut microbiota. Research outcomes from scientific studies from the few decades have emphasized the health benefits of higher intake of plant-based foods rich in bioactive compounds such as berries, cherries, tomatoes, colored capsicums, and vegetables, especially to counter chronic oxidative stress associated NCD (Xu et al., 2017). Most of these previous studies were focused on phenolic bioactives, due to their high antioxidant potential and wider distribution in common plant-based foods.

1.4.1. Phenolic Bioactives of Plant-Based Food

Plant-based foods are known to be excellent sources of phenolic bioactives with high antioxidant potentials, which includes diverse groups of phenolic acids, flavonoids, alkaloids, stilbenes, tannins, coumarins, and lignans. These phenolic bioactives are primarily derived from the shikimate and phenylpropanoid pathways in plants and divided into several classes mainly based on basic skeleton such as simple phenols (C6 skeleton include catechol and phloroglucinol), phenolic acids (C6-C1 skeleton includes gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic acids), hydroxycinnamic acid derivatives (C6-C3 skeleton includes p-coumarin, ferulic, caffeic, sinapic acids), and flavonoids (C6-C3-C6 skeleton includes anthocyanins). Phenolic bioactives from plant-based food sources are receiving increasing attention due to their several bioactive functionalities such as ability to limit lipid oxidation by acting as hydrogen donors, electron donors, singlet oxygen quenchers, enzyme inhibitors, and as

metal-chelating agents (Goiris et al., 2012). Additionally, they are most abundant and well distributed in plant- based foods such as tea, coffee, chocolate, wines and food matrices having higher amounts of fruits, vegetables and whole grains. Structurally about 8000 phenolics are known today and more than 4000 flavonoid compounds have been identified (Harborne and Williams, 2000). Phenolic compounds are extensively studied and received greater attention in past few decades as dietary supplements, nutraceuticals, and high value dietary components of functional foods and beverages with targeted potential to manage chronic oxidative stress that are commonly associated with NCDs. Specifically, these phenolic compounds of plant-based foods are an important source of dietary antioxidant and help to counter free radicals which are resultant of chronic oxidative stress in the body. Due to such high antioxidant potentials, phenolic compounds have been targeted and integrated in various commercial applications related to agriculture, food, cosmetics, and pharmaceutical sciences. The specific role of phenolic compounds to counter various NCD related pathogenesis are not studied extensively, but the overall benefits of higher intake of plant-based foods that are rich in phenolics to reduce the risk of NCDs are well documented (Xu et al., 2017).

1.4.2. NCD-linked Health Benefits of Plant Phenolics

Western dietary patterns that are rich in hyper-processed and calorie-dense foods rich in macronutrients are increasingly exposing people of all age groups around the globe to several dietary risk factors associated with NCDs. Many of these dietary risk factors are associated with chronic oxidative stress which is one of the primary underlying disease breakdown conditions of NCDs. Free radicals from dietary components and their interactions are generating chronic oxidative stress which are capable of damaging cells at rapid rates due to their unstoppable chain reactions by degenerating several biological processes. The degenerative processes associated

with oxidative stress induced cellular breakdowns lead to autoimmune diseases , progression of β-cell dysfunction (type 2 diabetes), clogging of arteries (hypertension and cardiovascular diseases), age-related macular degeneration, nervous system diseases (Alzheimer's disease, Parkinson's disease, epilepsy, cerebral palsy etc.), and several kinds of cancers (Kadenbach et al., 2009). However, healthy lifestyle choices and nutritionally balanced dietary patterns can help to mitigate many of the degenerative processes associated with chronic oxidative stress.

Therefore, adopting a healthy lifestyle with regular consumption of well-balanced plant-based foods enriched in phenolic bioactives can help to maintain redox homeostasis by protecting cells from oxidative stress and associated cellular damages (Goiris et al., 2012). The daily intake of foods rich in dietary antioxidants such as phenolic bioactives is critical and can be targeted in health-focused food solution strategies against chronic oxidative stress-induced NCDs

1.4.3. Oxidative Stress and Antioxidant Activity of Plant Phenolics

Being an indispensable part of eukaryotic life, oxygen is essential for aerobic respiration process and for cellular energy production (ATP). However, due to their highly unstable and chemically reactive nature, it can form free radicals, and these reactive oxygen species (ROS) can cause serious cellular damage (Cheeseman and Slater, 1993). In general, free radicals such as hydrogen peroxide, hydroxyl radical, singlet oxygen, hypochlorite, and nitric oxide radical act as either oxidants or reductants by donating or accepting electrons from other stable compounds. Therefore, either by donating or accepting electron free radicals can alter the structure and function of stable macromolecules such as DNA, RNA, proteins, lipids, and carbohydrates. Apart from the natural aerobic respiration process, exposure to different harsh environments such as UV radiation, X-rays, air pollutants, and toxic chemicals can also generate free radicals in the cells (Lobo et al., 2010). Generation of such free radicals is part of natural cellular and metabolic

processes and even essential for many signaling pathways. Under normal conditions, the endogenous antioxidant defense system can quench free radicals and help to maintain cellular redox balance. However, imbalance in the antioxidant defense system and excessive generation of free radicals lead to breakdown of cellular redox homeostasis and subsequent development of a chronic oxidative stress state. As chronic oxidative stress causes structural and molecular changes to macromolecules leading to several irreversible damage to the biological systems, exogenous intake and application of antioxidants are required to offset the damages. In this context, several natural (e.g. ascorbic acid, vitamin E, acrotenoids, anthocyanin, phenolics) and artificial (e.g. butylated hydroxyanisole and butylated hydroxytoluene) antioxidant compounds have been widely targeted. Which are used in processed food, cosmetics, supplements, and therapeutic products (Gupta et al., 2014; Van den Ende et al., 2011). However, many of these synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have adverse side effects on human health, whereas natural and dietary antioxidants of plant-based foods are safe, inexpensive, and sometimes more effective to counter chronic oxidative stress and associated disease pathogenesis (Huang, 2018). Mainly diets rich in vegetables, fruits, seeds, nuts, and whole grains are good source of dietary antioxidants such as phenolic bioactives which helps to maintain cellular redox balance without affecting regular physiological and metabolic processes. The antioxidant properties of phenolics are based on their unique chemical structure and ability to effectively quench/scavenge free radicals. Such high antioxidant properties of phenolic bioactives have significant relevance for prevention and management of chronic oxidative stress associated NCDs and related health risks. Additionally, phenolic bioactives of plant –based foods are used widely as bio-preservatives to reduce post-harvest oxidative breakdowns and related deterioration of food and nutritional qualities. These phenolic bioactives

also exhibit high anti-microbial properties and can be targeted for safe and diverse food safety relevant applications. Therefore, phenolic bioactives of plant-based foods with high antioxidant potentials have diverse human health relevant benefits and can be integrated in health-focused food solutions, especially to address emerging diet and lifestyle linked NCDs, such as type 2 diabetes, CVD, obesity, and neuro degenerative disease.

1.4.4. Role of Plant Phenolics to Improve Glucose Metabolism

Glucose is the main source of energy derived from three different sources in the body which includes intestinal absorption after consumption of food, glycogenolysis (i. e. breakdown of glycogen into glucose under fasting state), and gluconeogenesis (i. e. glucose derivation from amino acids and lactate under a prolonged fasting state) (Stephen et al., 2004). These mechanisms are controlled by a set of hormones in the body to maintain glucose homeostasis. Referred to as glucoregulatory hormones, which include insulin, glucagon, GLP-1, amylin, GIP (glucose-dependent insulinotropic peptide), cortisol, epinephrine, and growth hormone (Stephen et al., 2004). In addition to glucoregulatory hormones, several key enzymes like alpha-amylase, alpha- and beta-glucosidase play critical roles in carbohydrate digestion which includes breakdown of starch to maltose and mono- di- saccharides and thereby towards glucose conversion in the body. The diabetic and pre-diabetic condition, these simple carbohydrates enter the blood stream leading to increase in their concentration without sufficient secretion and impaired regulatory function of insulin resulting in chronic hyperglycemia. Poor management of such postprandial chronic hyperglycemia has been identified as key factor leading to the development of type 2 diabetes, atherosclerosis, and indirectly other NCDs such as kidney failure, retinal impairment and cardiovascular diseases/complications (Laasko and Kuusisto, 2014). In this context, plant-based foods, especially plant phytochemicals have inhibitory

properties against key glucose metabolism associated enzymes such as alpha-amylase and alpha-glucosidase, which are critical to slow down absorption of glucose in the bloodstream and to manage chronic hyperglycemia. There are many synthetic inhibitors such as acarbose, voglibose, and miglitol which have been targeted in therapeutic intervention to inhibit these key enzymes for managing postprandial glucose spike. However, these synthetic inhibitors also have harmful side effects like unusual tiredness, rectal bleeding, nausea, bloating, and dark urine. Therefore, screening and identification of natural food-based sources to manage postprandial glucose homeostasis is important for advancing safe and inexpensive intervention strategies. Overall, a combination of high alpha-glucosidase inhibitory activity coupled with moderate alpha-amylase inhibitory activity was reported as desirable to avoid stomach distention, diarrhea, and flatulence (Bischoff et al., 1985). Results of various *in vitro* and *in vivo* screening studies indicated high alpha-glucosidase and alpha-amylase enzyme inhibitory activities by several phenolic compounds such as caffeic acid, quercetin, coumaric acid, and gallic acid, and protocatechuic acid and plant based foods that are rich in these bioactives (Kwon et al., 2008; Matsui et al., 2001; McDougall et al., 2005). In an *in vitro* screening study, high anti-hyperglycemic functionalities coupled with high antioxidant and anti-hypertensive properties were observed in Asian spices (mustard, fenugreek, cinnamon, ginger, turmeric, fennel, cardamom,) and American vegetables (baby spinach, green peppers, broccoli sprouts, red pepper, string beans) (McCue et al., 2005). Therefore, higher intake of fruits, vegetables, and spices rich in phenolic bioactives is an effective dietary strategy to manage chronic hyperglycemia and associated health risks such as chronic inflammation and hypertension.

1.4.5. Anti-Hypertension

Along with managing chronic hyperglycemia, lowering high blood pressure/ hypertension has been highly recommended as one of the most effective prevention strategies to address the NCD-linked health challenges globally (WHO, 2013). Chronic and elevated blood pressure is considered as a high-risk factor for development of CVD, type 2 diabetes, cerebrovascular disease, and renal disease. A global pooled analysis of 1479 different studies which included 19.1 million adults have shown an increasing trend in number of individuals with blood pressure of 180 mm Hg systolic and higher or bottom of 120 mm Hg diastolic pressure from 594 million in 1975 to 1.13 billion in 2015 (Zhou et al., 2017). The increase in number of hypertensive patients was mostly observed in low-income and middle-income countries such as East and Southeast Asia, Sub Saharan Africa and Oceania (Mills et al., 2016). The International Society of Hypertension (Unger et al., 2020) suggested healthy lifestyle choices as major preventative strategy to delay the onset of hypertension in the individuals (Piepoli et al., 2016). Among healthy lifestyle choices, dietary diet plays an important role as whole grains, vegetables, fruits, and low-fat dairy foods, fish, nuts, beans have shown protetive functions against chronic hypertension (Xu et al., 2017). Specifically, higher intake of dietary polyphenols as part of daily whole plant-based diet is considered to have diverse NCD-linked health benefits such as anti-hypertensive, antioxidant, anti-hyperglycemic and anti-inflammatory functionalities. Furthermore, Dietary Approaches to Stop Hypertension (DASH) is highly recommended by National Cancer Institute (NCI) and American Heart association (AHA) to prevent and manage hypertension related disease risks (Bellows and Moore, 2013). This recommended DASH diet includes higher portion of fruits and vegetables and smaller portion of saturated fat. Several previously published studies have found positive impacts and benefits of consuming healthy diet

containing increased amount of fruits and vegetables for reducing blood pressure and subsequently preventing CVD (Du et al., 2016; Tsai et al., 2013; Wang et al., 2012). Wang et al. (2012) found a beneficial health impact of regular and higher consumption of fruits and vegetables on hypertension among 28,082 US health professional (women aged > 39 years). This large-scale cohort study was conducted over 12.9 years and observed a positive correlation between a higher intake of fruits and vegetables combined with regular physical activity and moderate prevention of hypertension among participants. Similarly, a population-based study in Brazil showed an inverse relationship between intake of plant-based foods rich in polyphenols and prevalence of hypertension among 2,691 individuals. The results of this study showed importance of a higher intake of plant-based foods, especially foods that are rich in alkylphenols, tyrosols, stilbenes, and lignans to prevent chronic hypertension (Miranda et al., 2016). The hypertension relevant benefits of a phenolic enriched diet on lowering blood pressure was explained by several researchers through different mechanisms which includes antioxidant and anti-inflammatory properties (Miranda et al., 2016), vasodilation (Suter et al., 2002), improving insulin sensitivity (Suter et al., 2002; Zhou et al., 2014), changes in plasma renin-angiotensin activity and inhibition of angiotensin-I-converting enzyme (Chen et al., 2012), decrease in blood velocity (McCarty, 2002), and positive influence on gut microbiome (Marques et al., 2017). All these beneficial functions of phenolic bioactives of plant-based foods are relevant for reducing the risks of developing hypertension and other NCDs.

1.4.6. Human Gut Health Benefits

Improved human gut health is essential for maintaining human health and wellbeing. Overall, microbiota present in the human gut, especially in the gastrointestinal (GI) tract is directly involved in the absorption of nutrients from consumed food and provides protection

against enteropathogenic organisms. Furthermore, several beneficial microorganisms help in metabolic degradation of consumed plant-based foods rich in phytochemicals and improve their bioavailability and functionalities (Russell and Duthie, 2011). Human gut microorganisms help to degrade complex secondary metabolites present in plant-based foods such as anthocyanins, procyanidins, flavonols, flavanones, isoflavones, and tannins to simple phenolic compounds. This degradation process of complex polyphenols to simple phenolic compounds offers better absorption of phytonutrients from various foods and improves human health relevant functionalities of phenolics including NCD-linked health benefits. Beyond their role in phenolic degradation, improved gut microbiota is also related to several human relevant functions including synthesis of vitamins, biotransformation of vital bile acids to maintaining cholesterol homeostasis, metabolization of certain dietary residues, oligosaccharides, and polysaccharides such as resistant starches, cellulose, hemicellulose, pectin and gums, fermentation of proteins, carbohydrates, lipids, improved dietary uptake of choline, and improvement in host protection and immune system development and maintenance (Bull and Plummer, 2014; Krishnan et al., 2005). Therefore, improving the gut microbiome is important for diverse metabolic functions, especially to reduce the risks of diet and lifestyle-linked NCDs. Overall, plant-based foods rich in dietary fiber, minerals and phenolic bioactives promote and improve the composition of beneficial microorganisms in the human gut and subsequently support critical metabolic regulations of human body. Also, improving nutritional and post-harvest preservation qualities of plant-based foods, especially fresh foods is critical for enhancing overall accessibility and affordability of these foods. Plant phenolics with high antioxidant property play and important role in improving nutritional qualities and post-harvest preservation qualities of highly perishable and human health-relevant fresh foods such as fruits and vegetables.

1.5. Role of Plant Phenolics to Improve Post-Harvest Preservation Qualities of Fresh and Healthy Foods

Globally, one third of agricultural fresh produce is unavailable for human consumption due to improper pre-harvest handling and post-harvest spoilage during storage, which accounts for 1.3 billion tons of wasted food every year. The economic cost of total food loss and spoilage is estimated at USD 310 annually, especially in developing countries (FAO, 2018). As most of these fresh and perishable foods are considered as an antidote to common NCDs, improving post-harvest preservation of these important agricultural products are essential to address food insecurity and related public health challenges (FAO, 2019). Several factors are involved in post-harvest spoilage of fresh produce including physical, physiological, biochemical (enzymatic and non-enzymatic), and microbial reactions. Additionally, mechanical damage like bruising, tearing, and puncturing during harvest and post-harvest stages can cause abnormal internal tissue damage and associated physiological and metabolic changes such as increase in respiration and transpiration rate, higher rates of dehydration, contamination and subsequent decay by fungal and bacterial species (Toivonen, 2010). Many of these tissue damages also lead to an increase in production of free radicals and oxidative breakdown, which affects nutritional, and sensory properties of stored fresh food. In this context, natural antioxidant compounds (phenolics and vitamins) such as thymol, eugenol, oregano, methyl jasmonate, cinnamaldehyde, ascorbic acid, vitamin C, and vitamin E are widely used as post-harvest treatment to prevent oxidative breakdown and redox imbalance in stored fresh foods. Additionally, these phenolic compounds also have antimicrobial properties and can protect fruits, vegetables and whole grains from microbial spoilage (Araya-cloutier et al., 2018, Gassara et al., 2016). However, excessive presence of highly oxidized phenolics in fruits and vegetables can lead to enzymatic browning

due to polymerization of free phenolics by phenolpolyphenol oxidase (PPO) in the presence of oxygen (Boekel et al., 2010). Higher content of procyanidins, monomeric and oligomeric forms of catechins in fresh plant-based foods generally leads to increased enzymatic browning when compared to other phenolic compounds (Lee and Jaworski, 1988; Radi et al., 1997). Whereas less oxidized phenolics can act as antioxidants and help to prevent oxidation reaction by quenching free radicals. Exogenous applications of natural antioxidants such as phenolics can also induce protective endogenous defense responses such as upregulation of redox-linked pentose phosphate pathway (PPP) and by stimulating associated antioxidant enzyme responses (Sarkar and Shetty, 2014; Shetty, 1997). Therefore, protective metabolic regulation of PPP is critical for biosynthesis of less oxidized phenolics and to enhance an antioxidant defense response for countering oxidative breakdowns and associated spoilage of fresh plant-based foods at post-harvest storage. The same metabolically driven strategy for enhancing phenolic bioactive content of plant-based foods is also relevant for improving nutritional qualities and human health relevant functionalities, especially NCD-linked health benefits of plant-based foods.

1.6. Role of Redox Protective Plant Phenolics in Wound-Healing Response of Damaged Plant Tissues

Addressing post-harvest spoilage and losses of nutritionally rich fruits and vegetables is extremely relevant for advancing food and nutritional security solutions. Wounding or mechanical damages to fresh fruits and vegetables during harvest, post-harvest storage, and transportation is a serious post-production challenge that not only just affect shelf-life and post-harvest preservation qualities, but also can cause significant deterioration of nutritional qualities of plant-based foods (Hodges et al., 2008). Potato, which is the leading vegetable/tuber crop in the world, is extremely susceptible to post-harvest wounding and subsequent spoilage (Reyes

and Cisneros-Zevallos, 2003). In general, wounded plant tissues such as damaged potato tubers follow endogenous defense responses by initiating a series of metabolic regulations and essential tissue structural adjustments (Lulai and Neubauer, 2014). In this wound-healing response, particularly for suberization processes, less-oxidized phenolic compounds including suberin polyphenolics (SPP) play critical biological roles and protect the wounded cell layer from further abiotic and biotic stress-induced damages (Lulai et al., 2016a, 2016b). Therefore, advancing metabolically-driven strategies to improve biosynthesis of protective phenolics through stimulation of redox-linked defense pathways, such as the pentose phosphate pathway (PPP) and associated anabolic responses has significant merit (Shetty and Wahlqvist, 2004; Sarkar and Shetty, 2014). The improvement of phenolic biosynthesis and associated antioxidant enzyme response is also relevant for enhancing human health relevant nutritional qualities of tuber crops such as potato and sweet potato.

1.7. Importance of Phenolic Bioactive Rich Sweet Potato as a Model Crop for Health-Focused Dietary Solutions

Sweet potato is a widely grown tuber crop with high adaptability to diverse climate and soil conditions. Due to such high resilience against extreme climate and soil, sweet potato is found in almost all continents and is an ideal choice to be targeted as part of climate change adaptation-linked food security solutions globally. In addition to wider adaptability to climate change, it also offers nutritional security solutions due to rich profiles of diverse bioactive compounds, essential minerals, and is a rich source of dietary fiber [(International Potato Centre) CIP, 2014]. Furthermore, sweet potato is a low resource input crop with high market opportunities, especially for developing value-added food products as well as for fresh cooked food for direct consumption and therefore potentially relevant to improve livelihood and incomes

of marginal and small farmers (CIP, 2018). The nutritional benefits of sweet potato are associated with its carbohydrate composition (resistant starch), dietary fiber profile, and high concentration of human health relevant bioactives (Kusano and Abe, 2000). In terms of human health relevant bioactives, sweet potato is rich source of beta carotene, anthocyanins, and other phenolic bioactives. Previous studies have shown benefits of beta-carotene rich sweet potatoes to combat night-blindness and malnutrition both in children and adults (Van Jaarsveld et al., 2005). Due to the superior micronutrient profile as well as easy availability and affordability, sweet potatoes become an integral part of a healthy diet for children and pregnant women in sub-Saharan African countries (Low et al., 2017). Additionally, sweet potato tubers contain indigestible sugars such as raffinose, verbascose, and stachyose that can support beneficial bacteria, which are therefore relevant for developing functional foods and beverages with prebiotic benefits (Koubala et al., 2014). The amylose content in sweet potato tubers is higher than amylopectin content when compared to potatoes, which is beneficial for postprandial blood glucose control and relevant for dietary support strategies against early stages of type 2 diabetes. Furthermore, sweet potatoes are recognized as superfood in terms of presence of nutraceuticals components, which showed antidiabetic, antioxidant, anticancer, anti-hypertensive, anti-ulcer properties along with beneficial role against cardiovascular diseases and immune system (Pochapski et al., 2011).

Therefore, the primary aim of this dissertation was to explore such bioactive nutrients-linked human health-relevant functionalities targeting healthy food design for expanding the uses of sweet potato as a functional food and to strengthen sweet potato production and processing industries globally. A secondary aim is to explore the relevance of related phenolic-linked

antioxidant functionality in wound healing in a model tuber crop such as potato as tuber model to then extend the wound healing rationale to all tuber crops.

CHAPTER 2. REVIEW OF LITERATURE

2.1. The Role of Vegetables in Health-Focused Dietary Design

Vegetables with high health protective compounds are important components of a heathy dietary pattern and can be rationally integrated in dietary interventions to reduce the risks of obesity, cancer, diabetes, stroke and heart disease. Lower consumption of complex carbohydrates, dietary fiber and health protective bioactives have been attributed as one of the primary health risks causing nearly 2.7 million NCD-linked deaths annually worldwide (WHO, 2017). Therefore, a higher proportion of diversity of vegetables in the daily diet is recommended to improve the daily consumption of dietary fiber, essential minerals, and health promoting bioactives and to reduce NCD-associated morbidity and mortality (Dias and Ryder, 2011). Higher consumption of vegetables has diverse health protective functions like improving glucose metabolism, human gut health, satiety, managing dyslipidemia, and improving cardiovascular health. Due to such wider health benefits, vegetables and vegetable-based food ingredients are ideal targets that can be integrated in functional food and beverage design, especially for NCD-linked food solution strategies.

2.2. Vegetable-Based Functional Foods and Beverages

Functional foods and beverages are described as a modified food that provide additional human health benefits beyond the basic nutritional needs when compared to conventional foods. The term “Functional” is widely used to cover a range of foods developed through food fortification and nutrient enrichment by adding specific health relevant dietary component such as omega-3-fatty acids, dietary fibers, minerals, and vitamins. The demand and market value of functional foods and beverages have increased exponentially over past few years. In 2017, the global functional foods market was estimated as $300 billion which is expected to grow to $ 440

billion by 2022 (Shahbandeh, 2018). Among different functional foods and beverages, a higher market trend was observed in probiotic beverages due to their favorable flavor and taste profile and higher consumer preferences. Due to such growing demand, food industries are also focusing on designing functional foods and beverages and developing high value products based on higher consumer preferences. Biotransformation of plant-based substrates, such as whole grains, fruits and vegetables using beneficial bacteria is one of the most common and effective strategies which is becoming extremely popular among food industries to develop probiotic functional beverages (Gurakan et al., 2009). In general, probiotics are described as beneficial microorganisms which confer beneficial effects to the host body such as increase in gut microbiome and subsequently enhancing immune responses, prevention of allergies, and reduction of level of serum cholesterol, risk of colon cancer, inflammable bowel disease symptoms, and lactose intolerance symptoms (FAO/WHO, 2002). Among different beneficial microorganisms, lactic acid bacteria (LAB) such as *L. plantarum*, *L. acidophilus*, *L. casei*, *L. rhamnosus* and *Bifidobacteria bifidum* are widely used both in traditional conventional foods and beverages and in functional beverages. In recent years, novel functional food and beverage design has targeted these beneficial LAB to transform plant-based substrates like vegetables, fruits and whole grain for enrichment of human health relevant bioactives and to enhance associated functionalities. Such novel food and beverage products include whole grain probiotic liquids, fruit juices, and vegetable blends (Saarela, 2009). Overall, vegetables are considered as excellent substrates for production of vegetable-based probiotic food products due to their excellent prebiotic properties by promoting growth of beneficial bacteria in the functional food and beverage matrix (Bernal et al., 2017). Several recent studies have highlighted the suitability of vegetable-based substrates for LAB-based fermentation and to improve bioactive-linked

human health relevant functionalities in probiotic food and beverages (Lavermicocca et al. 2005; Mallik et al. 2019; Yoon et al. 2004). Therefore, targeting a LAB-based fermentation strategy in combination with other post-harvest processing optimization for designing vegetable-based functional foods and beverages for NCD benefits-linked food solution has significant scientific merit and public health relevance.

2.3. Strategies to Improve Human Health Relevant Bioactives and Associated Functionalities of Vegetables and Vegetable-Based Foods

2.3.1. Rapid Screening of Vegetable Cultivars for Health Relevant Bioactives and Associated NCD-linked Functionalities

Plant-based foods that are rich in bioactives can be incorporated into new dietary interventions to prevent or delay emerging NCDs. However, the bioactive profiles and associated human health relevant functionalities of plant-based foods vary widely between varieties/cultivars, growing conditions, and based on different agricultural practices. Furthermore, post-harvest storage condition and food processing also influence the nutritional qualities and bioactive profiles of plant-based foods. Modern breeding strategies for developing new food plant cultivars mostly focus on improving yield and resistance to different pests, while largely neglecting the nutritional qualities of plant-based foods. The challenge for breeders is to optimize the critical distribution of photosynthate (carbon) between primary and secondary metabolism for maintaining good productivity, while also enhancing nutritional and end use quality of plant-based foods such as vegetables. Recently, vegetable breeders have been able to enhance the nutritional content of certain vegetable crops like beta-carotene and anthocyanin in sweet potatoes and essential amino acid composition in potatoes (Chakraborty et al., 2000). Additionally, biofortification to improve nutritional content of sweet potatoes have gained

greater attention around the world for food and nutritional insecurity solutions to millions of malnourished children, especially in the African continent and several developing countries. In this context, orange fleshed sweet potato cultivars rich in beta-carotene which mainly act as provitamin-A sources were developed and targeted to address early age blindness and malnutrition of children in Africa and Asia (Van Jaarsveld et al., 2005). Similarly, different colored cauliflower cultivars were developed for enhancing different micronutrients that are critical to address many food and nutritional insecurity associated public health challenges (Kalisz et al., 2018). However, it is also important to advance other strategies to screen and select existing vegetable cultivars with superior bioactive content and associated health benefits. Bioactive profile and associated nutritional qualities vary widely between different vegetable cultivars, so it is important to screen them based on metabolically-linked human health benefits prior to incorporating in dietary interventions and functional food designs. Therefore, metabolically-driven rapid screening of superior vegetable cultivars for high bioactive profile and associated human health relevant functionalities such as antioxidant, anti-hyperglycemic, and anti-hypertension properties is an effective strategy for health-focused food solutions targeting NCD-linked benefits.

2.3.2. Biotransformation Strategy-Fermentation

Advancing fermentation-based biotransformation by recruiting beneficial microorganisms in order to improve nutritional attributes coupled with organoleptic characteristics of plant-based food substrates is also an effective post-harvest strategy in the current context of emerging NCDs. Specifically, during the metabolic breakdown of plant-based substrates, beneficial microbes not only release products of fermentation (lactic acid and ethanol) but also improve bioavailability of other useful products like phenolic bioactives, peptides,

minerals, and enzymes (Machdo et al., 2004; Robison et al., 2001). A specific example is the bioconversion of glycosides to aglycones, a fermentation-linked metabolic breakdown of higher molecular compounds to lower molecular compounds (Joo et al., 2009). The bioconversion and subsequent improvement in bioavailability of specific compounds also improve human health related functionalities such as antioxidant, anti-inflammatory, anti-hyperglycemic, and anti-hypertensive properties in fermented foods and beverages (Liao and Wu, 2015; Rodriguez et al., 2009). Additionally, such a beneficial microorganism-based biotransformation improves shelf life, nutritional qualities, sensory qualities, and, organoleptic properties of bioprocessed plant-based foods and beverages. These fermented foods and beverages are especially important to improve human gut health due to the potential probiotic benefits. Therefore, the controlled fermentation process by recruiting different kinds of beneficial LAB species and strains such as *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, and Bafidobacterium longum* can be advanced to improve post-harvest preservation qualities and human health relevant nutritional qualities in plant-based foods such as fruits, vegetables and whole grains. Among these different beneficial LAB strains, *Lactobacillus plantarum* is widely found in the natural plant and plant-based food matrix and can be targeted to develop high-value fermented foods and beverages with probiotic and other human health benefits (Rodriguez et al., 2009). Previously, *Lactobacillus plantarum* –based fermentation was used to improve physicochemical properties of sweet potato starch and qualities of noodles derived from fermented sweet potato (Liao and Wu, 2015). Similarly, Yuliana et al. (2017) reported improvement of overall physicochemical properties of sweet potato flour after fermentation with beneficial LAB. Therefore, beneficial –LAB-based transformation of vegetables provides an excellent tool to improve human health relevant functionalities and to

design unique functional food and beverages from superior vegetable cultivars with a high bioactive profile.

2.3.3. Processing Optimization

Like the importance of selecting successful vegetable cultivars and advancing novel biotransformation strategy, optimization of post-harvest processing and cooking strategies is also essential for retaining and improving nutritional qualities of vegetable-based foods. In raw and fresh vegetables, a significant part of health relevant bioactive compounds remains in bound form, which are not always bioavailable when consumed as unprocessed and uncooked foods. Therefore, many vegetables need to go through processing and cooking prior to their consumption, especially to obtain their optimum health benefits. Several food processing strategies such as boiling, steaming, baking, microwave cooking, and frying can help to release of embedded bioactives and can enhance human health relevant functionalities such as antioxidants, anti-diabetic, and anti-hypertensive properties. However, certain food processing methods could lead to loss of nutrients including phenolic compounds as stability of such bioactives vary widely between different cooking temperatures, pressures, and due to rapid structural alterations of food matrix (Faller and Fialho, 2009). As food processing and cooking induce such physical and chemical changes in food the process sometimes leads to detrimental human health effects (Rehman et al., 2003). Therefore, optimization of food processing conditions is important for improving nutritional qualities and health benefits of vegetable-based foods and food ingredients. In this context, response surface methodology (RSM) is an effective statistical model to optimize processing conditions of food products under controlled experimental set-up. Additionally, the RSM model also helps to explain the relationship between independent (temperature, time, sample size) and dependent variables (quality determining

attributes of product) related to processing parameters in the experimental design (Olawoye, 2016). Such processing optimization strategy is an effective tool and can be targeted for designing novel vegetable-based food with higher nutritional qualities and human health benefits.

2.4. Sweet Potato: To Address Nutritional Insecurity-Linked Public Health Challenges

Food and nutritional insecurity-linked public health challenges, especially emerging diet and lifestyle-linked NCDs are the foremost global health challenge of the 21st century. In the Global Burden of Disease study, health effects of dietary risks in 195 countries between 1990 and 2017, and several dietary risk factors for NCDs, such as diets low in whole grains, vegetables, and fruits, in combination with high processed foods and sugar enriched beverages were assessed (Bisanzio and Shokraneh, 2018). The study concluded that a well-balanced and whole plant-based diet can reduce many of these NCD-risk factors, while also protecting and promoting human health and well-being (Dias and Ryder, 2011). Specifically, a whole plant-based diet with higher food diversity can reduce the risk of heart diseases hypertension, type 2 diabetes, dyslipidemia and some cases of cancer while also helping to address global food and nutritional insecurity challenges.

Sweet potato (*Ipomoea batatas* L.) is one of the most nutritionally rich root crops that can be integrated in health-focused dietary support strategies to address global nutritional insecurity and related public health challenges (Truong et al., 2018). Additionally, sweet potato is also resilient to constant environmental variations and suitable for growing in warmer tropical regions around the world. Due to such higher climate stress resiliency, it can produce tubers during extreme weather conditions and can serve as a main nutritional source when other food plants fail to survive. One such example was during 1999 flooding in Odisha, India, where sweet potato

was a major food and nutritional source for people who faced severe food scarcity, nutritional insecurity, and related public health challenges (Mukherjee et al., 2015). Similarly, stored sweet potatoes also helped to address food scarcity and nutritional insecurity challenges in cyclone affected areas of Philippines (CIP, 2014). Due to their dual benefits as climate resilient and having high nutritional qualities, the CIP research program has selected sweet potato as an important plant-based food source for nutritional insecurity-linked public health solutions in Africa and Asia.

To achieve this goal, dozens of nutritionally rich orange-fleshed sweet potato (OFSPs) cultivars were developed and distributed under a community based agri-food systems program in African and Asian countries (CIP, 2019). These pro-vitamin-A rich orange fleshed sweet potatoes were specifically targeted to improve vitamin-A deficiency of 140 million children under the age of 5 years in Sub-Saharan and Saharan Africa. Under this program, 6.5 million rural households achieved partial nutritional security. The success of this program also led to the development of strategic plan for improving agricultural income by 15 % and nutritional quality of the daily diet by 20 % to 15 million resource-poor rural people by 2023 (CIP, 2019). The nutritive value of sweet potatoes is based on diverse health relevant bioactive compounds and associated functional benefits like antidiabetic, antioxidant, anti-hypertensive, and anti-ulcer properties (Pochapski et al., 2011). Additionally, sweet potatoes are also rich sources of dietary fiber, anthocyanin, beta-carotene, copper, vitamin $B_{6,}$ and essential micronutrients such as potassium and magnesium (USDA, 2014).

China is the leading producer of sweet potato with 80% of world production, followed by Uganda and Nigeria (around 3%) (FAOSTAT, 2014). Although it is cultivated widely across the world, the global trade and market of sweet potato is only 1 % with Canada, United Kingdom,

Netherlands and Japan as major importing nations (FAOSTAT, 2012). Overall, sweet potatoes are considered as low-calorie foods with average caloric value of 90 calories/100g as compared to 70 calories/100g of potatoes *(Solanum tuberosum).* Most of the sweet potato production is used for human nutrition with small percentage going for industrial uses and cattle feed. Besides the tuberous roots of sweet potato, the fermented vines are also used as silage and waste byproducts for poultry feed in many Asian countries such as Indonesia, China and Vietnam due to their high-protein profile (Sheikha and Ray, 2017). However, due to diverse human health relevant nutritional qualities, the interest in sweet potato as a high value specialty crop is increasing rapidly, especially for health-focused food solution strategies.

2.5. Wider Use of Sweet Potato in Diverse Food-Related Applications

There has been a growing interest towards the colored flesh tubers of sweet potato for different industrial applications, such as purple-fleshed sweet potato tubers rich in anthocyanin content has been targeted by cosmetic industries as well as by food coloring industries (CIP, 2020). Additionally, high-value food and food ingredient applications of sweet potato such as starch derived industrial products, dried chips, bread, candy, juice, noodles, pasta, baby foods, sauces, and gravy have been explored by food industries worldwide (CIP, 2019). The major reason for processed utilization of sweet potato tubers in developing countries of Africa and East Asia is due to the lower shelf-life and high perishability of this crop. The fried items of sweet potato such as fried chips, crisps, and French fries also has high demand in Nigeria, United States, Japan, India, and Peru for their unique flavor and taste. A consumer preference study by Caetano et al. (2018) revealed higher acceptability of deep-fried products with greater purchase intention of consumers over oven-baked and air fried sweet potato chips. These processing strategies and post-harvest preservation are found essential to extend shelf life of harvested

tubers as well as to preserve health relevant bioactive compounds. Therefore, optimizing sweet potato processing strategies based on their bioactive profile and health relevant nutritional qualities has significant merit, especially for high-value food application.

2.6. Sweet Potato Bioactives

The nutritional and human health benefits of sweet potatoes are mainly due to the presence of diverse bioactive compounds such as flavonoids, alkaloids, coumarins, anthocianosides, glycolipids, triterpenes, and soluble phenolics (Jung et al., 2011). When consumed as part of a diet, these bioactive compounds of sweet potatoes could provide many physiologically and metabolically relevant functions such as protection against allergies, cardiovascular risks, asthma, stomach distress, type 2 diabetes, ageing-related disorders, and select types of cancers (Duke and Wain, 1981) (Figure 2.1). Sweet potatoes with orange flesh are considered as rich sources of beta-carotene, which are good sources for provitamin-A (100 g sweet potato tubers give 14187IU of vitamin A and 8509μg of β-carotene). The purple fleshed sweet potatoes have intense purple color in their tubers which indicates the presence of higher amounts of anthocyanins, which showed strong antioxidant activity in *in vivo* studies with mice, rats, and rabbit models (Jawi and Budiasa, 2011) as well as strong antimutagenic activities. Suprapta et al. (2012) has reported hypoglycemic and antioxidant activities of water extract of purple fleshed sweet potato in a diabetes induced rat model study. Even white fleshed sweet potatoes displayed high antioxidant activity and can be integrated in dietary strategies to counter chronic oxidative stress and associated health risks. Therefore, different flesh colored sweet potato cultivars have different human health relevant functional properties based on their bioactive and nutritional profile. Due to such wider variation in nutritional qualities, it is

important to screen and select cultivars with an optimum bioactive profile, especially for specific health targeted food applications.

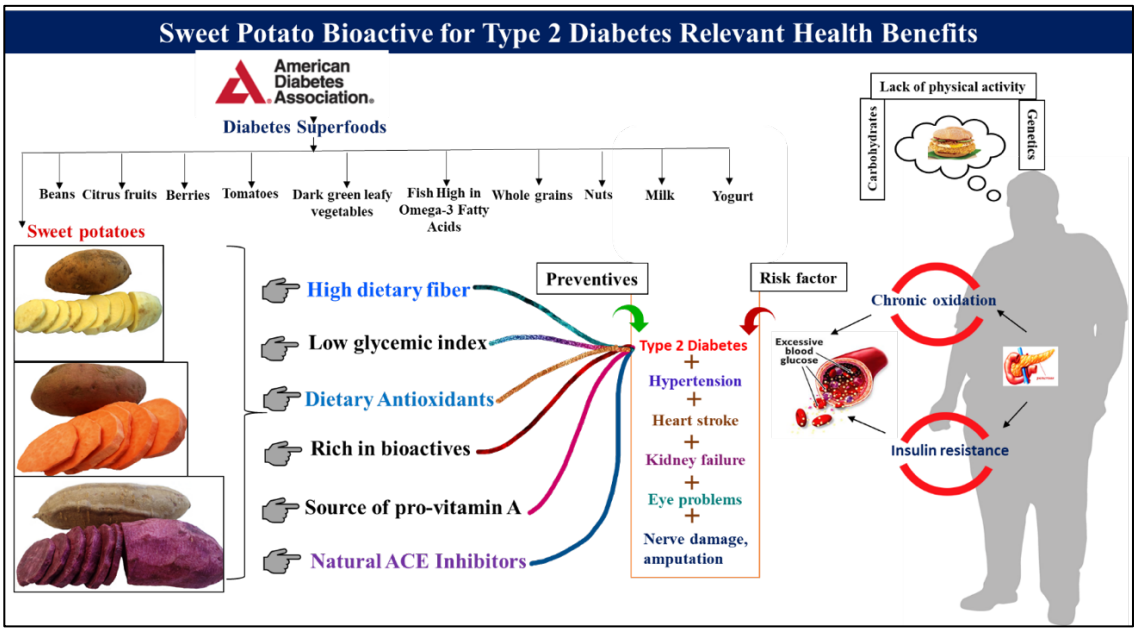

Figure 2.1. Human health benefits of sweet potatoes, specifically to reduce NCD-linked health risk factors.

2.7. Health Benefits of Sweet Potato

2.7.1. NCD-Linked Health Benefits

2.7.1.1. Anti-Inflammatory

Chronic oxidative stress is considered as a main cause for inflammation due to excessive production of highly reactive free radicles (Masaki, 2010). Plant-based dietary antioxidants are a safe and inexpensive source to counter chronic oxidative stress and associated inflammation (Mark, 1998). Among different plant-based food sources, sweet potatoes are a rich source of natural and dietary antioxidants, which can be integrated in dietary and therapeutic interventions to manage oxidative stress associated inflammation (Jung et al., 2011). Additionally, these bioactive compounds of sweet potato with high antioxidant potential can enhance human immune response, which also has potential to counter chronic inflammation and associated damages. Based on this rationale, sweet potatoes have been studied extensively for their anti-

inflammatory and select anti-cancer properties (Pochapski et al., 2011). Among different flesh colored sweet potatoes, purple fleshed cultivars received greater attention due to presence of anthocyanin compounds such as cyanidin, peonidin, and acylated derivatives. Overall, anthocyanins are widely studied for their pharmacological properties such as antioxidative (Jawi and Budiasa, 2011), anticancer (Wang et al., 2006), anti-inflammatory (Kang et al., 2014; Sugata et al., 2015), and anti-neurodegenerative functions (Wang et al., 2010). Recent studies with purple fleshed sweet potato Tainung 73 showed potential anti-inflammatory and anti-cancerous properties against human breast cancer, colon adenocarcinoma, and gastric cancer cells (Sugata et al., 2015). Similarly, Kang et al. (2014) observed a protective role of purple fleshed sweet potato leaf extracts on neuroinflammatory responses. This research finding of potential anti-neuroinflammatory effects of purple fleshed sweet potato were potentially due to its strong antioxidant activity and production of proinflammatory cytokines. In another study, neuroprotective effects of purple sweet potato extract were found in an LPS (lipopolysaccharide) treated mouse behavioral model, which indicated inhibition of proinflammatory molecules by sweet potato bioactives (Wang et al., 2010). Therefore, sweet potato and sweet potato based functional foods are a potentially safe dietary and therapeutic choice that can be targeted for countering chronic inflammation and associated NCDs, such as type 2 diabetes, obesity, and CVD.

2.7.1.2. Anti-Hyperglycemic

Glucose homeostasis is critical for keeping the blood glucose level less than 180 mg/dL (postprandial plasma glucose), which is essential for preventing hyperglycemia and subsequent development of type 2 diabetes (ADA, 2020). Any imbalance in glucose homeostasis results in either hypoglycemic (blood glucose level < 70 mg/dL) or hyperglycemic (blood glucose level >

150 mg/dL) conditions (ADA, 2020). Among two conditions, hyperglycemic condition is the most common cause for development and pathogenesis of type 2 diabetes. Chronic hyperglycemia for a prolonged period could lead to damage to vital organs such as the heart, liver, eyes, kidneys, and pancreas. In general, glucose molecules absorbed in the blood after digestion of food supply energy to every cell in the body with the help of peptide hormone insulin secreted by pancreatic cells (Wilcox, 2005). Insulin plays a pivotal role in carbohydrate metabolism along with other hormones like glucagon, glucocorticoids and catecholamines (Wilcox, 2005). Imbalance in production and impaired function of these hormones affects the glucose homeostasis resulting into chronic hyperglycemic. Additionally, chronic oxidative stress also affects pancreatic β-cells and their efficiency to produce insulin (Asmat et al., 2016). Therefore, imbalance between free radical generation and the endogenous antioxidant defense system lead to chronic oxidative stress, which is an underlying risk for chronic hyperglycemia (Bajaj and Khan, 2012). Therefore, plant-based foods rich in dietary antioxidants, such as sweet potato can be targeted for improving antioxidant defense to counter chronic oxidative stress and associated hyperglycemia (Bajaj and Khan, 2012; Hamilton et al., 2007). In addition to their antioxidant function, sweet potato bioactives such as phenolics are also relevant for direct anti-hyperglycemic function by inhibiting enzymes involved in carbohydrate metabolism. Such dual functional health benefits of sweet potato bioactives are important for their effective integration in dietary and therapeutic interventions to counter chronic hyperglycemia and chronic oxidative stress induced early stages of type 2 diabetes. Suprapta et al. (2012) observed antioxidant and anti-hyperglycemic properties in aqueous extracts of purple sweet potato on oxidative stress induced rats. This study suggested that anthocyanin rich purple sweet potato is a good dietary source for reducing blood glucose levels after a high oral dose of glucose in rat model.

Additionally, positive health effects of sweet potato antioxidants in preventing production of advanced glycation end products as a resultant of glucose oxidation with different proteins were also observed (Jawi et al. 2012; Maritim et al., 2003). In another study, antidiabetic activity of white skinned sweet potato was found in obese Zucker fatty rats, specifically by suppressing insulin resistance in rats while improving their carbohydrate and lipid metabolism (Kusano and Abe, 2000). Kusano et al. (2001) identified acidic glycoprotein of sweet potato as a main compound for suppressing insulin resistance and maintaining glucose homeostasis in rat models. Similarly, a white fleshed sweet potato, Caiapo showed promising effect on reducing cholesterol level and both fasting and postprandial plasma glucose levels in a clinical study with type 2 diabetes patients (Ludvik et al., 2004). Therefore, all these studies indicated that sweet potato is an ideal vegetable target to manage chronic hyperglycemia and chronic oxidative stress, which are commonly associated with type 2 diabetes pathogenesis.

2.7.1.3. Anti-Hypertension

Hypertension or high blood pressure is a major health risk factor for heart, kidney, type 2 diabetes, and eye diseases. The WHO (2020) estimated 1.13 billion people with hypertension worldwide and it is considered as one of the most serious medical conditions causing premature death by affecting 1 in 4 men and 1 in 5 women in developing and under-developed countries. Undiagnosed chronic hypertension for prolonged time can cause unrecoverable damages such as bursting/blocking of blood vessels, which leads to organ failure including brain damage. The role of multifunctional enzymes such as angiotensin-I-converting enzyme (ACE) in blood pressure regulation is critical as it involves in the conversion of blood angiotensin I to angiotensin II during blood circulation, which narrows blood vessels by increasing pressure on heart and angiotensin II also contributes to blood pressure rise. Therefore, to treat high blood pressure,

several synthetic ACE inhibitors (captopril, fosinopril, moexipril, perindopril, ramipril, trandolapril) have been used in therapeutic strategy (Andrews et al., 1985). However, many of these synthetic ACE inhibitors have harmful side effects such as vascular dysfunction and kidney failure. Therefore, finding ACE inhibitors from natural dietary sources are important for safe and effective control of chronic hypertension. The National Cancer Institute (NCI) and American Heart association (AHA) have widely recommended using dietary approaches to manage hypertension and related health risks (Bellows and Moore, 2013). A whole plant-based diet is part of such healthy dietary approaches, which helps to reduce risk of heart disease, type 2 diabetes, hypertension, and other NCDs. In this context, many plant based foods have generated wider interest due to the presence of natural angiotensin I-converting enzyme (ACE) inhibitors, which are beneficial to relax blood veins and arteries and subsequently decrease blood pressure (Lee et al., 2006; Yang et al., 2003; Yoshimoto et al., 2001). Ishiguro et al. (2012) found ACE inhibitory activity in sweet potato juice in a hypertensive rat model-based *in vivo* study. In this study, ACE inhibitory peptides with anti-hypertensive potential were identified in sweet potato. Similarly, a decrease in systolic and diastolic blood pressure in hypertensive rats were observed after administration of purple sweet potato-based fermented milk for 8 weeks (Tsai et al., 2013). In this study, researchers observed an increase in microflora in the hypertensive rats after consumption of purple sweet potato-based fermented milk. Interestingly, population of beneficial and probiotic microorganisms such as *Bifidobacterium spp.* and *Lactobacillus spp.* were increased, while diarrhea causing gram positive bacteria *Clostridium perfringens* decreased significantly. In another study, probiotic rich fermented purple sweet potato yogurt containing different beneficial lactic acid bacterial strains (*Lactobacillus acidophilus* BCRC 14065; *L. gasseri* BCRC 14619; and *L. delbrueckii subsp. lactis* BCRC 12256) showed enhanced cardiac

survival and activation of anti-apoptotic pathways in hypertensive rats (Lin et al., 2013). Activation of anti-apoptotic pathways are critical to reduce blood pressure in hypertensive individuals. Therefore, fermented and non-fermented sweet potato-based foods can be targeted as a dietary support strategy to manage hypertension and to improve cardiac health. However, metabolically driven *in vitro* screening and future clinical studies are required for effective integration of sweet potato-based foods in dietary intervention strategies targeting anti-hypertensive benefits.

2.7.1.4. Human Gut Health Benefits

Human gut is one of the most densely populated parts of the body with thousands of different microbial colonies (Clark and Coopersmith, 2007), mainly dominated by bacteria, Eukarya, and Archaea (Hentges, 2012). Among these three types of organisms, bacteria represent more than 90 % of gut microbiota (Bachead et al., 2005). Gut microbiota colonize in the nutrient rich environment of the gut, however changes in physicochemical conditions of the gut can adversely affect the microbial population. Therefore, inhabitation and composition of gut microbiota of an individual is determined by several factors especially dietary habits, living conditions and age, antibiotic therapy, chronic illness, maternal microbiota, and mode of delivery during birth (Wall et al., 2009). For instance, newborn babies acquire microbial colonies through various ways and the process is described as very gradual and dynamic (Wall et al., 2009). Infants fed with breast milk have different microbiota profile (higher levels of *Bifidobacterium spp.*) then infants fed with formulas (*Lactobacillus spp*., *Bacteroides spp*. and *Clostridium coccoides*) (Fallani et al., 2012). Furthermore, significant diversification of the gut microbiome was observed in individuals based on their dietary patterns (Heiman et al., 2016). The study conducted by Devkota and Chang (2015) showed gut microbiome can reshape, groom, and

modulate based on dietary choices and existing host environment. Maintaining a good microbial population is not only important for improved human gut health, but it also has significant relevance for diverse NCD-linked health benefits. Therefore, improved gut health is critical for many metabolic regulations and physiological functions of human body. Many published studies have linked altered gut microbiota and subsequent deterioration of human gut health with increased risks of several common NCDs (Gille et al., 2018; Noce et al., 2019; West et al., 2015). In general, a dietary pattern rich in whole plant-based foods is considered beneficial for human gut health, as it favors colonization of beneficial microorganism. Among different food components, dietary fibers, micronutrients, and bioactives of plant-based foods are critical for nurturing good microbial population and improving human gut health. Most common vegetables, including sweet potatoes are rich dietary sources for gut health promoting food components. Therefore, targeting and integrating sweet potato in dietary support strategies to improve human gut health and for related NCD-linked health benefits has significant scientific merit. Such improvement of gut health using dietary approaches is specifically relevant for maternal and child health benefits.

2.7.1.5. Maternal and Child Health Benefits

According to estimates by WHO (2010), one pregnant woman perishes every minute due to pregnancy related complications. Most of these pregnancy related deaths are reported in developing countries, especially in rural and low-income regions (United Nations Children's Fund, 2008). Poor diet, physical inactivity, and unhealthy lifestyle habits during pregnancy increase many health-related risks in mothers such as obesity, gestational diabetes, and hypertension. In general, obese mothers are more prone to pregnancy related complications with short term and long-term effects on newborn babies (WHO, 2017). Similarly, poor diet and

nutritional imbalance can lead to several maternal health risks such as gestational diabetes, chronic respiratory diseases, and cardiovascular diseases (WHO, 2016). The long term effect of gestational diabetes on mother and pre-born child can be severe as it increases chances of developing type 2 diabetes later in life both for mother and baby, abnormal weight gain for babies (fetal macrosomia), premature and stillbirth, higher chances of respiratory diseases, and hypoglycemia (low blood glucose) during pre-natal developmental stages (WHO, 2009). Therefore, improving the overall health condition of women before and during pregnancy is essential for healthy delivery of baby and to reduce the risks of having any pregnancy-related complications. Balanced diet, especially adequate supply of diverse beneficial nutrients is essential for healthy pregnancy and post birth health of mothers and babies during their neo-natal developmental stage. Both quantity and nutritional qualities of foods have significant impact on pregnant women and on their children (Clapp, 2002). As carbohydrate-based foods, especially cereal grains are main source of nutrients for the majority of pregnant women worldwide, it is very important to select high-quality carbohydrates with low to medium glycemic indexes, as part of balaned meals for avoiding a postprandial spike of blood glucose and maintaining glucose homeostasis. Additionally, it is also important to integrate other non-carbohydrate-based foods such as nuts and dairy products for achieving nutritional adequacy and to reduce risks of common NCDs during pregnancy. Low to medium glycemic food includes non-starchy vegetables (broccoli, sweet potatoes, spinach, peppers, mushrooms, onions, tomatoes, cabbage, beetroot, avocados, dry fruits), whole grains (brown rice, wheat, oats,), legumes (peas, beans, lentil,) and low-fat dairy foods. Sweet potatoes are considered as a medium to low glycemic index food, which also have high antioxidant, anti-hyperglycemic, and anti-hypertensive relevant functional properties (Allen et al., 2012). Allen et al. (2012) reported medium glycemic index in

differently processed sweet potatoes, which includes baked (64 ± 4.3), microwaved (66 ± 5.7), and steamed (63 ± 3.6). Whereas, low glycemic index was reported in raw and dehydrated sweet potatoes (32 ± 3.0 and 41 ± 4.0). Additionally, sweet potatoes are excellent sources of health promoting nutrients, such as dietary fiber, anthocyanins, beta carotene, copper, vitamin C, vitamin $B_{6,}$ vitamin E and rich in mineral content such as calcium, potassium, magnesium, sodium, and iron (USDA, 2014). Among different flesh colored sweet potatoes, orange fleshed sweet potatoes are considered as good sources of β-carotene (a baked sweet potato tuber with skin contains 1400 mcg of Retinol Activity Equivalents (RAE)) (USDA, 2019). Consuming a whole orange fleshed sweet potato can help to meet daily recommended intake of vitamin A (750 mcg RAE for pregnant women and 1200 mcg RAE at lactation stage). Furthermore, intake of foods with higher amount of bioavailable β--carotene is recommended to prevent pregnancy related mortality and deficiency of vitamin A in both pregnant women and infants (xeropthalmia) (Van Den Broek et al., 2010). A long-term study showed significant increase in intake of vitamin A through regular consumption of orange fleshed sweet potatoes and subsequent reduction in risk of low retinol binding protein among pregnant women (Girard et al., 2017). Therefore, sweet potatoes rich in β—carotene and other bioactives are excellent dietary choice to improve nutritional adequacy, while also preventing diet-linked NCDs such as gestational diabetes, obesity and hypertension in pregnant women. Such dietary choices are critical for improving health and well-being of both mother and child.

2.8. Different Flesh Colored Sweet Potato: Cultivar Differences

Different flesh colored such as white, off-white, yellowish, yellowish-orange, orange, and purple colored sweet potatoes are widely available in different parts of world. The nutritional qualities and human health relevant benefits of sweet potatoes vary widely between different

flesh colored and among different cultivars. Sweet potato cultivars with white to light yellowish flesh colored tubers are believed to be less sweet and having higher moisture content when compared with dark fleshed cultivars (Milind and Monika, 2015). Similarly, sweet potatoes with yellowish orange to orange fleshed are considered as rich sources of β-carotene, which is relevant for reducing vitamin A deficiency and improving retinal health (Ambrosio et al., 2011). In general, Vitamin A plays a critical role in protecting eye health and cell membranes from lipid peroxidative damage. Deficiency of vitamin A is very common in South Asia and Sub Saharan Africa causing severe blindness, and increased mortality from measles and diarrhea. Despite the world-wide vitamin-A supplementation programs, prevalence of vitamin-A deficiency did not change much in several regions of the world (Stevens et al., 2015). Therefore, integrating orange fleshed sweet potato with higher bioavailability of vitamin A in dietary interventions is an effective strategy to improve vision and to fight malnutrition associated diarrhea and other infections. The purple fleshed sweet potatoes are rich in anthocyanins with high antioxidant potentials (Jawi and Budiasa, 2011), Therefore purple fleshed sweet potatoes have diverse human health benefits such as antioxidant, antimutagenic,(Oki, 2002), anti-diabetic, and anti-hypertensive properties (Ray and Tomlins, 2009) enhancing memory function (Wu et al., 2008), , and inhibiting the growth of select cancer cells (Wang et al., 2006). Not only just different flesh colored, but different cultivars from same flesh colored sweet potato also vary based on their total phenolic content, individual phenolic acid profile, and associated antioxidant properties. Therefore, screening of different flesh colored sweet potato cultivars (white, orange, and purple) for phenolic bioactive-linked antioxidant, antihyperglycemic and antihypertensive properties using rapid and inexpensive *in vitro* assay models has significant relevance for their effective integration in health-focused food solution strategies (Figure 2.2).

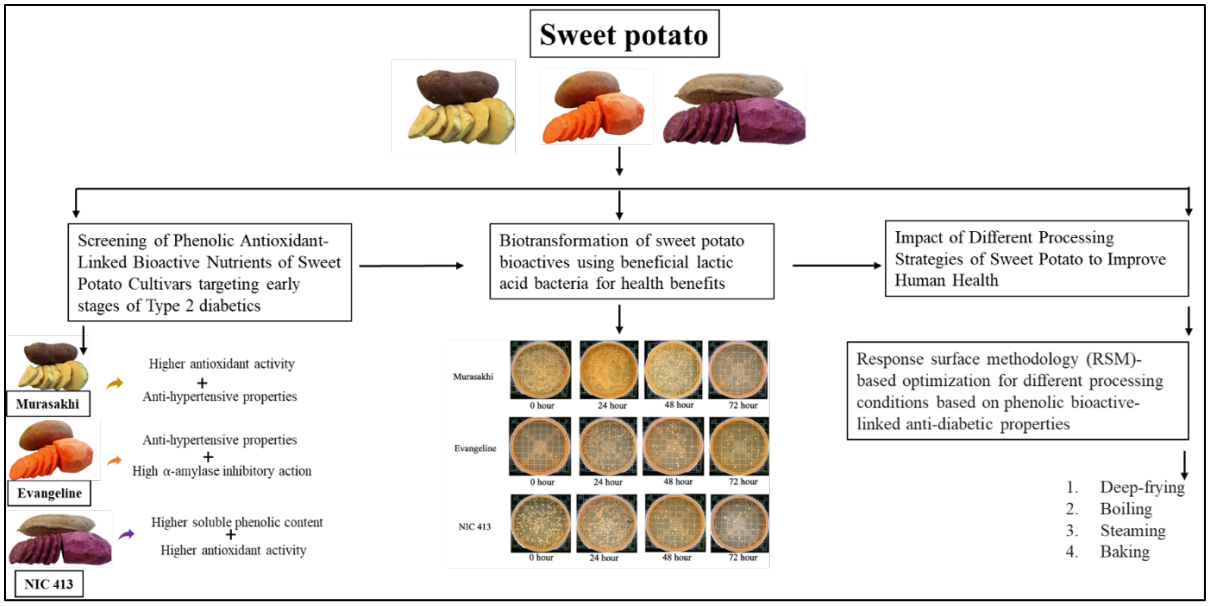

Figure 2.2. Metabolically driven cultivar screening and food processing optimization to improve human health relevant phenolic bioactives in sweet potatoes.

2.9. Biotransformation of Sweet Potato

In order to improve nutritional qualities coupled with organoleptic characteristics of plant-based food substrates, fermentation using beneficial and probiotic bacteria is an effective strategy. Fermented foods and beverages are becoming extremely popular among consumers due to several food and human health relevant attributes such as improved shelf life, enhanced nutritional, sensory, and organoleptic properties (Kabak and Dobson, 2011). Fermentation of many plant-based substrates also leads to release of bioactive compounds such as bound phenolics in food matrix and improve their bioavailability and functionalities (Acosta-Estrada et al., 2014). Additionally, it also helps in higher retention and stability of these health relevant compounds with controlled fermentation (Poutanen et al., 2009). Fermented foods and beverages are considered beneficial for human gut health due to the probiotic nature of fermenting bacteria (Acosta-Estrada et al., 2014). Not only just human gut health, fermented foods and beverages also support other metabolic regulations, which are essential for prevention and management of diet and lifestyle-linked NCDs (Sarkar and Shetty, 2018). Most of the plant-based fermented

foods and beverages are produced through controlled fermentation process by different kinds of beneficial bacteria, especially lactic acid bacteria strains such as *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, and Bifidobacterium longum*. Among these fermenting bacterial species, *Lactobacillus plantarum*, which is ubiquitous in natural plant and food matrix, are widely used to develop plant-based fermented foods and beverages (Rodriguez et al., 2009). Potential health benefits of *Lactobacillus plantarum* fermented sweet potato starch on physicochemical properties of flour and noodles derived from fermented flour was observed (Liao and Wu, 2015). Similarly, Yuliana et al. (2017) reported improvement of physicochemical properties of sweet potato flour after 7 days of fermentation with pickled brine and *Lactobacillus plantarum*. However, not many studies have investigated human health relevant bioactives and their optimum functionalities after controlled fermentation of sweet potato substrates. Therefore, recruiting beneficial LAB based fermentation of different flesh-colored sweet potato to optimize beneficial bioactive-linked nutritional qualities has significant merit. Such controlled fermentation strategy can help to design novel sweet potato-based fermented foods and beverages with superior nutritional profile and associated health benefits (Figure 2.2).

2.10. Processing of Sweet Potato

Sweet potato tubers are highly perishable due to their high moisture content (60-70%), low dry matter (Tomlins et al., 2010), and delicate cell membrane structure (Woolfe, 1992), which cause serious post-harvest loss during storage. Additionally, pre-harvest growing conditions, handling during harvest, and biochemical composition of tubers also influence post-harvest shelf-life of sweet potato. Therefore, post-harvest processing of sweet potato tuber is highly encouraged to improve shelf-life and for higher retention of nutritional qualities. In the

African countries, majority of harvested sweet potato tubers are processed into dried chips or flour for preservation and year-round consumption (Truong et al., 2018). Whereas, in Philippines tubers are processed as dried cubes which are later used in cooking with coconut milk, brown sugar, and rice for daily consumption (Miller et al., 1946). A special snack food 'Keropok' made from mixture of orange fleshed sweet potato flour and cassava flour are generally consumed with fish, prawn or squid paste in Malaysia (Padmaja et al., 2012). Similarly, sweet potato pickles are common in Thailand, Bangladesh, Philippines, and India. Additionally, sweet potato is also widely used as baby food in many countries due to balanced composition of macro and micro-nutrients, rich health protective bioactives profile, and their easy to digest attributes (Mayer et al., 2018). In countries like Nigeria, deep fried sweet potato products have high commercial value, as such processing improves shelf-life and minimize post-harvest losses (Odebode et al. 2008). Deep frying is described as high mass and heat transfer processing method, which leads to physical and biochemical changes in the fried product and potentially improves its aroma, flavor, taste, and textural appearances (golden brown) (Hindra et al., 2006). However, the effect of these processing methods on bioactive profile and nutritional qualities of sweet potato-based foods is largely unknown. Therefore, it is important to optimize such processing strategies and cooking methods based on the bioactive profile and associated health benefits for designing sweet potato-based functional foods and beverages and for its effective integration in health-focused food solutions (Figure 2.2).

2.11. Sweet Potato-Based Functional Food and Beverage Design

Sweet potato is fast-growing, short duration (3-4 months) tuber crop with abundant source of starch, high dietary fiber, and diverse bioactive compounds (Kusano and Abe, 2000). Additionally, sweet potato tubers contain several indigestible sugars such as raffinose,

verbascose, and stachyose, which promotes growth of beneficial microorganism and therefore beneficial to design probiotic foods and beverages (Koubala et al., 2014). Therefore, sweet potatoes are ideal targets to address both undernourishment and imbalanced nutrition related chronic diseases due to their unique nutritional profile, which includes high quality carbohydrates, dietary fiber, anthocyanins, beta-carotene, vitamins and essential minerals (USDA, 2014). The carbohydrate composition (amylose: amylopectin) of sweet potato is beneficial for postprandial blood glucose control and relevant against chronic hyperglycemia induced early stages of type 2 diabetes (T2D) (Bjorck et al., 1994). Additionally, unique nutritional profile of sweet potato is also relevant for other NCD-linked health benefits, and can be targeted as dietary antidotes against hypertension, cardiovascular disease, respiratory disease and retinal dysfunctions (Pochapski et al., 2011). Therefore, exploring such bioactive nutrients-linked human health-relevant functionalities targeting healthy food design would help to expand uses of sweet potatoes in functional food and beverage design.

Due to rapidly emerging diet-linked NCDs and overall improvement of consumer awareness on nutritional profile of different foods, the interest on vegetable-based processed and functional food is also growing rapidly. Such growing consumers' demand also forced food industries to find alternative food formulation and processing strategies and to advance innovation for novel integration of vegetable-based foods and food ingredients in traditional food formulations. In this context, sweet potatoes can be used in the preparation of breads, candy, noodles, juice, snacks, fufu, alcohol and diverse range of healthy beverages. There is also urgent need to develop culturally and ecologically relevant ethnic food models that will provide balanced nutrition and protection against diet linked chronic NCDs to communities facing higher economic and health disparities. To address such health disparities and food insecurities of

communities, functional and texture-modified foods from orange and purple fleshed sweet potatoes fortified with functional protein, functional sugars and oligosaccharides would be a good compliment to overall diets. These functional sweet potato-based foods with added human health benefits would also provide dietary support and will help to address nutritional insecurity-linked health challenges of mother and child.

There are also challenges to design novel foods that provide balanced nutrition, while still palatable, easy to eat, and easy to digest. In this context, sweet potatoes and sweet potato derived foods can be both acceptable by the consumers as well as healthy alternative due to their preferable texture, flavor, and health protective functionalities. Protective functional pigments like anthocyanins and carotenoids from purple and orange fleshed sweet potatoes could also be used for development of intermediary food products like food thickeners which have applications for the functional food industry (Padmaja et al., 2012). Further, exploring promising leads like development of weaning foods and intermediary food products from orange fleshed and purple fleshed sweet potatoes would create multiple applications for the functional food industry and will support value added employment opportunities in the processing sector. Therefore, selecting sweet potatoes as healthy food choice and their value-added integration in functional food and beverage design targeting NCD-linked health benefits has significant relevance in food security and public health solutions. However, the human health relevant nutritional qualities of sweet potatoes vary widely among different flesh colored, between cultivars, and due to pre-harvest growing conditions, post-harvest storage, and different processing and cooking strategies. Therefore, it is important to screen sweet potato cultivars with better nutritional profile and to optimize different processing strategies based on metabolically driven health rationale prior to design high-value and novel functional foods form sweet potatoes. Such cultivar screening and

processing optimization is important for integrating sweet potatoes in safe, inexpensive, and effective dietary support strategies to address both undernourishment and imbalanced nutritional linked chronic disease challenges and to achieve food and nutritional security worldwide.

CHAPTER 3. OBJECTIVES

Based on these above scientific background and rationale and needs the aim of this dissertation was to screen different sweet potato cultivars (white, off-white, orange and purple) for phenolic bioactive-linked antioxidant, anti-hyperglycemic, and anti-hypertensive functionalities using rapid *in vitro* assay models. Additionally, beneficial LAB based fermentation was also advanced to improve stability and retention of human health relevant bioactives and associated functionalities for designing sweet potato based functional and probiotic beverages. Furthermore, different processing strategies (frying, baking, boiling and steaming) of select sweet potatoes were optimized based on phenolic bioactive-linked health benefits for their effective integration in traditional as well as novel dietary approaches. The wider goal is to develop low cost, nutritionally balanced, and human health benefits relevant food products from sweet potatoes for wider health-focused food solution strategies, especially to address rapidly emerging diet and lifestyle-linked NCD epidemic. A secondary aim was also to explore related phenolic antioxidant-linked wound healing response in a tuber model such as potato which can be integrated in post-harvest applications across tuber storage, including sweet potato.

The specific objectives derived from above broad objective are

1. To screen different flesh colored sweet potato cultivars for phenolic bioactive-linked antioxidant, anti-hyperglycemic, and anti-hypertensive properties using rapid *in vitro* assay models.
2. To recruit beneficial LAB based fermentation strategy to design sweet potato-based functional beverages with improved phenolic-linked nutritional qualities.

3. To optimize deep frying methods of select sweet potatoes based on their phenolic content and associated antioxidant potentials using Response Surface Methodology (RSM) statistical model.
4. To optimize steaming and boiling processing strategies for higher retention and stability of phenolic bioactives and associated human health relevant functionalities in sweet potato-based foods.
5. To optimize different baking related variables based on phenolic-linked health benefits of select sweet potatoes.
6. To explore the relevance of stress protective phenolic-linked antioxidant response for improving wound-healing processes in a tuber crop model like potato that can also be relevant to tubers like sweet potato.

CHAPTER 4. SCREENING OF SWEET POTATO CULTIVARS FOR PHENOLIC BIOACTIVE-LINKED HEALTH BENEFITS TARGETING EARLY STAGES OF TYPE 2 DIABETES

4.1. Abstract

Sweet potato is a rich source of dietary antioxidants such as β-carotene, vitamin C, anthocyanins, xanthophylls, and range of other functional bioactives. Due to such rich sources of dietary antioxidants and health protective bioactive compounds it has potential for protection against chronic oxidative stress commonly associated with pathogenesis of non-communicable chronic diseases (NCDs). when consumed either as fresh or processed food. Therefore, targeting sweet potato in ethnic or functional foods to address chronic oxidative stress-linked NCD challenges such as early stages of type 2 diabetes and its associated complications has significant merit. However, the bioactive profile, associated antioxidant properties and other health relevant functional benefits of sweet potato vary widely between flesh color and among cultivars. Thus, the aim of this study was to screen seven sweet potato cultivars with three different flesh colors (white-yellow, orange, and purple) for total soluble phenolic (TSP) content, total antioxidant activity, anti-hyperglycemic function relevant α-amylase, α-glucosidase, and anti-hypertensive function related to angiotensin-I-converting enzyme (ACE) inhibitory activities using rapid *in vitro* assay models. Among the different cultivars, the purple-fleshed cultivar (NIC 413) had significantly higher phenolic-linked antioxidant activity. High to moderate α-amylase (84.42 to 74.84 %) and α-glucosidase (60.33 to 21.41 %) enzyme inhibitory activities were observed in all sweet potato cultivars. Additionally, moderate anti-hypertensive relevant ACE inhibitory activity was recorded in select sweet potato cultivars (e.g. Murasaki, Covington). The results of this initial *in vitro* screening study suggest that select sweet potato cultivars such as

NIC 413, Murasaki, Evangeline, and Covington should be integrated in dietary support strategies targeting early stages of type 2 diabetes and associated anti-hypertensive benefits.

Keywords: Antioxidants, Anti-diabetic, Anti-hypertension, Sweet Potato Cultivars, Phenolic Bioactives.

4.2. Introduction

Dietary and lifestyle interventions by integrating whole food-based and nutritionally balanced diets combined with regular physical activities is recommended as an effective preventative strategy against the early stages of type 2 diabetes and associated health complications (ADA, 2020). Insulin resistance, impaired glucose tolerance, impaired insulin function, central adiposity, β cell dysfunctions, hypertension, retinopathy, and nephropathy are health risks related to type 2 diabetes development and progression. Due to such complex physiological and metabolic dysfunctions, type 2 diabetes is now one of the leading causes for increased NCD-linked mortality rate worldwide (ADA, 2020). The high mortality and morbidity of type 2 diabetes is primarily from increased risks of heart diseases, kidney failure, and liver damage [World Health Organization (WHO), 2020]. Managing blood glucose homeostasis and chronic oxidative stress at the early stages of disease development are essential to prevent or delay type 2 diabetes pathogenesis and to avoid associated mortality. Due to the complex progression and adverse effect of type 2 diabetes on micro- and macro-vascular systems. It is important to advance more holistic dietary and therapeutic preventative strategies for better health outcomes (ADA, 2020).

Dietary interventions by integrating antioxidant rich plant-based foods are highly recommended by American Diabetes Association (2007) to prevent and counter the adult on-set of type 2 diabetes and its associated health risks. World Health Organization (2019) also

highlighted the health benefits of higher daily intake of fruits and vegetables rich in dietary antioxidants, dietary fiber, vitamins, and minerals, especially to manage diet and lifestyle-linked NCD challenges. Furthermore, several epidemiological and clinical studies confirmed the positive health impact of a higher portion size of vegetable and fruit intake on lowering the health risks associated with common NCDs such as early stages of type 2 diabetes and cardiovascular diseases (Bauer et al., 2013; Eshak et al., 2013). Specifically, plant-based foods that are rich in phenolic bioactive compounds such as phenolic acids, flavonoids, tannins, coumarins, and stilbenes have shown improvement of overall health through diverse health protective functions such as antioxidative, anti-diabetic, anti-hypertensive, and by improving beneficial gut microbiota (Ozcan et al., 2014; Shahidi and Ambigaipalan, 2015). Common vegetables and fruits are rich source of these health protective bioactives and when consumed as part of diet, can provide protection against metabolic and physiological dysfunctions related to early stages of type 2 diabetes and other NCDs (Sarkar and Shetty, 2014). However, many starch-based vegetables are not recommended for managing type 2 diabetes as they can potentially increase post-prandial blood glucose level with immediate breakdown and intestinal absorption of glucose after the meal. Therefore, finding starch –based vegetables with low glycemic index, slow release of glucose, and other health protective functions are important for their effective integration in type 2 diabetes related dietary interventions.

Among different starch-based vegetables, sweet potato is becoming increasingly popular among consumers due to its unique nutritional profile and associated health benefits. Sweet potato is a rich source of dietary fibers, antioxidants, minerals, vitamins and other health-promoting bioactive compounds (Ellong et al., 2014). Additionally, the wide diversity of sweet potato genotypes mainly due to different flesh color (white, yellow, orange, and purple) from

unique land races and newly developed cultivars within each flesh color group make sweet potato a promising and effective integration in several ethnic as well as modern functional food designs. The nutritional profile, especially functional bioactives associated with health benefits of sweet potatoes vary widely between different flesh color and among cultivars/varieties. The orange-fleshed sweet potatoes for example are rich source of β-carotene and ideal targets for managing vitamin A deficiency and chronic oxidative stress. Therefore, orange sweet potatoes have been a key dietary target in WHO's nutrition mission to fight malnutrition and blindness in several African and Asian countries, where undernutrition and imbalanced nutrition-linked public health challenges co-exist (Fetuga et al., 2013; WHO, 2020).

Sweet potatoes known for their high level of beta-carotene, but are also rich in anthocyanin, flavonoids, dietary fiber, and essential minerals. The purple cultivars are rich in anthocyanins and have shown to have strong antioxidant properties in *in vivo* mice, rats, and rabbit models and can be integrated in dietary support strategies to counter chronic oxidative stresses induced by metabolic breakdowns commonly associated with NCDs (Jawi and Budiasa, 2011; Oki, 2002). Improvement of memory function (Wu et al., 2008), and reduction of post-prandial blood glucose level were also recorded with administration of purple-fleshed sweet potato-based foods (Li et al., 2013; Ray and Tomlins, 2009). The composition and profile of starch in sweet potatoes, such as higher amylose content is beneficial for postprandial blood glucose control and to improve insulin response which are critical for prevention and management of early stages of type 2 diabetes (Zakir et al., 2008). Furthermore, the presence of polysaccharides (PSWP, PSAP-1 and PSAP-2) consisting monosaccharide units of arabinose, galactose, glucose, and rhamnose from sweet potato consumption have hepatoprotective properties (Sun et al., 2018). Therefore, targeting sweet potatoes in safe and inexpensive dietary

support strategies to prevent and manage early stages of type 2 diabetes and related health risks has significant relevance. All sweet potato cultivars regardless of flesh color, may vary in their total phenolic content, individual phenolic acid profile, and associated antioxidant and other health relevant functional properties. Thus, prior to integrating a dietary and therapeutic interventions, it is important to advance metabolically-targeted rapid screening of different cultivars of sweet potatoes for their bioactive profile and associated nutritional and health benefits. Based on this rationale, seven sweet potato cultivars with three different flesh color (white, orange, and purple) were screened for phenolic bioactive linked antioxidant, anti-hyperglycemic and anti-hypertensive properties using rapid *in vitro* assay models. The wider objective of this study was to screen and select superior sweet potato cultivar with high antioxidant, anti-hyperglycemic and anti-hypertensive properties, which could be then integrated in ethnic and functional food design for early stages of type 2 diabetes and anti-hypertension benefits.

4.3. Materials and Methods

4.3.1. Sample Collection and Extraction

Five sweet potato cultivars [(white fleshed-Bonita and off white fleshed-Murasaki), (orange fleshed-Covington and Evangeline), (purple fleshed-NIC 413)] were generously provided by North Carolina State University (USA) sweet potato breeding program. Two cultivars (orange fleshed-Burgundy and Orleans) grown under organic cultivation practices were obtained from Whitewater Gardens Farm (Altura, MN, USA). Four sweet potato tubers from each cultivar were randomly selected and used for each replicate in the *in vitro* assays. Tubers were cleaned, peeled, and chopped into small pieces for extraction. Approximately, 40 g of chopped sweet potato was weighed and blended separately with 100 mL 12% ethanol and cold

water for 5 min using a blender (Waring commercial blender, Connecticut, USA) set at high speed. Then extracts were centrifuged (Sorvall Biofuge Primo centrifuge, Thermo scientific, USA) at 8000rpm for 20 min, repeated two more times and the supernatant was collected and stored in the refrigerator for further biochemical analysis.

4.3.2. Chemicals Used

Porcine pancreatic alpha–amylase (EC 3.2.1.1), rat intestinal alpha–glucosidase (EC 3.2.1.20), 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, 2, 2- Azobis (2- amidinopropane) dihydrochloride (AAPH), and phenolic acid standards were purchased from Sigma Chemical Co (St. Louis, MO).

4.3.3. Total Soluble Phenolic Content

The total soluble phenolic (TSP) content of aqueous and ethanol (12%) extracts of sweet potatoes was determined based on the protocol described by Shetty et al. (1995). The assay was carried out by placing 0.5 mL of the sweet potato extracts into the sample test tubes and diluted for 2 times by adding 0.5 mL of distilled water. For measuring blank, 0.5 mL of distilled water was added instead of the sample extracts. In each test tube, 1 mL of 95 % ethanol, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent, and 5 mL of distilled water were added. Then after adding 1 mL of 5% sodium carbonate, the contents were mixed well by using a vortex mixer (Digital vortex mixer, Fisher Scientific, Pittsburgh, USA) and incubated in the dark for 60 min. Samples remixed again before reading the absorbance using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY) set at 725nm. The absorbance values were converted to total soluble phenolic content and expressed in milligrams gallic acid equivalent (GAE) per gram of fresh weight (FW) based on a standard curve that was established using different concentrations of gallic acid in 95% ethanol.

4.3.4. Total Antioxidant Activity

The total antioxidant activity of each sweet potato extract was measured using two different assays: 2, 2-Dipheny-1—picryl hydrazyl (DPPH) free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay. For the DPPH assay as described by Kwon et al. (2006), 0.25 mL sweet potato sample was added to 1.5 mL centrifuge tubes, while 0.25 mL 95 % ethanol was added to the control tube. Then 1.25 mL of 60 mM DPPH (in 95% ethanol) was added to all centrifuge tubes mixed well by vortexing incubating for 5 min. The mixture was then centrifuged at 13,000 rpm for 1 min pelletize the precipitate. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). For the ABTS assay the same procedure by Re et al. (1999) was followed except that 1 mL of ABTS (in 95% ethanol) was added to 0.05 mL of sweet potato sample or control (95% ethanol) and the absorbance was measured at 734nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). Based on the absorbance readings, the percentage of inhibition for both DPPH and ABTS radicals were calculated using the equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \text{ x } 100 \qquad (4.1)$$

The inhibition percentage from the DPPH and ABTS radical scavenging assays was then expressed as mM Trolox equivalents (TE) per gram of sample based on the Trolox standard curve.

4.3.5. α-Amylase Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the Worthington Enzyme Manual (1993a). Undiluted, half-diluted, and one-fifth diluted sample extracts were used to determine potential dose dependence in the α-amylase enzyme inhibitory activity. The dilutions were made

using distilled water. The buffer used was 0.1M sodium phosphate (pH 6.9) with 0.006M sodium chloride added to it. A volume of 500 µL of each sweet potato sample extract was added to test tubes while the control tubes had 500 µL of buffer only. Additionally, each sweet potato sample extract had a corresponding sample blank tube which contained 500 µL of the sample extract. Then 500 µL of porcine pancreatic amylase (0.5 mg/ mL buffer) was added to all the tubes, with the exception of sample blank and incubated for 10 min at 25°C. After incubation, 500 µL of 1% starch (1 g/100 mL buffer) was added to all tubes and incubated for 10 min. The reaction was then stopped by the addition of 1 mL of 3, 5 dinitro salicylic acid before placing tubes in a boiling water bath for 10 min removed and allowed to cool at room temperature. The reaction mixture in each tube was then diluted by adding 10 mL of distilled water to adjust the absorbance of the control to 1.0±0.02 before absorbance was measured at 540 nm using a UV-VIS Genesys spectrophotometer (Genesys UV- visible, Milton Roy Inc, Rochester, NY)

The inhibition percentage of α amylase enzyme activity was calculated based on the absorbance readings and using the formula:

$$\%\ \text{inhibition} = \frac{\text{Abs control} - (\text{Abs extract} - \text{Abs sample blank})}{\text{Abs control}} \text{ x } 100 \quad (4.2)$$

4.3.6. α-Glucosidase Enzyme Inhibitory Activity

The α-glucosidase enzyme inhibitory activity assay was conducted based on the protocol from the Worthington Enzyme Manual (1993b) with some modifications taken from McCue et al. (2005). A volume of 50 µL (undiluted), 25 µL (half-diluted) and 10 µL (one-fifth diluted) of each sweet potato sample (for dose dependent response) extracts were pipetted into 96 well microtiter plates (Falcon, Fisher Scientific, USA) respectively. The half and one-fifth dilutions contained total of 50 µL in volume fronm the addition of 25 µL and 40 µL of 0.1M potassium phosphate buffer (pH 6.9) respectively. Each sweet potato sample extract had a corresponding

control with 50 µL of phosphate buffer instead of the sample. Finally, the volume in all the wells was made up-to 100 µL by the addition of 50 µL of phosphate buffer in each well including the control and then 100 µL of 0.1 M potassium phosphate buffer (pH 6.9) containing α-glucosidase enzyme (1 U/mL) before incubating for 10 min at 25°C. After this, 50 µL of 5 mM p-nitrophenyl-α-D- glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals (after 10 min incubation period at room temperature). The reaction mixtures were then incubated foe 5 min at 25ºC. Absorbance readings were taken before (0 min) and after (5 min) the incubation period using a microplate reader (Thermomax, Molecular device Co., Virginia, USA) set at 405 nm.

The inhibition percentage of α-glucosidase enzyme activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs control 5min} - \text{Abs control 0 min}) - (\text{Abs extract 5min} - \text{Abs extract 0min})}{(\text{Abs control 5min} - \text{Abs control 0min})} \text{ x } 100 \quad (4.3)$$

4.3.7. Angiotensin I-Converting Enzyme (ACE) Inhibition Assay

The assay protocol used in this study was adapted from the method modified by Kwon et al. (2006). The enzyme ACE-1 and substrate hippuryl-L-histidyl-L-leucine were purchased from sigma Aldrich (St. Louis, MO, USA). The 50 µL extract sample was added to micro-centrifuge tubes and mixed with 200 µL 2 mU of ACE enzyme solution (in 1 M NaCl-borate buffer, pH 8.3) and incubated for 10 min at 37º C. This was followed by addition of 100 µL hippuryl-L-histidyl-L-leucine and placement in a 37 ºC water bath for 60 min. The reaction was stopped by the addition of 150 µL 0.5 N HCl. The ACE inhibitory activity was measured by quantifying the liberated hippuric acid using a high-performance liquid chromatography (HPLC) (Agilent 1260 series equipped with autosampler and DAD 1100 diode array detector, Agilent Technologies,

Palo Alto, CA, USA) protocol. The solvents used for the gradient were 10 mM Ortho-phosphoric acid (pH 2.5) and 100 % methanol. The analytical column used was Agilent Zorbax SB-C18, 250- 4.6 mm i.d. with packing material of 5 µm (column temperature 22-24 ^{o}C). The inhibition percentage was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{AREA control} - (\text{AREA sample} - \text{AREA sample blank})}{\text{AREA control} - \text{AREA blank}} \text{ x } 100 \qquad (4.4)$$

4.3.8. Total Protein Content

The total protein content of the sweet potato tuber samples was measured using a modified version of the Bradford assay (Bradford, 1976). The dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted to a solutuion 1:4 with distilled water after which 5 mL of the diluted dye was added to 50 µL of the sweet potato extract samples, mixed thoroughly and incubated in the dark for 5 min at room temperature. For the blank, 50 µL of distilled water was used instead of the sweet potato extract sample. All samples were analyzed in triplicates and the absorbance was then measured at 595 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). A standard curve was prepared using bovine serum albumin (BSA) in different concentrations and the protein content was expressed as mg/g fresh weight (FW).

4.3.9. HPLC Analysis of Phenolic Acid Profiles

Each 2 mL of sweet potato extract sample was centrifuged for 5 min before 5 µL of the sample was injected using Agilent ALS 1100 auto sampler into Agilent 1260 series HPLC (Agilent Technologies, Palo Alto, CA equipped with DAD 1100 diode array detector). The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% over the next 7 min, then decreased to 0% for the next 3 min and maintained for the next 7 min

(total run time, 25 min). The analytical column used was Agilent Zorbax SB-C18 250x4.6 mm i.d., with packing material of 5 µm particle size at a flow rate of 1 mL/min at ambient temperature. During each run, the chromatogram was recorded at 306 nm, 333 nm, 540 nm, and 580 nm and integrated using Agilent Chem station enhanced integrator. Pure standards of gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, quercetin derivatives, resveratrol and m-coumaric acid (Sigma Chemical Co., St. Louis, MO) in 100% methanol were used to calibrate the standard curve and retention times.

4.3.10. Statistical Analyses

The entire experiment was repeated two times. Analysis at every time point from each experiment was carried out in triplicates. Means, standard errors, and standard deviations were calculated from replicates within the experiments and analyses were done using Microsoft Excel XP. The data were analyzed for analysis of variance (ANOVA) using Statistical Analytical Software (SAS version 9.4; SAS Institute, Cary, NC). Significant statistical differences between sweet potato cultivars, type of extractions (cold water and 12% ethanol extraction) and sweet potato sample × extraction interactions for all *in-vitro* assays were determined using Tukey's least square means separation at the 0.05 probability level. Additionally, in order to measure the strength of the linear association between total soluble phenolic content and antioxidant and anti-hyperglycemic functionalities, a Pearson's correlation test was carried out using Microsoft Excel.

4.4. Results and Discussion

4.4.1. Total Soluble Phenolic Content (TSP)

Total soluble phenolic (TSP) content can be targeted as a key initial overall biomarker to understand the human health relevant nutritional qualities and diverse health protective functions of plant-based foods. Overall, high phenolic content was observed in all sweet potato cultivars,

which were screened in this study. There were no statistically significant differences in TSP content of sweet potato cultivars between two extractions (aqueous and 12 % ethanol). Among sweet potato cultivars, the purple-fleshed cultivar (NIC 413) had ($p<0.05$) highest TSP content that ranged from 2.1-2.84 mg GAE (gallic acid equivalent)/100 g FW in 12 % ethanol and aqueous extracts and it was statistically significant ($p<0.05$) when compared to TSP content of white-fleshed and orange-fleshed cultivars (Fig. 4.1). However, the TSP content of white-fleshed and orange-fleshed sweet potato cultivars were statistically at par. The lowest TSP content was observed in aqueous and ethanol (12%) extract of white -fleshed cultivar (Bonita) (0.39 and 0.38 mg GAE/100 g FW, respectively). Interestingly, TSP content of off-white cultivar (Murasaki) was 0.50 mg GAE/100 g FW in aqueous extracts and 0.49 mg GAE/100 g FW in ethanol (12%) extracts and it was statistically at par with orange-fleshed cultivars such as Evangeline, Burgundy, and Orleans.

In a previous study, Teow et al. (2007) reported TSP content of 0.38 mg GAE/100 g to 0.50 mg GAE/100g FW in white and orange-fleshed sweet potato cultivars. However, Vizzotto et al. (2017) observed significantly higher total phenolic content in purple-fleshed sweet potato followed by orange-fleshed and white-fleshed cultivars. Rumbaoa et al. (2009) also found high phenolic content and proportionately high antioxidant activity in a purple-fleshed sweet potato cultivar (*Dakol*). The screening results of TSP content of sweet potato cultivars found in our study was similar to the previous findings (Teow et al., 2007; Vizzotto et al., 2012) and suggest that more the high TSP content of purple-fleshed sweet potatoes and the associated high antioxidant property has relevance for their potential integration in diverse health-focused food and therapeutic solutions. The cultivar screening results of our study indicated that sweet potatoes, especially purple-fleshed cultivars (NIC 413) are good source of soluble phenolics and

can be incorporated in ethnic and functional food design for human health benefits. However, not only just TSP content, but it is also important to find the composition and profile of phenolic compounds in different flesh colored sweet potato cultivars for their effective and high-value food and health applications.

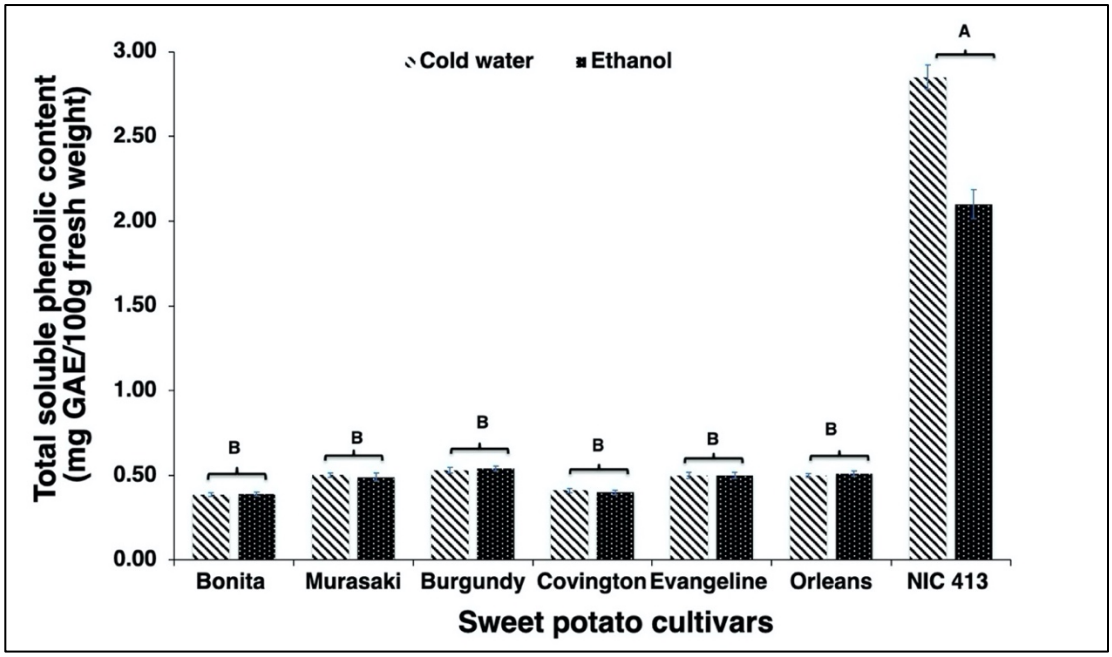

Figure 4.1. Total soluble phenolic content (mg GAE/100g FW) of aqueous and 12% ethanol extracts of seven sweet potato cultivars. Different letters represent significant differences between cultivars (main effect) at $p<0.05$.

4.4.2. Analysis of Phenolic Compounds Using HPLC

The major phenolic acids found in seven sweet potato cultivars were gallic acid, ferulic acid, dihydroxy benzoic acid, chlorogenic acid, and an important flavanol catechin (Table 4.1). Significant differences in composition of phenolic acids between cultivars and different flesh color were observed. Presence of chlorogenic acid was detected in only purple-fleshed NIC 413 sweet potato cultivar. Similarly, ferulic acid was found in off white-fleshed cultivar (Murasaki) and orange-fleshed cultivars (Evangeline, Covington, Burgundy). Among all phenolic metabolites measured gallic acid was the most predominant across all sweet potato cultivars. Previously, Padda and Picha (2008) reported chlorogenic acid and 3,5-dicaffeoylquinic acid as

major phenolic compounds in sweet potato tubers. Similarly, Park et al. (2016) found hydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, and sinapic acid in different flesh color sweet potatoes. They have also observed significant differences in phenolic composition between different cultivars. In the present study, high chlorogenic acid was found in purple-fleshed cultivar (NIC 413), especially in the ethanolic (12%) extracts (4.56 µg/g FW). Additionally, significantly high gallic acid and catchin were present in purple-fleshed cultivar (NIC 413). Previous studies (Esatbeyoglu et al., 2017; Oki et al., 2002; Padda and Picha, 2008) also reported higher concentration of different phenolic acids in purple-fleshed sweet potato cultivars.

The presence of chlorogenic acid in purple-fleshed cultivar might have significant human health protective relevance as Frank et al. (2003) observed reduction of cholesterol level and reduced risk of cardiovascular disease in rat model after oral administration of chlorogenic acid. One of the most important human health protective function of phenolic acids of plant-based foods is the antioxidant property. All phenolic acids found in our study are very potent as antioxidants and therefore targeting these sweet potato cultivars in dietary interventions to manage chronic oxidative stress and to reduce associated NCD risks have significant merit. However, it is also important to investigate the potential relationship between phenolic and antioxidant properties of sweet potato cultivars prior to integrating them in health-focused dietary solutions.

Table 4.1. Major phenolic compounds (µg/g FW) (n = 5) detected in sweet potato cultivars

Sweet potato cultivar	Extraction method	Phenolic acids (µg g−1 fresh weight)				
		Catechin	Gallic acid	Dihydroxybenzoic acid	Ferulic acid	Chlorogenic acid
Bonita	CW	0.67^{h}	3.05^{b}	0.08^{gh}	nd	nd
	ET	0.95^{g}	2.81^{c}	0.19^{b}	nd	nd
Murasaki	CW	1.61^{de}	2.21^{d}	0.10^{e}	0.12^{d}	nd
	ET	3.06^{b}	3.23^{ab}	0.19^{bc}	0.19^{c}	nd
Evangeline	CW	1.37^{f}	2.87^{c}	0.06^{i}	1.19^{a}	nd
	ET	1.68^{de}	1.02^{h}	nd	1.04^{b}	nd
Orleans	CW	0.54^{h}	1.24^{g}	0.07^{hi}	0.17^{cd}	nd
	ET	0.60^{h}	1.39^{g}	0.18^{c}	0.17^{cd}	nd
Covington	CW	2.21^{c}	1.79^{e}	0.09^{fg}	nd	nd
	ET	2.11^{c}	1.57^{f}	0.22^{a}	nd	nd
Burgundy	CW	1.51^{e}	3.60^{a}	0.06^{i}	0.12^{e}	nd
	ET	1.77^{d}	2.83^{c}	0.14^{d}	0.09^{e}	nd
NIC 413	CW	3.34^{a}	1.82^{e}	nd	nd	2.74^{b}
	ET	2.91^{b}	1.58^{f}	0.10^{ef}	nd	4.56^{a}

nd- not detected,

CW-Cold water extract, ET- 12% ethanol extract

Different letters represent significant differences in individual phenolic content due to cultivar × extraction type interaction at 95% level of confidence interval ($p < 0.05$).

4.4.3. Total Antioxidant Activity

The total antioxidant activity of the sweet potato cultivars varied with the extraction method for the DPPH free radical scavenging assay, and ABTS radical cation assay. The DPPH free radical scavenging assay is one of the most widely used antioxidant assay to estimate the antioxidant properties of plant-based foods (Teow et al., 2007). In this present study, antioxidant activity of sweet potato cultivars based on DPPH free radical scavenging assay ranged from 0.045 mM Trolox/g FW (Bonita cold water extract) to 0.389 mM Trolox/g FW (NIC 413 cold water extract). The antioxidant activity results of sweet potato cultivars positively correlated with the results of the TSP content. Among seven sweet potato cultivars, the purple-fleshed cultivar (NIC 413) had approximatelely twice the (DPPH-based) antioxidant activity in both aqueous and ethanol (12%) extracts (0.389 and 0.387 mM Trolox/g FW respectively), which was significantly greater than the antioxidant activity from the other cultivars (Fig 4.2) followed by the orange-fleshed cultivar Evangeline (0.121 and 0.129 mM Trolox/g FW), off-white-fleshed cultivar Murasaki (0.128 and 0.111 mM Trolox/g FW). Similar to the results of the TSP content, the white fleshed cultivar Bonita (0.045 and 0.054 mM Trolox/g fw) had significantly ($p<0.005$) lower total antioxidant activity compared other sweet potato cultivars in the study. Previously, Teow et al. (2007) reported similar level of antioxidant activity in different sweet potato cultivars such as higher antioxidant activity in purple fleshed cultivars and lower in white fleshed cultivars. In the present study, antioxidant activity of aqueous and ethanol extracts of sweet potato cultivars based on ABTS free radical scavenging assay ranged from 0.424 mM to 0.670 mM Trolox/g FW (Fig. 4.3). In general, higher antioxidant activity was observed with ABTS free radical assay when compared with the results of the DPPH-based assay, which was attributed to the varied affinity of these free radicals under water soluble food matrix (Arnao,

2000). Similar to the results of TSP content and DPPH-assay, the purple-fleshed cultivar (NIC 413) had ($p<0.005$) significantly higher ABTS antioxidant activity in both aqueous (0.61 mM Trolox/g FW) and ethanol extracts (0.60 mM Trolox/g FW), followed by the orange-fleshed cultivars (Evangeline and Covington). Previously, Vizzotto et al. (2017) also found high antioxidant activity in purple-fleshed sweet potato cultivars. Higher antioxidant activity of purple fleshed NIC 413 when compared to other flesh colored cultivars might be due to the presence of higher soluble phenolic content, specific phenolic acid composition (chlorogenic acid) coupled with higher acylated anthocyanins. Additionally, polysaccharides in purple sweet potato cultivars such as PSWP, PSAP-1 and PSAP-2 also showed strong antioxidant activity coupled with hepatoprotective effects in *in vivo* analysis (Sun et al., 2018).

Overall, sweet potato cultivars in the present study with higher total antioxidant activity can be targeted to develop novel food products as well as nutraceuticals and can help to replace synthetic antioxidant compounds in the food and supplement formulations (Engin et al., 2011). Furthermore, these sweet potato cultivars with high antioxidant potentials can be potentially integrated as whole food or functional foods in dietary interventions to counter NCD-linked oxidative breakdowns and to reduce the associated health risks. Plant –based foods rich in phenolic bioactives are not only just good source of dietary antioxidants, but when consumed they also provide other health-protective functions such as anti-hyperglycemic, anti-hypertensive, anti-mutagenic, and cardio-protective properties.

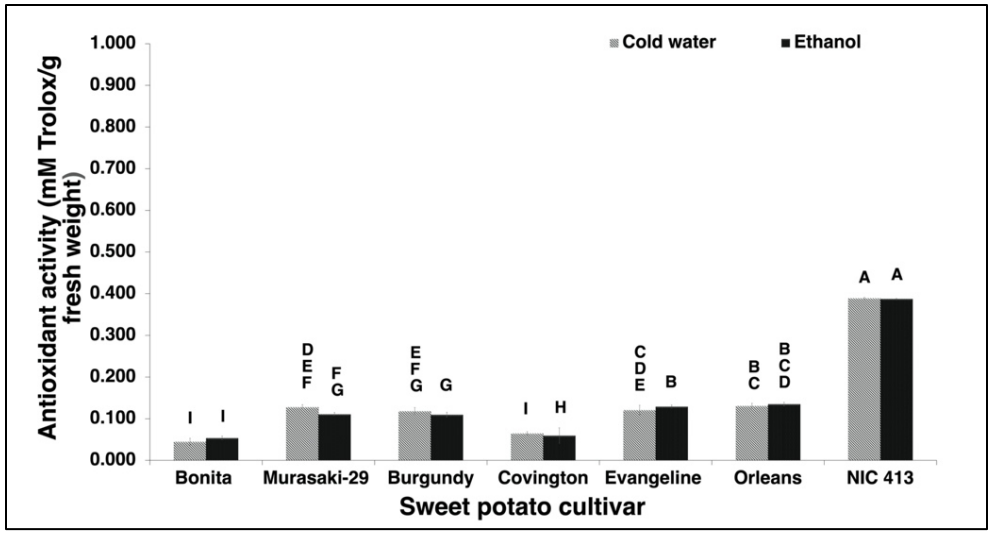

Figure 4.2. DPPH free radical scavenging assay-based antioxidant activity (mM Trolox/g FW) of aqueous and 12% ethanol extracts of sweet potato cultivars. Different letters represent significant differences between cultivar × extraction type interaction at $p<0.05$

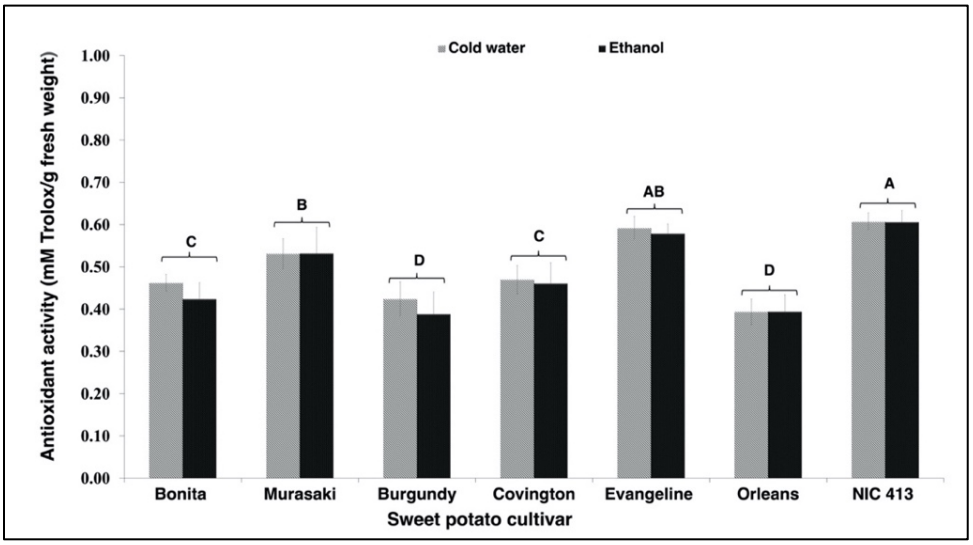

Figure 4.3. ABTS free radical scavenging assay-based antioxidant activity (mM Trolox/g FW) of aqueous and 12% ethanol extracts of sweet potato cultivars. Different letters represent significant differences between cultivars at $p<0.05$

4.4.4. Alpha-Amylase Enzyme Inhibitory Activities

Alpha-amylase inhibitors, especially synthetic inhibitors such as Acarbose [4,6-dideoxy-4-{[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-(hydroxymethyl)cyclohex-2-en-1-yl]amino}-alpha-D-glucopyranosyl-(1->4)-alpha-D-glucopyranosyl-(1->4)-D-glucopyranose] and Miglitol [(2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol] are considered as a

starch digestion blocker because they play critical role in prolonging digestion of dietary starch and subsequently help to regulate postprandial blood glucose spikes (Chiasson et al., 2003). Deceleration of starch digestion has been recommended as one of the effective therapeutic strategies to maintain blood glucose homeostasis and to manage post-prandial hyperglycemia (Oboh et al., 2016). However, many synthetic α-amylase inhibitors commonly used in type 2 diabetes therapy have shown adverse side effects such as flatulence, liver problems, and digestion related disorders (Lee et al., 2014). Therefore, finding naturally occurring and safe α-amylase inhibitors from dietary sources, especially from plant-based food sources are essential for managing chronic hyperglycemia commonly associated with early stages of type 2 diabetes.

Many plant-based foods and food derivatives such as common beans (*Phaseolus vulgaris*), wheat albumin (Kodama et al., 2005; Obiro et al., 2008), sweet potato, taro (Rekha and Padmaja, 2002), select potato cultivars (Kalita et al., 2018) and true yam (*Dioscorea bulbifera*) tubers (Ghosh et al., 2014) have shown significant *in vitro* α-amylase enzyme inhibitory activity. In the present study, moderate to high α-amylase enzyme inhibitory activity was observed in all sweet potato cultivars (Fig. 4.4 A, B, and C). Furthermore, significant dose dependent response for α-amylase enzyme inhibitory activity was also observed in aqueous and ethanol (12%) extracts of sweet potato cultivars. Overall, α-amylase enzyme inhibitory activity of sweet potato cultivars ranged from 59.08% to 84.82% in undiluted sample of aqueous extracts, while in ethanol (12%) extracts of undiluted sample, the α-amylase enzyme inhibitory activity ranged between 56.85% and 97.78% inhibition. In the present study, sweet potato cultivar Covington had highest α-amylase enzyme inhibitory activity in all dilutions. However, it was statistically similar with other orange, white, and purple-fleshed sweet potato cultivars. Previously, Esatbeyoglu et al. (2017) reported moderate (40%) α-amylase enzyme inhibitory activity in a

purple-fleshed sweet potato cultivar. Whereas in the present study, we observed moderate to high α-amylase enzyme inhibitory activity (around 77% in undiluted sample) in a sweet potato cultivar (NIC-413). Therefore, purple-fleshed sweet potato cultivar NIC-413 with high phenolic content, high antioxidant activity, and moderate to high α-amylase enzyme inhibitory activity is potentially an ideal and safe dietary target which can be incorporated in health-focused food solution for managing chronic hyperglycemia and chronic oxidative stress commonly associated with early stages of type 2 diabetes and other NCDs. Additionally, results of our study also suggested that orange-fleshed (Covington, Evangeline, and Burgundy) and white-fleshed (Bonita) and off-white-fleshed (Murasaki) sweet potato cultivars are also good choice for type 2 diabetes relevant dietary interventions. In a previous clinical study, blood glucose lowering property was observed in response to sweet potatoes (Zakir et al., 2008). The results of our cultivar screening study provide biochemical rationale, that suggest one should target superior sweet potato cultivar with anti-diabetic property in future *in vivo* assay model-based and associated clinical studies.

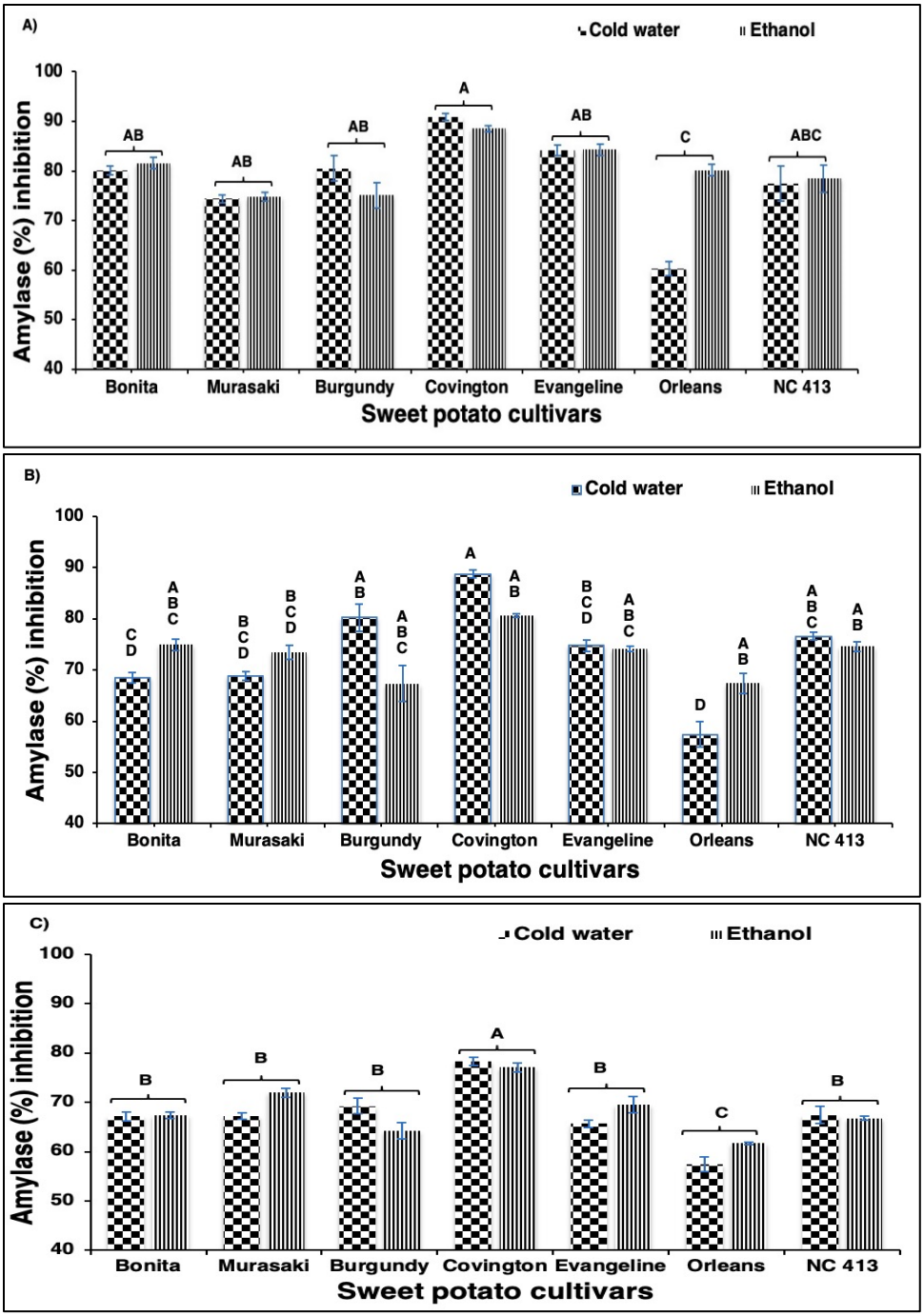

Figure 4.4.α-Amylase enzyme inhibitory activity (%) of aqueous and 12% ethanol extract of sweet potato cultivars at no dilution (A), half dilution (B), and one fifth dilution (°C). Different letters represent significant differences between cultivars (A & C) and cultivar × extraction type interaction (B) at $p<0.05$ (separately run for each dilution).

4.4.5. Alpha-Glucosidase Enzyme Inhibitory Activity

Alpha glucosidase is another key therapeutic target to manage chronic hyperglycemia and inhibition of this enzyme represents slowing down of initially digested carbohydrate breakdown and final uptake of blood glucose at the intestinal enterocyte. In this present study, low to moderate α-glucosidase enzyme inhibitory activity was observed in all sweet potato cultivars and with two extraction types (aqueous and 12% ethanol) (Fig. 4.5 A, B, and C). There was no significant statistical difference in α-glucosidase enzyme inhibitory activity between two extraction types. Furthermore, across all sweet potato cultivars, significant dose dependent response (without dilution, half-dilution, and one-fifth dilution) was observed in α-glucosidase enzyme inhibitory activity, which reduced proportionately with increased dilutions. Overall, orange-fleshed cultivars, Orleans and Burgundy had significantly ($p<0.05$) higher α-glucosidase enzyme inhibitory activity followed by Bonita, NIC 413, Covington, Evangeline, and Murasaki in both extraction types. The results of this study indicated that sweet potato cultivars with moderate to high α-amylase and moderate α-glucosidase enzyme inhibitory activities are potentially good dietary targets to add and manage post-prandial blood glucose level in diabetic and pre-diabetic population. Plant-based foods containing natural α-glucosidase enzyme inhibitors are safe dietary target to slowdown the breakdown and absorption of consumed carbohydrates without adverse side effects, which is an important food-based therapeutic approach to manage type 2 diabetes associated chronic hyperglycemia (Hanhineva et al., 2010).

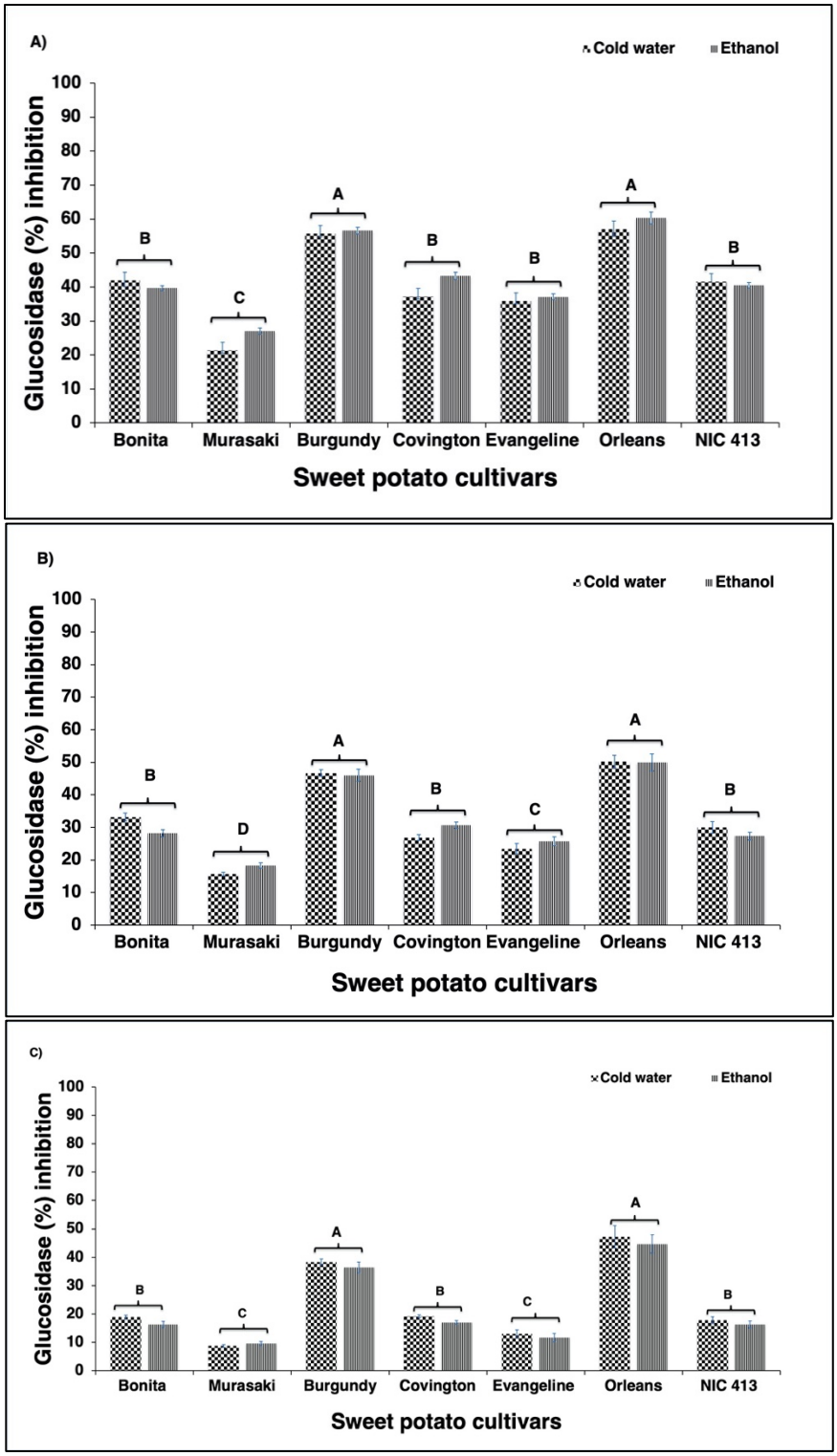

Figure 4.5.α-Glucosidase inhibition (%) of aqueous and 12% ethanol extract of sweet potato cultivars at no dilution (A), half dilution (B), and one fifth dilution (C). Different letters represent significant differences between cultivars at $p<0.05$ (separately run for each dilution).

4.4.6. Angiotensin-I-Converting Enzyme (ACE) Inhibition Assay

Like chronic hyperglycemia, chronic hypertension is a serious health risk factor for type 2 diabetes, cardiovascular disease, and other NCDs. One of the most effective therapeutic approaches to manage chronic hypertension is the use of synthetic angiotensin-I-converting enzyme (ACE) inhibitors. However, many synthetic drugs targeting ACE inhibition such as Captopril (D-3-Mercapto-2-methylpropanoyl-L-proline), Enalapril [(S)-1-(N-(1-(Ethoxycarbonyl)-3-phenylpropyl)-L-alanyl)-L-proline], and Lisinopril [[N2-[(S)-1-Carboxy-3-Phenylpropyl]-L-Lysyl-L-Proline] are believed to be not safe due to long-term adverse health effects. Therefore, finding a dietary source with natural ACE inhibitors is essential to manage chronic hypertension, which is commonly associated with several NCDs such as type 2 diabetes. Many plant-based foods such as edible mushrooms, sweet potatoes, potatoes, flax seed, rapeseed, canola, pea, common beans, garlic, ginger have shown ACE inhibitory activity mainly due to the presence of diverse bioactive compounds like phenolics and peptides (Pihlanto and Makinen, 2013).

Previously, Ishiguro et al. (2012) purified ACE enzyme inhibitory peptide from sweet potato juice using isoelectric precipitation method and demonstrated its hypotensive effect in spontaneously stimulated hypertensive rat model. Similarly, Huang et al. (2011) reported ACE enzyme inhibitory activity in active recombinant thioredoxin h protein derived from sweet potato and overexpressed in *E. coli*. In this present study, ACE enzyme inhibitory activity of seven sweet potato cultivars from three different flesh color was investigated using an *in vitro* assay based protocol described by Kwon et al. (2006). Overall, moderate to high ACE enzyme inhibitory activity was found in orange, white-fleshed, and purple-fleshed sweet potato cultivars (Fig. 4.6). Among different sweet potato cultivars, off-white-fleshed Murasaki (88.81 % in

ethanol extract) and orange-fleshed Covington (80.73 % in aqueous extract and 85.91 % in ethanol extract) had significantly ($p<0.05$) higher ACE enzyme inhibitory activity followed by Burgundy, Orleans, NIC 413, Bonita and Evangeline.

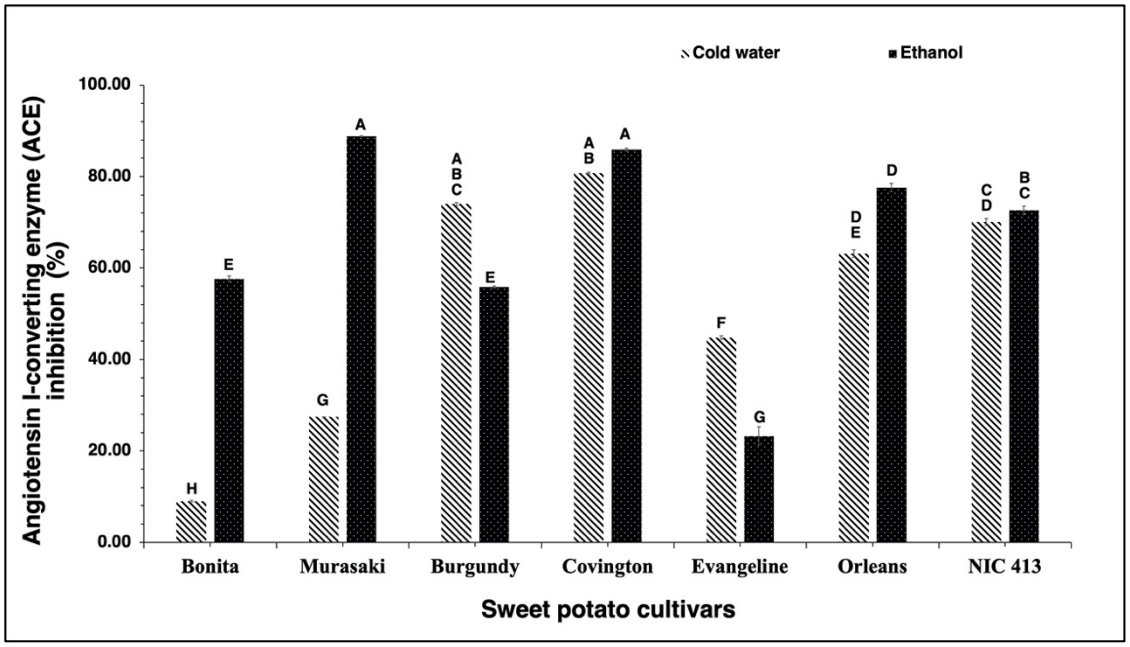

Figure 4.6. Angiotensin-I-converting enzyme (ACE) inhibitory activity of aqueous and ethanol (12%) extracts of different sweet potato cultivars. Different letters represent significant differences between cultivar × extraction type interaction at $p<0.05$.

Such differences in ACE inhibitory activity between different flesh color and among sweet potato cultivars are probably due to variation in their bioactive profile. Based on our phenolic profile characterization, ferulic acid was found in Murasaki and Covington, which also had high ACE inhibitory activity in previous published studies (Ardiansyah et al., 2008; Hou et al., 2004). Previously, Hou et al. (2004) observed inhibition of angiotensin II –induced vascular smooth muscle cell with pure ferulic acid standard. In another study, Ardiansyah et al. (2008) found ACE inhibition and lowering of blood pressure after single administration of ferulic acid to stroke-prone spontaneously hypertensive rats. However, some previous studies (Ishiguro et al., 2012; Tasi et al., 2013) highlighted the anti-hypertensive function of bioactive peptides of sweet potatoes. Overall, irrespective of their bioactive profiles and associated function, results of this

present study suggested that sweet potato cultivars such as Murasaki and Covington are potentially good dietary targets to address chronic hypertension and related health risks such as type 2 diabetes and cardiovascular diseases.

4.4.7. Total Protein Content

Like health protective phenolic compounds, proteins from plant-based food sources are also considered as important dietary antidotes against type 2 diabetes and other NCDs. Therefore, determining protein content of plant-based food is important for their effective integration in dietary interventions and for designing functional foods with human health benefits. Additionally, understanding protein content of sweet potato cultivars are also critical for advancing sweet potato-based foods to address malnutrition and imbalanced-nutrition associated public health challenges (both infectious and non-infectious diseases) (Kwak, 2019). Previous studies (Aina et al., 2009; Krochmal-Marczak et al., 2014; Senanayake et al., 2013) reported 1-4% crude protein content in sweet potato tubers. In this present study, for some cultivars (Evangeline, Murasaki and NIC 413) higher protein content was observed in aqueous extracts when compared to ethanol extracts (12%) of same sweet potato cultivar (Table 4.2). However, for Bonita and Orleans, ethanol extracts (12%) had higher protein content than aquoes extracts. Among seven cultivars, NIC 413 (1.67 and 1.46 mg/g FW in aqueous and ethanol extracts respectively) had significantly higher protein content followed by Murasakhi, Evangeline, and Covington (0.88, 0.79, 0.74 mg/g FW in aqueous extracts). Whereas lower protein content was observed in Orleans and Bonita (0.23 and 0.40 mg/g FW in aqueous extracts). Previously, Ji et al. (2015) observed high protein content in purple-fleshed sweet potato cultivar (Jizi 01). The higher protein content of purple-fleshed sweet potato cultivar (NIC 413) along with their high phenolic content and antioxidant activity has significant relevance for targeting and utilizing this

sweet potato cultivar in dietary support strategies to address both malnutrition and imbalanced nutrition-linked NCDs such as type 2 diabetes, cardiovascular disease, and obesity.

Table 4.2. Total protein content of sweet potato cultivars (n=7)

	Average concentration (mg/g fresh weight)	
Cultivars	**Aqueous[a]**	**Ethanol (12%)[b]**
Bonita	0.40 [j]	0.60 [g]
Burgundy	0.54 [i]	0.56 [h]
Covington	0.74 [e]	0.73 [f]
Evangeline	0.79 [d]	0.76 [e]
Murasaki	0.88 [c]	0.40 [j]
NIC 413	1.67 [a]	1.46 [b]
Orleans	0.23 [k]	0.56 [h]

[a]Cold water
[b]Ethanol
Different letters represent significant differences in total protein content due to cultivar × extraction types interactions at 95% confidence level ($p < 0.05$).

4.5. Conclusion

Overall, the results of the present study suggested that sweet potatoes are good source of dietary phenolics and protein with diverse human health relevant functionalities such as antioxidant, anti-hyperglycemic, and anti-hypertensive properties. However, the bioactive profiles and associated human health related functionalities of sweet potatoes varied widely between different flesh color and across different cultivars. The purple-fleshed sweet potato cultivar (NIC 413) had high phenolic content, protein content, high antioxidant activity, and moderate anti-hyperglycemic and anti-hypertensive properties, while orange-fleshed cultivars such as Covington, Burgundy, Evangeline, and Orleans had high anti-hyperglycemic and moderate antioxidant property. Additionally, moderate to high anti-hypertensive property (ACE inhibition) was observed in off-white-fleshed (Murasaki) and orange-fleshed (Covington) and purple-fleshed (NIC413) cultivars. Presence of abundant anthocyanins and unique phenolic acid profile in purple-fleshed sweet potato cultivar (NIC 413) has added advantage due their water-soluble nature, which support their integration into aqueous based food matrix, especially to

design functional foods and beverages with human health benefits. Similarly, orange-fleshed and white-fleshed sweet potato cultivars can be potentially incorporated in dietary interventions to address chronic hyperglycemia and chronic hypertension, which are two major risk factors for type 2 diabetes and other NCDs.

CHAPTER 5. BENEFICIAL LACTIC ACID BACTERIA-BASED BIOTRANSFORMATION OF SWEET POTATO FOR NUTRITIONAL BENEFITS

5.1. Abstract

Sweet potatoes are a rich source of diverse bioactive compounds, essential minerals, and dietary fibers and can be rationally targeted for wider human health-focused dietary solutions, especially to address diet-linked non-communicable chronic disease (NCD) challenges. However, high perishability and deterioration of nutritional qualities at post-harvest stages is a major constraint for targeting value added NCD- linked food applications. Therefore, using beneficial lactic acid bacteria (LAB) based biotransformation to preserve and improve sweet potato based human health relevant nutritional qualities for health-targeted food application is essential. Based on this rationale, beneficial LAB (*Lactobacillus plantarum*) based fermentation was evaluated as a preservative to improve phenolic bioactive-linked antioxidant and anti-hyperglycemic functionalities. Three different flesh colored sweet potato cultivars [Murasaki (off-white-fleshed cultivar), Evangeline (orange-fleshed) and NIC 413 (purple-fleshed cultivar)] were selected for initial cultivar screening based on their high baseline phenolic content and associated health benefits (*in vitro* assay-based antioxidant, anti-diabetic, and anti-hypertensive properties). Cold water extracts relevant for food grade applications were fermented for 72 h at 37°C with *Lactobacillus plantarum*. At 0-, 24-, 48-, and 72-h time points the phenolic content, antioxidant activity, and anti-hyperglycemic properties of fermented and unfermented sweet potato extracts were determined. Overall, high total soluble phenolic (TSP) content and high total antioxidant activity were observed at 24 h and remained at a similar content even after 72 h of fermentation. Moderate to high anti-hyperglycemic activity relevant α-amylase (62.1 %) and α-glucosidase (76.3 %) enzyme inhibitory activities were measured in fermented and

unfermented extracts. Among the three evaluated cultivars, fermented and unfermented extracts of purple flesh-colored NIC 413 had higher total soluble phenolic content (TSP) and associated antioxidant activity, while off-white flesh colored Murasaki had high α-amylase enzyme inhibitory activity. Results suggested that beneficial LAB-based fermentation strategy was an effective post-harvest processing strategy for higher retention of phenolic bioactives and associated antioxidant and anti-hyperglycemic functionalities in sweet potato. Additionally, such LAB-fermented sweet potato with prebiotic potential can be integrated in health-focused dietary solution strategies, especially to support and improve human gut health and to mitigate chronic oxidative stress-linked NCD challenges.

Keywords: Antioxidants; Anti-diabetic, Anti-hypertension, Fermented sweet potato, Phenolics**.**

5.2. Introduction

Sweet potato is resilient to wide range of environmental and soil conditions, which makes it an ideal food crop choice for climate change adaptation-linked food security solutions globally (Placide et al., 2015). In addition to wide adaptability to climate change, it also offers nutritional benefits due to rich profiles of diverse bioactive compounds, essential minerals, and dietary fibers (CIP, 2014) for public health solutions. Sweet potato overall market and consumer demand of sweet potato is increasing rapidly, due to potential economic advantages, especially opportunities to improve livelihood and incomes of marginal and small-scale farmers worldwide (CIP, 2018). However, sweet potatoconsumer demand and overall market value largely depends on its nutritional profile and related high-value food applications, which must be improved. Its nutritional benefits of sweet potato are largely associated with its carbohydrate composition (resistant starch), dietary fiber profile, mineral content, and higher concentration of human health relevant bioactives (Kusano and Abe, 2000). Sweet potatoes are also suitable substrates that can

support the growth of beneficial bacteria in food matrix and therefore relevant for developing functional foods and beverages with probiotic and other human health benefits. Due to their rich source of dietary fiber and undigestible sugars such as raffinose, verbascose, and stachyose (Koubala et al., 2014).

Advancing biotransformation by utilizing beneficial microorganisms such as lactic acid bacteria-based fermentation in order to improve nutritional attributes coupled with organoleptic characteristics of sweet potatoes has significant merit. Beneficial microorganism-based biotransformation strategies improve shelf-life, nutritional qualities, sensory qualities, and organoleptic properties of appropriately fermented plant-based foods and beverages. These fermented foods and beverages are especially important to improve human gut health due to potential probiotic benefits. Therefore, controlled fermentation processes using several beneficial lactic acid bacteria (LAB) strains such as *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, and Bafidobacterium longum* can be improve post-harvest preservation qualities and human health relevant nutritional qualities in plant-based foods such as sweet potato.

Among different beneficial LAB strains, *Lactobacillus plantarum* is widely found in natural plant and plant-based food matrixes and can be targeted to develop high-value fermented foods and beverages with probiotic and other human health benefits associated nutritional qualities (Rodriguez et al., 2009). Previously, *Lactobacillus plantarum* –based fermentation was used to improve physicochemical properties of sweet potato starch and qualities of noodles derived from fermented sweet potato (Liao and Wu, 2015). Similarly, Yuliana et al. (2017) reported improvement of overall physicochemical properties of sweet potato flour after fermentation with beneficial LAB. Wu et al. (2012) found higher antioxidant activity, higher

organic acid content, and anthocyanin content in purple-fleshed sweet potato fermented milk with different strains of LABs such as *L. acidophilus, L. gasseri,* and *L. delbrueckii* subsp. *lactis*. These beneficial bacterial strains are known for releasing bioactive peptides, which are believed to be effective angiotensin-I-converting enzyme (ACE) inhibitors, a key dietary and therapeutic target for managing chronic hypertension (Rubak et al., 2020). Previous studies have also found presence of gamma aminobutyric acid (GABA) in LAB fermented food products, which is being considered an important food component with anti-hypertensive functions (Hayakawa et al., 2004). Additionally, LAB strains can act as strong biotransforming agents by releasing health promoting compounds that are generally bound in the food matrix through fermentation process. Previous studies have shown biotransformation of phenolic compounds, such as chlorogenic acid to caffeic acid by enzymes released from *Lactobacillus acidophilus* (Shen et al., 2018). During the biotransformation process, extracellular cellulolytic enzymes such as ferulic acid esterases (FAEs) impact the de-esterification of bound phenolics which are ester-linked to polysaccharides, lipids, organic acids, and proteins in the cell wall matrix (Szwajgier, 2010). Therefore, LAB-based fermentation is an effective liquid-state biotransformation strategy to improve stability, retention and functionalities of bioactive compounds and associated nutritional benefits of plant- based foods and beverages.

However, the potential role of beneficial LAB-based fermentation to improve phenolic bioactive-linked health benefits of fermented sweet potatoes, especially nutritional qualities that improve type 2 diabetes and associated health benefits has important relevance for public health needs. The main objective of this study was to advance and optimize beneficial LAB (*Lactobacillus plantarum*)-based fermentation to improve phenolic bioactive-linked antioxidant, and anti-hyperglycemic properties for three different flesh colored sweet potato cultivars, which

would provide the key biochemical rationale to develop sweet potato-based and health –targeted functional foods and beverages.

5.3. Materials and Methods

5.3.1. Sample Preparation

Three cultivars of sweet potato selected for this study were Murasaki (off-white-fleshed cultivar), Evangeline (orange-fleshed cultivar), and NIC 413 (purple-fleshed cultivar). These three cultivars were selected from our previous cultivar screening study and based on their high baseline phenolic content and associated health benefits (*in vitro* assay-based antioxidant and anti-diabetic properties). Beneficial LAB strain (*Lactobacillus plantarum*) was targeted to ferment aqueous extracts of selected sweet potato cultivars. For fermentation, four healthy tubers were randomly selected per cultivar, cleaned thoroughly, peeled, and chopped into small pieces. Approximately 40 g samples were weighed, added to 100 mL cold water and blended (Waring, commercial blender, Connecticut, USA) for 5 min at low speed. The solution was centrifuged (Sorvall Biofuge Primo centrifuge, Thermo scientific, USA) at 8000 rpm for 20 min twice and the supernatant was collected and stored in the refrigerator until fermentation.

5.3.2. Agar Media and MRS (Man, Rogosa, Sharpe) Broth Preparation

Agar media was prepared in a 500 mL Erlenmeyer flask to prevent overflowing of 17.5 g agar dissolved in 250 mL distilled water. Flasks were sealed tightly with aluminum foil and mixed thoroughly on 200 °C preheated hot plate using magnetic bar for 15 min. Samples were then incubated at 100^{o}C for 10 min before autoclaving. After autoclaving flasks, the agar medias were maintained at 55 ^{o}C for bacterial growth plate preparation. MRS Broth preparation was carried out in a beaker, where 8.25 g MRS was added to 150 mL distilled water and mixed well.

Then 10 mL broth was added to the test tubes and covered with steel cap. The test tubes containing broth were then autoclaved.

5.3.3. Lactic Acid Bacteria Strains and Inoculum Preparation

Fermentation was conducted in tightly sealed Erlenmeyer flasks (covered with aluminum foil) containing 45 mL of sweet potato extracts. Intially lactic acid bacterial strain (*Lactobacillus plantarum*) were inoculated into test tubes containing 10 mL Difco Lactobacilli MRS Broth (Becton, Dickson and Company Soarks, MD, USA) and then incubated for 24 h at 37 ^{o}C. This was followed by re-inoculation of 100 μL of the grown strain with 10 mL MRS broth for another 24 h at 37 ^{o}C. Then 5 mL bacterial culture after 48 h growth was added to each 45 mL sweet potato extract and incubated for 72 h at 37 ^{o}C. A control (only sweet potato extract) without bacterial culture was also used to compare with the fermented sample. Sampling (from fermented and control flasks) for biochemical analysis at 0, 24, 48, and 72 h time points during fermentation. Different serial dilutions (10^{-1}, 10^{-2}, $10^{-3,}$ 10^{-4},10^{-5}) were made using 100 μL of grown strain from broth and sterile water to determine the bacterial growth. Serially diluted samples were plated on MS agar media (Difco) and plates were incubated anaerobically in GasPak jars with anaerobe sachets (produces less than 1% of oxygen) for 48 h at 37 ^{o}C to determine the colony forming units of LAB.

5.3.4. Viable Cell Counts

Fermented sweet potato extracts were serially diluted to 10^{-5} followed by 100 μL aliquots of each dilution plated on duplicated MRS agar plates by the spread plate method. Later, these plates were incubated for 48 h at 37 ^{o}C. Plates containing viable cells of *Lactobacillus plantarum* at 0, 24, 48, 72 h incubation were recorded as Log CFU (colony forming units)/ mL.

5.3.5. Chemical Used

Porcine pancreatic alpha–amylase (EC 3.2.1.1), rat intestinal alpha–glucosidase (EC 3.2.1.20), 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Trolox, 2, 2- Azobis (2- amidinopropane) dihydrochloride (AAPH)). All chemicals were purchased from Sigma Chemical Co (St. Louis, MO).

5.3.6. Total Soluble Phenolic Content

The total soluble phenolic (TSP) content of aqueous extracts of unfermented and LAB fermented sweet potato samples was determined based on the protocol described by Shetty et al. (1995). The assay was carried out by adding 0.5 mL of fermented and unfermented sweet potato extract to 10 mL test tubes. For the control tubes, 0.5 mL of distilled water was added instead of a sweet potato extract. Each test tube also received 1 mL of 95 % ethanol, 5 mL of distilled water, and 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent. This was followed by the addition of 1 mL 5% sodium carbonate and the mixing of contents using a vortex mixer (Digital vortex mixer, Fisher Scientific, PA, USA) and then incubated for 60 min in the dark. After incubation, samples were remixed before reading the absorbance using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY) set at 725nm. The absorbance values were converted to total soluble phenolic content and expressed in milligrams gallic acid equivalent (GAE) per gram of fresh weight (FW) based on a standard curve that was established using a gallic acid concentration gradient when mixed in 95% ethanol.

5.3.7. Total Antioxidant Activity

The total antioxidant activity of unfermented and LAB fermented sweet potato samples were measured using two different assays: 2, 2-Dipheny-1—Picrylhydrazyl (DPPH) free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical

cation assay. The DPPH assay, described by Kwon et al. (2006) was used where 0.25 mL sample was added 1.5 mL centrifuge tubes followed by the addition of 1.25 mL 60 mM DPPH (in 95% ethanol) and mixing with a vortex mixer (Digital vortex mixer, Fisher Scientific, PA, USA) to ensure proper mixing prior to incubating for 4 min. Incubation, mixtures were centrifuged for 1 min at 13,000 rpm to pelletize the precipitate. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). Each sample had a corresponding control which contained 0.25 mL 95% ethanol instead of the sample. For the ABTS assay (Re et al., 1999) the same procedure was followed except that 1 mL of ABTS (matured stock adjusted with 95% ethanol) was added to 0.05 mL of fermented and unfermented sweet potato samples and the absorbance was measured at 734nm using a UV-visible spectrophotometer (Genesys 10S UV- VIS spectrophotometer, Thermo Scientific, NY). A control using 95% ethanol instead of sample was also used. Based on the absorbance readings, the inhibition percentages for both DPPH and ABTS radicals were calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \text{ x } 100 \qquad (5.1)$$

The inhibition percentage obtained from the DPPH and ABTS radical scavenging assays were expressed as mM Trolox equivalents (TE) per gram of sample based on Trolox standard curve.

5.3.8. α-Amylase Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the Worthington Enzyme Manual (1993a). Undiluted, half-diluted, and one-fifth diluted sample extracts were used to determine potential dose dependent response in α-amylase enzyme inhibitory activity. The dilutions were carried out using distilled water. The buffer used was 0.1M sodium phosphate (pH 6.9) with

0.006 M sodium chloride added to it. A volume of 500 µL, 250 µL and 100 µL sweet potato sample extract for the undiluted, halfdiluted, and one-fifth diluted, respectively was added to test tubes while the control tubes had 500 µL of buffer only. Additionally, each sweet potato sample extract had a corresponding sample blank tube which contained 500 µL of the sample extract. Then 500 µL of porcine pancreatic amylase (0.5 mg/ mL buffer) was added to all the tubes except for the sample blank and blank tubes and incubated for 10 min at 25°C. After incubation, 500 µL of 1% starch (1 g/100 mL buffer) was added to all the tubes and incubated for 10 min. The reaction was then stopped by the addition of 1 mL of 3, 5 dinitro salicylic acid (DNS) and the tubes were placed in a boiling water bath for 10 min at 100 °C after which the tubes were taken out and cooled at room temperature. The reaction mixture in the tubes was then diluted by adding 10 mL of distilled water to adjust the absorbance of the control to 1.0±0.02 and the absorbance was measured at 540 nm using a UV-VIS Genesys spectrophotometer (Genesys UV-visible, Milton Roy Inc, Rochester, NY)

The inhibition percentage of α amylase enzyme inhibitory activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - (\text{Abs extract} - \text{Abs sample blank})}{\text{Abs control}} \text{ x } 100 \qquad (5.2)$$

5.3.9. α-Glucosidase Enzyme Inhibitory Activity

The α-glucosidase enzyme inhibitory activity assay of unfermented and LAB fermented sweet potato samples was determined based on the protocol from Worthington Enzyme Manual (1993b) with some modifications taken from McCue et al. (2005). A volume of 50 µL (undiluted), 25 µL (half-diluted) and 10 µL (one-fifth diluted) of each sweet potato sample (for dose dependent response) extracts were pipetted into 96 well microtiter plates respectively. The half and one-fifth dilutions were made up to a total of 50 µL in volume by adding 25 µL and 40

µL of 0.1M potassium phosphate buffer (pH 6.9) respectively. Each sweet potato sample extract had a corresponding control with 50 µL of phosphate buffer instead of the sample. Finally, the volume in all the wells was made up-to 100 µL by the addition of 50 µL of phosphate buffer in each well including the control. Then 100 µL of 0.1M potassium phosphate buffer (pH 6.9) containing α-glucosidase enzyme (1 U/mL) was added to each well and incubated at 25°C for 10 min. After that, 50 µL of 5 mM p-nitrophenyl-α-D- glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were then incubated at 25°C for 5 min. Absorbance readings were taken before (0 min) and after (5 min) incubation period using a microplate reader (Thermomax, Molecular device Co., Virginia, USA) set at 405 nm.

The percentage of inhibition of α-glucosidase enzyme inhibitory activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs control 5min} - \text{Abs control 0min}) - (\text{Abs extract 5min} - \text{Abs extract omin}}{2\text{Abs control 5min} - \text{Abs control 0min}} \text{ x } 100 \quad (5.3)$$

5.3.10. Data Analysis

The entire LAB-based fermentation experiment was repeated two times. Each time Analysis at every time point from each experiment was carried out in triplicates. Means, standard errors, and standard deviations were calculated from replicates within the experiments and analyses were done using Microsoft Excel XP. The data were analyzed for analysis of variance (ANOVA) using Statistical Analytical Software (SAS version 9.4; SAS Institute, Cary, NC). Significant statistical differences between sweet potato cultivars, fermentation time points, and sweet potato cultivars × fermentation time point interactions for all *in-vitro* assays were determined using Tukey's least square means separation at the 0.05 probability level.

5.4. Results and Discussion

5.4.1. Total Soluble Phenolic Content

High perishability and spoilage of vegetables is a major post-harvest challenge that hinders their effective integration either as fresh or as processed food in health-focused nutritional solution strategies. Not only does high perishability occur at storage, but deterioration of nutritional qualities due to the chemical changes of bioactive compounds in stored vegetables is also a major constraint. Specifically, composition and biological activity of phenolic compounds undergo significant changes during post-harvest handling and storage. Additionally, different food processing methods affect the content and bioactivity of phenolic compounds in processed foods. Therefore, advancing strategies to improve stability and retention of phenolic metabolites and associated nutritional and health protective qualities of vegetables such as sweet potatoes is important for advancing high-value food and beverage applications. Fermentation using beneficial microorganism is a traditional food processing method, which improves post-harvest preservation qualities and often health relevant nutritional qualities of plant-based food substrates can be enhanced.

In this current study, beneficial LAB (*Lactobacillus plantarum*) strain was rationally recruited to bio-transform aqueous extracts of sweet potato in order to improve stability and retention of phenolic bioactive compounds and associated human health benefits. Three different flesh colored sweet potato cultivars were selected for LAB-based liquid state fermentation (72 h). The total soluble phenolic (TSP) content of fermented and unfermented sweet potato was determined using Folin-Ciocalteu reagent-based method. Additionally, the effect of changes in pH during fermentation on TSP content was also determined by comparing sweet potato extracts with natural pH and after adjusting to neutral pH (6.0) at all fermentation time points. Overall,

higher retention of total soluble phenolic (TSP) content was observed among all 3 sweet potato cultivars during 72 hours of fermentation (Table 5.1). Interestingly, the TSP content of fermented sweet potato was generally similar to the unfermented (control) sample even after adjusting to the neutral pH. Analysis of variance showed statistically significant difference in TSP content among 3 sweet potato cultivars, while the fermentation and interaction between cultivar × fermentation had no statistically significant effect. The differences in TSP content among cultivars might be due to different flesh colors, which was earlier observed in our cultivar screening study (Chapter 4). Among different sweet potato cultivars, the purple fleshed NIC 413 had significantly ($p<0.05$) higher TSP content across all fermentation time points.

Table 5.1. Total soluble phenolic (TSP) content (mg GAE/100g fresh weight) of unfermented and fermented (pH adjusted and unadjusted) sample of three sweet potato cultivars at different fermentation time points

Sweet potato unfermented and fermented samples	**Total soluble phenolic content (mg GAE/100g fresh weight) at different fermentation time points**			
	0 hour	**24 hours**	**48 hours**	**72 hours**
Murasaki control	0.74 ± 0.02^{abc}	0.80 ± 0.02^{abc}	0.67 ± 0.01^{abc}	0.60 ± 0.01^{bc}
Murasaki unadjusted pH	0.64 ± 0.01^{abc}	0.66 ± 0.01^{abc}	0.56 ± 0.01^{abc}	0.56 ± 0.00^{c}
Murasaki adjusted pH	0.67 ± 0.01^{abc}	0.70 ± 0.01^{bc}	0.62 ± 0.01^{abc}	0.58 ± 0.01^{bc}
Evangeline control	0.67 ± 0.01^{abc}	0.67 ± 0.01^{bc}	0.59 ± 0.01^{bc}	0.57 ± 0.01^{c}
Evangeline unadjusted pH	0.63 ± 0.01^{abc}	0.66 ± 0.00^{bc}	0.59 ± 0.01^{bc}	0.54 ± 0.01^{c}
Evangeline adjusted pH	0.68 ± 0.01^{abc}	0.69 ± 0.01^{abc}	0.64 ± 0.00^{abc}	0.59 ± 0.00^{bc}
NIC 413 control	1.84 ± 0.03^{a}	2.05 ± 0.02^{a}	1.64 ± 0.02^{ab}	1.59 ± 0.02^{ab}
NIC 413 unadjusted pH	1.58 ± 0.02^{ab}	1.66 ± 0.03^{abc}	1.14 ± 0.00^{abc}	1.12 ± 0.01^{abc}
NIC 413 adjusted pH	1.80 ± 0.01^{a}	1.93 ± 0.02^{ab}	1.11 ± 0.03^{abc}	1.09 ± 0.02^{ab}

± Standard error; n=12. Different letters represent significant differences in TSP content between cultivars × fermentation time point interactions at 95% probability level.

The slight improvement of TSP content was observed in purple-fleshed cultivar after 24 h fermentation and then it gradually reduced from 24-72 h. However, the reduction of TSP content was not statistically different when compared to the results of 0 h and unfermented sample. Previously, Wu et al. (2012) reported increased phenolic acid content in sweet potato (purple-fleshed) based fermented milk. In another study, an increase in phenolic content was reported for

fermented sweet potato when compared to the unfermented raw and boiled sample (Shen et al., 2018). However, the changes in phenolic content in these studies might be related to the changes in phenolic composition, specifically the release of some bound fractions after fermentation. The current TSP content results and those from previous studies suggest that LAB-based fermentation is an effective strategy to improve or retain phenolic content of sweet potato and can be targeted to design sweet potato-based functional foods and beverages with probiotic benefits. It is also important to investigate the effect of LAB based fermentation on human health relevant functionalities such as antioxidant, anti-hyperglycemic, and anti-hypertensive properties of sweet potato based fermented foods.

5.4.2. Total Antioxidant Activity

The total antioxidant activity of unfermented and LAB-fermented sweet potato samples was determined using two different assays: 2, 2-Dipheny-1—Picrylhydrazyl (DPPH) free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay. Statistically significant ($p<0.05$) differences in antioxidant activity (DPPH assay) between sweet potato cultivars, fermentation time points, and interactions between cultivars x fermentation time points were observed (Table 5.2). The highest total antioxidant activity (DPPH-based) was observed in the fermented and unadjusted pH sample of purple-fleshed (NIC-413) (83.82 %) sweet potato followed by unfermented (80.52 %) and fermented with adjusted pH (70.8 %) of same cultivar after 24 h of fermentation. However, for the other two sweet potato cultivars, high antioxidant activity was observed in 0 h sample when compared to other fermentation time points. Interestingly, even after 72 h fermentation, fermented sweet potato sample of Murasaki and Evangeline showed similar antioxidant activity (DPPH-based) as 24 h and 48 h time points. Therefore, results of this present study indicated that LAB-based

fermentation is an effective strategy to retain and even to improve antioxidant activity of sweet potatoes.

Table 5.2. Total antioxidant activity (DPPH free radical scavenging capacity-based assay) of unfermented and fermented (pH adjusted and unadjusted) sample of three sweet potato cultivars

Sweet potato unfermented and fermented samples	**Total antioxidant activity (%) at different fermentation time points**			
	0 hour	**24 hours**	**48 hours**	**72 hours**
Murasaki control	55.54 ± 0.52^{efghijk}	42.29 ± 0.52^{klmno}	34.59 ± 1.32^{mno}	29.31 ± 0.29^{o}
Murasaki unadjusted pH	46.05 ± 0.71^{ijklmno}	38.08 ± 0.72^{klmno}	30.90 ± 1.30^{o}	34.22 ± 0.54^{on}
Murasaki adjusted pH	51.90 ± 0.38^{ghijklmn}	45.26 ± 0.73^{jklmno}	41.19 ± 0.56^{klmno}	40.25 ± 0.4^{klmno}
Evangeline control	64.67 ± 0.39^{bcdefgh}	52.71 ± 0.54^{jklmno}	49.32 ± 0.60^{hijklmn}	37.36 ± 0.34^{lmno}
Evangeline unadjusted pH	60.82 ± 0.18^{defighj}	42.92 ± 0.43^{mnopqr}	44.42 ± 0.31^{jklmno}	43.48 ± 0.76^{jklmno}
Evangeline adjusted pH	62.70 ± 0.45^{cdefghi}	52.56 ± 0.38^{jkl}	50.42 ± 0.90^{ghijklmn}	49.94 ± 0.43^{ghijklmn}
NIC 413 control	76.70 ± 0.07^{abcd}	80.52 ± 0.27^{abc}	74.67 ± 0.12^{abcd}	74.75 ± 0.06^{abcd}
NIC 413 unadjusted pH	78.30 ± 0.99^{ab}	83.83 ± 0.32^{a}	79.74 ± 0.53^{abc}	79.81 ± 0.68^{abc}
NIC 413 adjusted pH	75.61 ± 0.12^{abcdefg}	78.68 ± 0.30^{abcd}	71.76 ± 0.05^{abcde}	70.31 ± 0.71^{abcdef}

± Standard error; n=12. Different letters represent significant differences in antioxidant activity between cultivars x fermentation time points interactions at 95% probability level.

Previously, many *in vitro* studies have found high antioxidant activities in anthocyanins rich purple sweet potatoes along with ameliorative effects and antihypertensive properties (Teow et al., 2007; Yamakoshi et al., 2007). Wu et al. (2011) reported significantly high ($p<0.01$) free radical scavenging activity in Chingshey purple sweet potato after fermentation with three different strains of *Lactobacillus* such as *L. gasseri, L. acidophilus,* and *L. delbrueckii subsp. lactis.* In this above study, improved antioxidant activities, higher GABA (gamma-aminobutyric acid) concentration, anthocyanins, and organic acids content were observed in fermented milk developed from Chingshey purple sweet potato. Similarly, Zhong-hue and Jie (2015) observed

high superoxide anion radicles scavenging activity and high Fe^{+3} ions reducing capacity in wine derived from purple fleshed sweet potato.

Table 5.3. Total antioxidant activity (ABTS free radical scavenging capacity-based assay) of unfermented and fermented (pH adjusted and unadjusted) sample of three sweet potato cultivars

Sweet potato unfermented and fermented samples	**Total antioxidant activity (%) at different fermentation time points**			
	0 hour	**24 hours**	**48 hours**	**72 hours**
Murasaki control	99.00±0.1^{a}	98.71±0.82^{a}	95.51±0.16^{ab}	71.45±0.25^{e}
Murasaki unadjusted pH	90.91±0.13^{abcd}	84.01±0.52^{bcde}	81.68±0.25^{cde}	81.22±0.07^{de}
Murasaki adjusted pH	98.86±0.66^{a}	98.48±1.93^{a}	98.24±0.10^{ab}	89.50±0.14^{abcd}
Evangeline control	99.10±2.17^{a}	99.06±0.14^{a}	98.72±0.61^{a}	98.43±0.12^{a}
Evangeline unadjusted pH	99.25±0.11^{a}	93.82±0.08^{abcd}	94.27±0.38^{abc}	89.29±0.17^{abcd}
Evangeline adjusted pH	99.30±0.36^{a}	99.08±0.11^{a}	98.74±1.75^{a}	98.45±0.18^{a}
NIC 413 control	98.98±0.22^{a}	98.66±0.21^{a}	98.07±0.13^{ab}	98.14±0.09^{ab}
NIC 413 unadjusted pH	98.99±2.68^{a}	99.04±0.12^{a}	98.95±0.13^{a}	98.96±0.11^{a}
NIC 413 adjusted pH	98.97±0.58^{a}	98.90±0.11^{a}	98.47±0.33^{a}	98.64±0.10^{a}

± Standard error; n=12. Different letters represent significant differences in antioxidant activity between cultivars x fermentation time points interactions at 95% probability level.

Like the results of TSP content and DPPH-based antioxidant activity, statistically significant ($p<0.05$) differences in antioxidant activity (ABTS-based assay) was observed between sweet potato cultivars x fermentation time points (Table 5.3). In general, even after 72 h fermentation, high antioxidant activity was observed in purple-fleshed (NIC 413) and orange-fleshed (Evangeline) sweet potato sample, while mean antioxidant activity was reduced in the off-white-fleshed (Murasaki) sweet potato sample.

However, the fermented sample of Murasaki (pH adjusted) had significantly higher antioxidant activity when compared to unfermented control sample after 72 h fermentation. Therefore, results of this study indicated that LAB based fermentation had positive effect on retaining and improving antioxidant activity of sweet potatoes and such strategy can be targeted for designing sweet potato-based functional foods and beverages.

5.4.3. Alpha-Amylase Enzyme Inhibitory Activity (%)

To understand the potential anti-hyperglycemic property of fermented sweet potato sample, α-amylase enzyme inhibitory activity was determined using *in vitro* assay model. Moderate to high α-amylase enzyme inhibitory activity (ranging from 67.76 % to 89.93 %) was observed in fermented and unfermented sweet potato samples of three cultivars with different flesh color (Table 5.4).

Significant ($p<0.05$) dose-dependent response [undiluted, half-diluted with water, and one-fifth diluted (data not presented because of zero inhibition was recorded for all the samples) with water] α-amylase enzyme inhibitory activity was also observed in fermented and unfermented samples. Overall, cultivar differences, fermentation, and pH of the sample had statistically significant effect on α-amylase enzyme inhibitory activity ($p<0.05$), however no statistically significant difference was observed between cultivars x fermentation time point interactions. The α-amylase enzyme inhibitory activity gradually decreased from 0 to 72 h in most samples. The rate of reduction was significantly higher for purple-fleshed sweet potato cultivar (NIC 413). The fermented sweet potato sample with natural pH had higher α-amylase enzyme inhibitory activity in most time points, and this was primarily due the interference of acidic condition. Among three sweet potato cultivars, fermented sample of Murasaki had higher α-amylase enzyme inhibitory activity even after 48 and 72 h fermentation. Therefore, for anti-hyperglycemic benefits, LAB-based fermentation can only be targeted for select sweet potato cultivars. Previously, reduction in α-amylase enzyme inhibitory activity was observed in fermented yam (Medoua et al., 2008). However, higher phenolic-linked antioxidant activity may potentially contribute to compensate for the lower α-amylase enzyme inhibitory activity of fermented purple-fleshed sweet potato. Additionally, it is also important to investigate other anti-

Table 5.4. α-Amylase enzyme inhibitory (%) activity (undiluted and half diluted) of unfermented and fermented (pH adjusted and unadjusted) sweet potato samples

Sweet potato unfermented and fermented samples	**Alpha-amylase enzyme inhibitory (%) activity at different fermentation time points**							
	0 hour		**24 hours**		**48 hours**		**72 hours**	
	Undiluted	**Half-diluted**	**Undiluted**	**Half-diluted**	**Undiluted**	**Half-diluted**	**Undiluted**	**Half-diluted**
Murasaki control	88.39^{ab}	2.71^{abc}	76.21^{abc}	26.70^{ab}	81.90^{abc}	6.95^{abc}	72.44^{ab}	4.73^{abc}
Murasaki unadjusted pH	77.31^{abc}	1.79^{abc}	80.06^{abc}	19.4^{ab}	94.87^{a}	28.29^{a}	47.85^{abc}	32.68^{a}
Murasaki adjusted pH	88.84^{ab}	10.35^{abc}	55.03^{abc}	14.88^{ab}	89.89^{ab}	4.87^{abc}	46.84^{abc}	4.61^{abc}
Evangeline control	95.33^{a}	6.26^{abc}	76.55^{abc}	31.48^{a}	84.31^{abc}	12.89^{ab}	53.23^{abc}	17.18^{ab}
Evangeline unadjusted pH	83.95^{abc}	6.29^{abc}	69.38^{abc}	18.64^{ab}	88.17^{ab}	22.31^{ab}	78.05^{ab}	22.42^{ab}
Evangeline adjusted pH	59.32^{abc}	13.09^{ab}	63.01^{abc}	6.35^{abc}	47.09^{abc}	8.02^{abc}	21.48^{bc}	8.45^{abc}
NIC 413 control	83.79^{abc}	9.67^{abc}	82.88^{abc}	37.18^{a}	65.11^{abc}	15.67^{ab}	80.38^{ab}	21.76^{ab}
NIC 413 unadjusted pH	66.01^{abc}	3.56^{abc}	67.22^{abc}	23.28^{ab}	73.28^{abc}	42.81^{a}	69.51^{abc}	17.44^{ab}
NIC 413 adjusted pH	78.01^{abc}	10.05^{abc}	14.26^{c}	10.89^{abc}	26.86^{abc}	10.97^{abc}	28.87^{abc}	10.77^{abc}

± Standard error; n=12. Tukey grouping for LS-Means were done separately for each dilution. Different letters represent significant differences in α-amylase enzyme inhibitory activity between fermented and unfermented sweet potato samples at 95% probability level (separate for both dilutions).

hyperglycemic relevant functionalities of LAB-fermented sweet potatoes in order to understand the overall impact of fermentation and potential health benefits.

5.4.4. Alpha Glucosidase Enzyme Inhibitory Activity

The potential inhibitory activity of another key anti-hyperglycemic relevant enzyme, α-glucosidase, of fermented and unfermented sweet potato sample was also determined using *in vitro* assay model. In this study, low to moderate (13- 55%) α-glucosidase enzyme inhibitory activity was observed in fermented and unfermented sweet potato samples (Table 5.5). Additionally, dose-dependent [undiluted, half-diluted with water, and one-fifth diluted (data not presented) with water] response in α-glucosidase enzyme inhibitory activity was also found in fermented sweet potato sample. Interestingly, α-glucosidase enzyme inhibitory activity of Murasaki (off-white-fleshed) sweet potato improved after 72 h fermentation, while for orange-fleshed (Evangeline) and purple-fleshed (NIC-413) cultivars, it remained statistically at par between 0 to 72 h fermentation time points. Overall, the results of α-glucosidase enzyme inhibitory activity showed statistically significant ($p<0.05$) differences among cultivars, between fermentation time points, and cultivars x fermentation time points interactions of in undiluted samples. The moderate α-amylase and α-glucosidase enzyme inhibitory activity at 72 h in fermented Murasaki sweet potato sample has significant relevance for its potential anti-hyperglycemic focused food and beverage application. The higher retention of α-glucosidase enzyme inhibitory activity along with high phenolic-linked antioxidant activity in select sweet potatoes after 48 and 72 h LAB-based fermentation are key findings of our study. The higher retention and stability of phenolic content might also have relevance in retaining and improving α-glucosidase enzyme inhibitory activity in fermented sweet potato sample.

Table 5.5. α-Glucosidase enzyme inhibitory (%) activity (undiluted and half diluted) of unfermented and fermented (pH adjusted and unadjusted) sweet potato samples

Sweet potato unfermented and fermented samples	Alpha-glucosidase enzyme inhibitory (%) activity at different fermentation time points							
	0 hour		24 hours		48 hours		72 hours	
	Undiluted	Half-diluted	Undiluted	Half-diluted	Undiluted	Half-diluted	Undiluted	Half-diluted
Murasaki control	42.35^{fghi}	28.12^{abcd}	41.19^{ghij}	30.93^{a}	43.77^{efghi}	31.89^{a}	48.90^{abcdef}	32.63^{a}
Murasaki unadjusted pH	44.37^{efghi}	29.22^{abcd}	50.48^{abcde}	32.11^{a}	55.62^{abc}	34.67^{a}	50.17^{bcde}	30.30^{ab}
Murasaki adjusted pH	34.55^{jklm}	18.32^{gh}	29.53^{m}	27.21^{abcde}	30.04^{lm}	19.53^{efgh}	55.07^{abc}	33.86^{a}
Evangeline control	48.22^{cdefg}	32.30^{a}	47.03^{defgh}	33.55^{a}	47.39^{defg}	34.84^{a}	47.18^{defg}	30.55^{a}
Evangeline unadjusted pH	44.92^{efghi}	30.11^{ab}	44.65^{efghi}	27.92^{abcd}	48.59^{cdefg}	31.12^{a}	56.22^{ab}	32.92^{a}
Evangeline adjusted pH	37.76^{ijkl}	26.94^{abcdef}	34.08^{jklm}	21.61^{defgh}	34.80^{jklm}	22.64^{bcdefg}	32.66^{klm}	18.97^{fgh}
NIC 413 control	48.37^{cdefg}	32.56^{a}	47.58^{defg}	30.53^{a}	48.68^{cdef}	33.71^{a}	48.82^{bcdef}	32.37^{a}
NIC 413 unadjusted pH	48.38^{cdefg}	31.21^{a}	53.64^{abcd}	29.97^{abc}	55.66^{abc}	13.91^{h}	57.88^{a}	30.52^{a}
NIC 413 adjusted pH	38.81^{ijk}	26.92^{abcdef}	39.65^{kl}	22.05^{cdefg}	28.02^{m}	13.74^{h}	30.04^{m}	14.94^{gh}

Tukey grouping for LS-Means were done separately for each dilution. Different letters represent significant differences in α-glucosidase enzyme inhibitory activity between cultivars x fermentation time points interactions at 95% probability level.

Previously, presence of specific phenolic compounds such as 6-O-caffeoylsophorose in combination with acylation of phenolics to sugar in fermented sweet potato were found to be associated with reduced postprandial glucose levels in rat model (Matsui et al., 2004). Therefore, not only just phenolic content, but the changes in composition of phenolic acids during fermentation through release of bound phenolics might have significant impact, which potentially determines anti-hyperglycemic and other human health relevant functionalities of fermented foods and beverages. The results of the present study suggested that LAB-based fermentation can be targeted to improve stability and retention of phenolic bioactive linked antioxidant and anti-hyperglycemic functionalities in select sweet potato cultivars, especially for off-white-fleshed sweet potato cultivars (Murasaki).

5.4.5. Viable cell count of Lactobacillus plantarum fermented sweet potato cultivars

The presence of active LAB counts in fermented food and beverage sample is important for their potential probiotic and other gut health benefits. In this present study, viable cell counts of *Lactobacillus plantarum* in fermented sweet potato sample was determined at different time points such as 0, 24, 48, and 72 h during fermentation. Maximum counts of *L. plantarum* was observed during initial stages of fermentation (0 and 24 h) when compared to later periods (48 and 72 h), which could be due to the presence of more nutrients and phenolic compounds at initial fermentation stage (Table 5.6 and Figure 5.1). Overall, there was reduction in colony forming units of LAB during fermentation from 24 -72 h. The decrease in the viable cell counts might be due to the differences in pH or due to declining growth of lactic acid bacteria after consumption of all available nutrients that are required to sustain their growth. Therefore, for probiotic application, 24 h fermentation of sweet potato might be optimum, while longer period of LAB fermentation can be targeted for other health-focused food and therapeutic applications.

Furthermore, other plant-based food with different fiber and bioactive profile can also be integrated with fermented sweet potato for improving the growth of LAB and to enhance the probiotic and human gut health benefits. Such fermentation strategy and food synergies can be targeted to design novel sweet potato-based fermented foods and beverages with diverse health benefits.

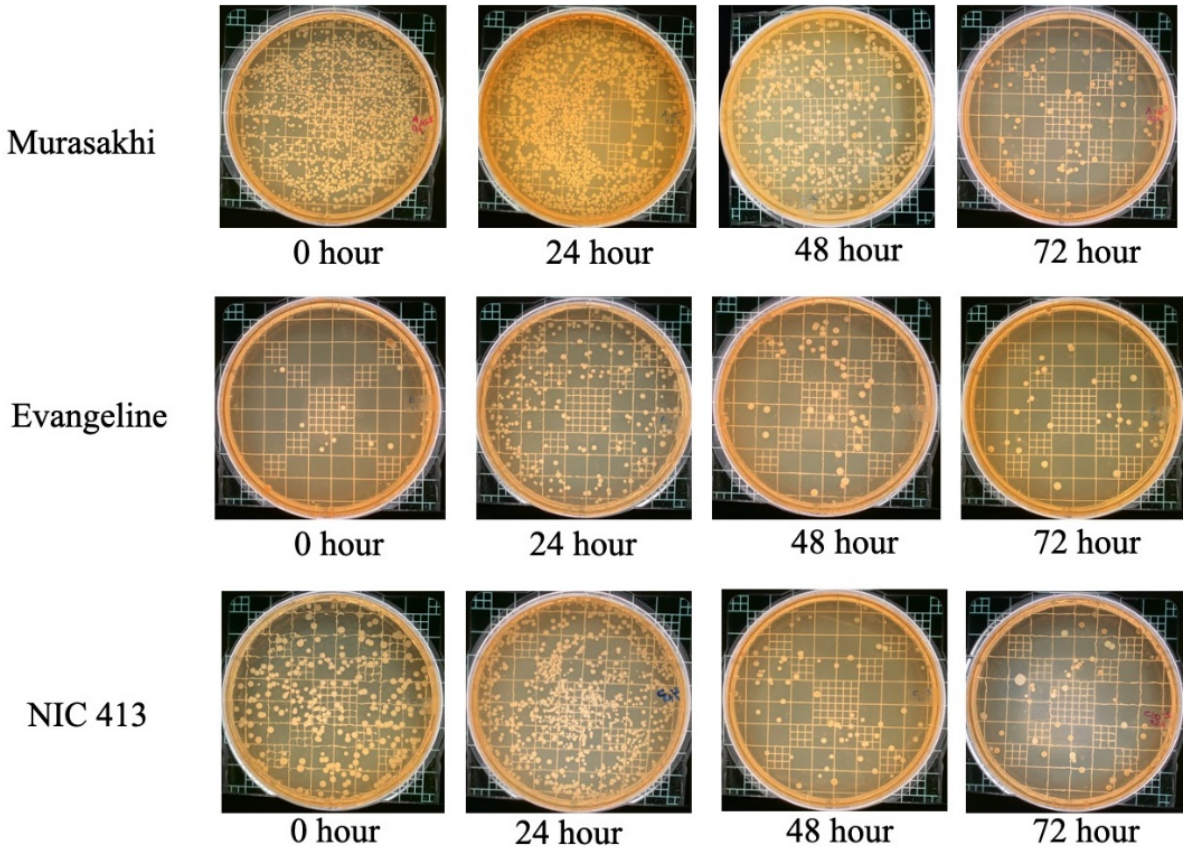

Figure 5.1. Viable cell count of *Lactobacillus plantarum* (at 10^{-3} dilution) in fermented sweet potato cultivars; Murasaki, Evangeline, and NIC 413 at 0, 24, 48, 72 h incubation (37 °C)

Table 5.6. Viable cell count of *Lactobacillus plantarum* in fermented sweet potato samples at 0, 24, 48, 72 hours of incubation (37 °C)

Fermentation incubation interval	**Sweet potato cultivar**	**Dilution**	**Viable cell count (Log CFU/mL)**
0 hour	Murasaki	10^{-3}	5.89
	Murasaki	10^{-4}	5.26
	Murasaki	10^{-5}	0
	Evangeline	10^{-3}	2.21
	Evangeline	10^{-4}	2.02
	Evangeline	10^{-5}	0
	NIC 413	10^{-3}	6.23
	NIC 413	10^{-4}	5.49
	NIC 413	10^{-5}	0
24 hours	Murasaki	10^{-3}	6.68
	Murasaki	10^{-4}	6.47
	Murasaki	10^{-5}	6.12
	Evangeline	10^{-3}	4.28
	Evangeline	10^{-4}	3.95
	Evangeline	10^{-5}	3.34
	NIC 413	10^{-3}	6.72
	NIC 413	10^{-4}	5.9
	NIC 413	10^{-5}	6.15
48 hours	Murasaki	10^{-3}	4.10
	Murasaki	10^{-4}	3.94
	Murasaki	10^{-5}	3.21
	Evangeline	10^{-3}	2.45
	Evangeline	10^{-4}	1.91
	Evangeline	10^{-5}	0
	NIC 413	10^{-3}	4.61
	NIC 413	10^{-4}	3.27
	NIC 413	10^{-5}	0
72 hours	Murasaki	10^{-3}	3.78
	Murasaki	10^{-4}	3.22
	Murasaki	10^{-5}	2.96
	Evangeline	10^{-3}	2.71
	Evangeline	10^{-4}	1.92
	Evangeline	10^{-5}	1.04
	NIC 413	10^{-3}	3.60
	NIC 413	10^{-4}	2.67
	NIC 413	10^{-5}	1.64

5.5. Conclusions

In the present study, higher retention of phenolic bioactive-linked antioxidant and anti-hyperglycemic functionalities were observed in fermented sweet potato samples. High total soluble phenolic (TSP) content and high total antioxidant activity were observed at 24 h and it remained statistically similar even after 72 h of fermentation. Moderate to high anti-hyperglycemic property relevant α-amylase (69.51 %) and α-glucosidase (57.88 %) enzyme inhibitory activities were also observed in fermented sweet potato extracts. Among the three sweet potato cultivars, fermented and unfermented extracts of purple-fleshed colored NIC 413 had higher TSP content and associated antioxidant activity, while off-white fleshed colored Murasaki showed higher anti-hyperglycemic benefit relevant α-amylase and α-glucosidase enzyme inhibitory activities. Therefore, LAB based fermentation can be targeted for improving retention of phenolic bioactives and associated functionalities in sweet potato-based foods and beverages. Additionally, such LAB-fermented sweet potato with prebiotic potential can be integrated in health-focused dietary solution strategies, especially to support and improve human gut health and to mitigate chronic oxidative stress-linked early stages of type 2 diabetes and other NCDs. Improving bioavailability and mobilization of soluble phenolics of sweet potato using LAB-based fermentation is an exciting strategy for the development of functional food ingredients and value-added probiotic beverages. This can provide improved nutritional and health benefits and will create new opportunities for designing novel sweet potato based value-added products, which can be rationally integrated in dietary support strategies to counter diet-linked type 2 diabetes and other non-communicable chronic diseases (NCDs).

CHAPTER 6. DEEP-FRYING PROCESS OPTIMIZATION OF SWEET POTATO BASED ON PHENOLIC BIOACTIVES-LINKED ANTIOXIDANT AND ANTI-HYPERGLYCEMIC PROPERTIES USING RESPONSE SURFACE METHODOLOGY (RSM) MODEL

6.1. Abstract

Sweet potato-based deep-fried foods, such as French fries and chips are becoming increasingly popular among consumers worldwide. However, nutritional and human health-relevant bioactive qualities of sweet potatoes change rapidly during the deep fat frying process. Therefore, optimizing the deep-frying processing of sweet potatoes based on their bioactive profile and associated health benefits has significant merit, specifically to address increasing health concerns of consumers about the health benefits of deep-fried food products. In this study, a well-established mathematical model, response surface methodology (RSM) based central composite orthogonal design (CCOD) was used to optimize deep-frying processing variables of orange-fleshed sweet potato. This deep-frying process optimization design (CCOD), included key independent variables, such as frying temperature (140 to 170°C), frying time (4 to 5 min), and sample size (different cube sizes of 1 to 2 cm), and their impacts on retention of nutritionally-relevant response variables (total soluble phenolics, total antioxidant activity, and type 2 diabetes relevant anti-hyperglycemic properties, protein content, color difference, and weight loss percent) was investigated. Results of this study indicated that the optimum deep-frying processing conditions of sweet potato were frying temperature of 155 °C, frying time of 4 min 30 sec, and sample size of 1.5 cm, which resulted in the higher retention of phenolic bioactives and associated health benefits. Under such optimized deep-frying conditions, processed sweet potato had higher retention of total soluble phenolic content [8.42 mg GAE/g

dry weight(DW)], protein content (15.32 mg/g DW), high antioxidant activity (1.08 mM Trolox/g DW-DPPH based assay & 1.18 mM Trolox/g DW ABTS-based assay), and moderate anti-hyperglycemic properties (37.43 %, α-amylase and 42.06% α-glucosidase enzyme inhibitory activities). Overall, the results of this study not only revealed the impact of deep-frying variables (frying temperature, frying time, and sample size) on phenolic-linked health benefits but also optimized the processing conditions for deep-frying to develop commercial sweet potato-based fried food products. Such superior phenolic antioxidant-linked bioactive qualities of processed sweet potato have relevance as anti-hyperglycemic diets for potential targeted management of early stages of type 2 diabetes.

6.2. Introduction

Deep-frying processing is considered as one of the oldest food processing methods that has been widely used in food preparation around the globe (Sanchez-Muniz and Bastida, 2006). Many plant-based foods such as banana, potato, sweet potato, cassava, jackfruit, leafy vegetables, onions, and chickpea are commonly deep-fried for regular consumption worldwide. The popularity of deep-fried foods among consumers is mainly due to their unique taste, textures, and appealing color. Additionally, simplicity during food preparation and less cooking time also make it as an ideal processing method for both household cooking and commercial production. Furthermore, due to rapid heat and mass transfer between food and oil at high temperature, deep-fried foods also have higher shelf-life. During deep-frying, food components go through different structural and physicochemical changes such as absorption of fats, dehydration of food surface, texture modification and generation of new organoleptic, and sensory characteristics (Krokida et al., 2000). A study conducted by Manjunathan et al. (2014) demonstrated frying kinetics of mass transfer during Gethi (*Dioscorea kamoonensis Kunth*)

deep-frying processing and observed a significant decrease in moisture content and increase in oil absorption proportionately with increase in deep-frying temperature. Specifically, removal of moisture content from fried food product is important to extend shelf-life of the processed food, as water activity of food dictate the microbial contamination, growth, and subsequent spoilage (Abdullah et al., 2000).

Therefore, deep frying is a common food processing method to extend shelf-life of highly perishable fruits and vegetables such as sweet potato, banana, potato, and cassava. In this context, sweet potato (*Ipomoea batatas* L.), a nutritionally rich tropical tuber crop (Truong et al., 2018) is widely consumed as deep-fried food across the globe. In most of the African countries, the majority of harvested sweet potato tubers are processed into dried chips or flour for long-term storage and year-round consumption. Sweet potato-based processed foods, such as deep-fried, vacuum fried, air fried, boiled, baked, and steam cooked are very popular among consumers due to their unique flavor, taste, and texture. A consumer preference study by Caetano et al. (2018) found higher acceptability of deep-fried sweet potatoes with greater purchase intention by consumers when compared to oven-baked and air fried sweet potato chips. In another study, faster cell wall breakdown and quicker absorption of nutrients during *in vitro* gastric digestion was observed with deep-fried sweet potato when compared to steam-cooked sweet potato products (Somaratne et al., 2020). However, during the deep frying process, human health protective functional properties of sweet potato may alter substantially, which could affect overall nutritional and health relevant bioactive qualities of deep-fried foods.

Therefore, to retain the nutritional and bioactive qualities and associated health benefits, it is important to optimize different processing parameters/conditions of deep fat frying of sweet potatoes, especially to produce healthy snacks with acceptable sensory qualities. Additionally, it

is also important to optimize specific deep-frying processing variables (frying temperature, frying time, sample size) to get uniform and highly desirable end products (less oily, nutritious fried products). In this context, response surface methodology (RSM) is an effective statistical model, which helps to optimize different processing conditions of food products under controlled experimental set-up (Bas and Boyaci, 2007). Additionally, RSM model helps to explain the relationship between independent (frying temperature, frying time, sample size) and dependent variables (quality determining attributes of product) related to specific food processing parameters. Among several RSM models, central composite orthogonal design (CCOD) is a factorial/fractional-factorial design which allows the optimization (includes maximize, minimize, or attain a specific target) of response variables with group of axial points and center points and minimizes the variations in the regression coefficients. Furthermore, CCOD allows the experimental designer to better understand about extreme levels of independent factors influencing outcomes of the experiment (other than experimental points/runs) (Olawoye, 2016).

There are several previous studies which explored process optimization of deep fat frying conditions based on food quality attributes (to minimize oil content) (Oladejo et al., 2017; Rady et al., 2019; Stastny et al., 2014a and Stastny et al., 2014b; Timalsina et al., 2019), however very few studies have attempted to investigate the impact of different processing variables on phenolic bioactive-linked antioxidant and anti-diabetic functionalities of vegetable-based deep-fried foods. Therefore, the overarching goal of this study was to optimize deep frying processing conditions by investigating the impact of different processing variables on phenolic bioactives and associated health benefits such as antioxidant and anti-hyperglycemic functionalities of deep-fried sweet potatoes. The specific objective of this study was to optimize different processing variables such a frying temperature, frying time, and sweet potato sample size based

on their impacts on total soluble phenolic content (TSP), antioxidant properties, and anti-hyperglycemic/type 2 diabetes relevant functionalities of deep fried sweet potato, which was determined using *in vitro* assay methods.

6.3. Materials and Methods

6.3.1. Sweet Potato Tuber Processing

Orange-fleshed sweet potato tubers were purchased from a local grocery store (Hornbacher's, Fargo, USA). The selection of an orange-fleshed cultivar was based on higher consumer preference and wider use of this sweet potato type in processed foods. Fresh tuber samples were stored at 10 °C for acclimatization prior to conducting deep frying experiments. The sweet potato tubers were washed, manually peeled with a hand peeler, and cut into three different cube sizes (1, 1.5, 2 cm as shown in Figure 6.1).

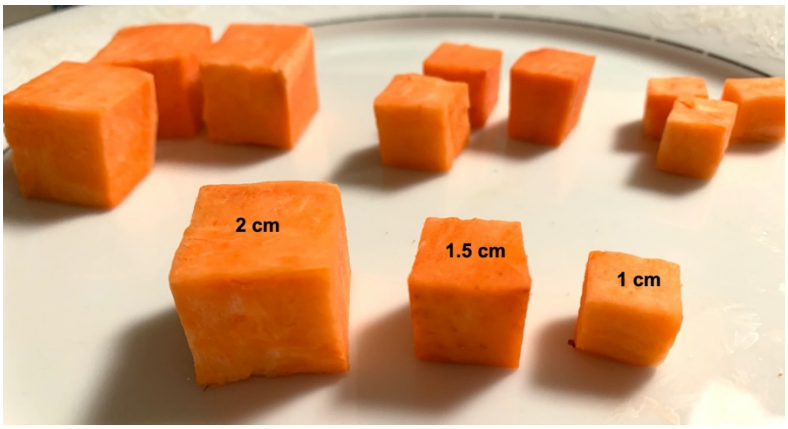

Figure 6.1. Different dimensions of sweet potato cubes used in the experiment

6.3.2. Deep-Frying Processing

Deep-frying experiments were conducted in a stainless steel deep-fryer (model: Cuisinart CDF-200P1, Stamford, CT, USA) purchased from Amazon.com. A series of deep-frying experiments were carried out as listed in Table 6.2. For each experimental run, sample size of 200 g sweet potato cubes was fried in 3-liters mixed vegetable oil. Fried cubes were removed

from oil and excess oil was drained out keeping cubes in heavy stainless-steel basket. Additionally, excess oil was removed by placing fried cubes on paper towel for 5 min and allowed them to cool. After cooling, deep-fried sweet potato cubes were packed and stored in labelled low-density polyethylene (LDPE) zip-top bags for wet lab and biochemical analysis.

6.3.3. Hot Water Extracts of Deep-Fried Sweet Potato

Deep fried sweet potato cubes were extracted with hot water for an *in vitro* model based biochemical analysis. Deep-fried sweet potato cubes of 40 g were mixed with hot water (200 mL of boiled 100 °C) and blended for 5 min using a blender (Waring, USA) at HIGH speed setting. The blended mixture was then transferred to 50 mL tubes and centrifuged at 8000 rpm for 20 min. This centrifugation step was repeated two times and the supernatant was collected and stored in at 2-4 °C.

6.3.4. Chemical Used

Porcine pancreatic alpha–amylase (EC 3.2.1.1), rat intestinal alpha–glucosidase (EC 3.2.1.20), 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Bio-rad protein dye, and Trolox, 2, 2- Azobis (2- amidinopropane) dihydrochloride (AAPH)). These chemicals were purchased from Sigma Chemical Co (St. Louis, MO).

6.3.5. Total Soluble Phenolic Content

A modified protocol described by Shetty et al. (1995) was used to determine the total soluble phenolic (TSP) content of deep-fried sweet potato extracts. Assay was carried out by taking 0.5 mL of hot water extracts of deep-fried sweet potato into 10 mL glass test tubes. For measuring blank, 0.5 mL of distilled water was added instead of the sample. Then 1 mL of 95 % ethanol, 5 mL of distilled water, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent, and 1 mL 5%

sodium carbonate were added sequentially. Then all reagents were mixed thoroughly by using a vortex mixer and kept under dark condition for 60 min incubation. After incubation, samples were taken out and mixed again before reading the absorbance using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY) set at 725 nm. The absorbance values were converted to total soluble phenolic content (TSP) expressed in milligrams gallic acid equivalent (GAE) per gram of dry weight (DW) based on a standard curve that was established using different concentrations of gallic acid in 95% ethanol.

6.3.6. Total Antioxidant Activity

The total antioxidant activity of the deep-fried sweet potato extracts was measured using two different assays: 2, 2-Dipheny-1—Picrylhydrazyl (DPPH) free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay. The DPPH assay as described by Kwon et al. (2006) was used in which 0.25 mL sample was taken in 1.5 mL centrifuge tubes. Then 1.25 mL of 60 mM DPPH (in 95% ethanol) was added to micro-centrifuge tubes containing sample extract. The tubes were mixed well using a vortex mixer to ensure proper mixing and then incubated for 5 min. After that, mixtures were centrifuged at 13000 rpm for 1 min to pellet down the precipitate. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). Each sample had a corresponding control which contained 0.25 mL of 95% ethanol instead of the sample. For the ABTS assay (Re et al., 1999), the same procedure was followed except in this case 1 mL of ABTS (matured stock adjusted with 95% ethanol) was added to 0.05 mL of sweet potato extracts and the absorbance was measured at 734 nm using a UV-visible spectrophotometer (Genesys 10S UV- VIS spectrophotometer, Thermo Scientific, NY). A control using 95% ethanol instead of sample was

also used. Based on the absorbance readings, the percentage of inhibition for both DPPH and ABTS radicals was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \text{ x } 100 \qquad (6.1)$$

6.3.7. α-Amylase Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the Worthington Enzyme Manual (1993a). The buffer used was 0.1M sodium phosphate (pH 6.9) with 0.006M sodium chloride added to it. A volume of 500 µL of each sample extract was added to test tubes while the control tubes had 500 µL of buffer instead of the sample. Additionally, each sample extract had a corresponding sample blank tube which contained 500 µL of the sample extract. Then 500 µL of porcine pancreatic amylase (0.5 mg/ mL buffer) enzyme solution was added to all the tubes except for the sample blank and incubated at 25 ℃ for 10 min. After incubation, 500 µL of 1% starch (1 g/100 mL buffer) was added to all the tubes and incubated for another 10 min. The reaction was then stopped by the addition of 1 mL of 3,5-dinitro salicylic acid and the tubes were placed in a boiling water bath for 10 min. After that, tubes were taken out and cooled at room temperature. The reaction mixture in the tubes was then diluted by adding 12 mL of distilled water to adjust the absorbance of the control to absorbance reading 1.0 and the absorbance was measured at 540 nm using a UV-VIS Genesys spectrophotometer (Genesys UV- visible, Milton Roy Inc, Rochester, NY)

The percentage of inhibition of α amylase enzyme inhibitory activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - (\text{Abs extract} - \text{Abs sample blank})}{\text{Abs control}} \text{ x } 100 \qquad (6.2)$$

6.3.8. α-Glucosidase Enzyme Inhibitory Activity

The α-glucosidase enzyme inhibitory activity assay was conducted based on the protocol from Worthington Enzyme Manual (1993b) with some modifications taken from McCue et al. (2005). A volume of 50 µL (undiluted), 25 µL (half-diluted) and 10 µL (one-fifth diluted) of each deep-fried sweet potato sample (for dose dependent response) extracts were pipetted into 96 well microtiter plates respectively. The half dilution and 1/5th dilutions were made up to a total of 50 µL in volume by adding 25 µL and 40 µL of 0.1M potassium phosphate buffer (pH 6.9), respectively. Each sample extract had a corresponding control of 50 µL of phosphate buffer. Finally, the volume in all the wells was made up to 100 µL by the addition of 50 µL of phosphate buffer in each well including the control. Then 100 µL of 0.1M potassium phosphate buffer (pH 6.9) containing α-glucosidase enzyme (1 U/mL) was added to each well and incubated at 25 °C for 10 min. After this 50 µL of 5 mM p-nitrophenyl-α-D- glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were then incubated at 25 °C for 5 min. Absorbance readings were taken before (0 min) and after (5 min) incubation period using a micro plate reader (Thermomax, Molecular device Co., Virginia, USA) set at 405 nm.

The percentage of inhibition of α-glucosidase enzyme inhibitory activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs control 5min} - \text{Abs control 0min}) - (\text{Abs extract 5min} - \text{Abs extract omin}}{2\text{Abs control 5min} - \text{Abs control 0min}} \text{ x } 100 \quad (6.3)$$

6.3.9. Total Protein Content

The total protein content of the deep-fried sweet potato extracts was measured using a modified version of the Bradford assay (Bradford, 1976). The protein dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted 1:4 with distilled water.

Then 5 mL of the diluted dye was added to 50 µL of the sweet potato samples, mixed thoroughly and incubated in the dark for 5 min. For the blank tube 50 µL of distilled water was used instead of the sweet potato sample extracts. All samples were analyzed in triplicates and the absorbance was then measured at 595 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, MA). A standard curve was prepared using bovine serum albumin (BSA) in different concentrations and the protein content was expressed as µg/g dry weight.

6.3.10. Color Measurements

Color difference of deep-fried sweet potato was calculated from L, a*, and b* values measured using a Macbeth, color-eye 7000 Reference spectrophotometer (X-Rite, Massachusetts, USA). The software used was ProPalette 5.0. Colors were recorded according to the CIE coordinates system (L, a*, and b* values), in which L values indicate lightness/darkness, a* values represent redness/greenness, and b* values indicate blueness/yellowness of deep-fried foods respectively. Total color difference (ΔE) of deep-fried sweet potato samples was calculated from the following equation (Altunakar et al., 2004).

$$\Delta E = \sqrt{[(\mathrm{L}^{*} - \mathrm{L}^{*}_{\mathrm{standard}})^2 + [(\mathrm{a}^{*} - \mathrm{a}^{*}_{\mathrm{standard}})^2 + [(\mathrm{b}^{*} - \mathrm{b}^{*}_{\mathrm{standard}})^2]} \qquad (6.4)$$

Where, standard values referred to raw sweet potato L, a*, and b* values. Triplicates values were recorded at room temperature and mean values were derived.

6.3.11. Weight Loss Determination

The weight loss per cent of deep-fried sweet potato samples were determined by weighing before and after the deep-frying process and packing them into respective labelled zip-top bags. The weigh loss per cent was calculated according to the following formula,

$$\text{Weight loss ratio (\%)} = (W1 - W2) \times 100\%/W1 \qquad (6.5)$$

Where,

W1= weight of selected sweet potato cubes before deep-frying.

W2= Weight of selected sweet potato cubes after deep-frying.

6.3.12. Statistical Modeling for Optimization of Deep-Frying Variable of Sweet Potato

A central composite orthogonal design (CCOD) (Box and Wilson, 1951) was set up to investigate the impact of three deep-frying variables on phenolic content, antioxidant properties, and anti-hyperglycemic functionalities of deep-fried sweet potato. The range for processing parameters were set up based on the results of preliminary experiments. The three selected independent variables examined under this study were, X1: frying temperatures (140 -170 °C), X2: frying time (4 to 5 min), and X3: sample size ($1^3 – 2^3$ cm cubes). The experimental design yielded 23 experimental runs in completely random order as per three variables of CCOD as shown in Table 6.2. The design included eight factorial points, six axial points, and nine replicated center points (Mason et al., 2003) and five levels for coding were determined as -1.68, -1, 0, +1 and +1.68 (Table 6.1).

Table 6.1. Codes derived from CCOD model for deep frying process of sweet potato cubes

Code	**Temperature (°C)**	**Time (min)**	**Size-cubes (cm^3)**
-1.68	129.77	3.67	0.659
-1	140	4:00	1
0	155	4:30	1.5
+1	170	5:00	2
+1.68	180.22	5:34	2.34

Additionally, optimal processing conditions were predicted using a second-order polynomial function to correlate selected independent and dependent variables. Analysis of variance and regression coefficients for the response variables listed in Table 6.3 were interpreted to evaluate the statistical significance of the terms and fitness of the developed model at $p<0.005$ level of significance. Three-dimensional response plots were generated to evaluate

and interpret the interaction effects between selected independent variables on respective response variables.

6.3.13. Data Analysis

Data analysis was carried out assuming a second order polynomial equation for each of the response variables as a function of independent variables of frying temperature (X1), frying time (X2), and size of sweet potato cubes (X3). The generation of experimental runs and optimization of selected variables was done using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The data were expressed as the mean, and statistically significant difference at $p < 0.05$ was determined. All data were mean of 12 observations (n=12) for each biochemical assay.

6.4. Results and Discussion

6.4.1. Regression Modeling for Data Analysis

Worldwide, consumers are becoming more health conscious and educated on the nutritional qualities of fresh and processed foods. Responding to consumer awareness and higher demands of healthy foods, food industries are also increasing efforts to develop appropriate and effective processing strategies for improving the retention of bioactive compounds and associated nutritional qualities of processed foods. In the context of this study, beta carotene present in orange fleshed sweet potatoes are more bio-accessible in fried products than boiled orange fleshed sweet potato (Tumuhimbise et al., 2009). However, beyond basic nutritional contents the phenolic bioactives-based health targeted benefits of sweet potato based deep-fried foods have been not studied extensively. Therefore, understanding the impact of different frying variables on phenolic bioactives- linked health benefits of sweet potato relevant to post-prandial glycemic control in the context of early stages of type 2 diabetes has significant public and consumer health relevance (Kwon et al., 2006; Rekha and Padmaja, 2002).

In this study, deep frying process optimization of orange-fleshed sweet potato cubes were carried out based on results of different response variables, which was summarized in Table 6.2. The response surface methodology (RSM) based CCOD model was used to evaluate the effect of the different independent variables such as frying temperatures, frying times, and sizes of sweet potato cubes on the phenolic bioactives- linked antioxidant and anti-hyperglycemic functionalities of fried cubes, which were selected as response variables (Table 6.2).

The effect of each independent variable on each response variable are presented as three-dimensional response surface plots. Sweet potato cubes fried at a high temperature (170 °C) for long frying time (4 min 30 sec and 5 min) showed darker brown color. This result indicated that sweet potato cubes were burned quickly at higher frying temperature and especially with longer frying time which indicated potential Maillard reaction-based changes. The regression model as shown in Table 6.3 explained variation in each selected response variable as a function of selected independent variables. Most of the regression models for selected response variables were in the range of 80 to 89 %, except for ABTS based antioxidant and anti-hyperglycemic relevant α-amylase enzyme inhibitory activity (found insignificant at $p < 0.05$ level).

6.4.2. The Effects of Deep-Frying Parameters on Selected Response Variables

6.4.2.1. Protein Content

The protein content of processed foods is an important nutritional marker, which indicates their potential human health-linked nutritional benefits. In general, proteins are heat sensitive and susceptible to denaturation under high temperature. However, many studies suggested that deep frying had little or no impact on the protein and mineral content of fried foods (Bordin et al., 2013; Fillion and Henry, 1998). As deep-frying processing also dehydrates the food, it sometimes increases the concentration of nutritional components such as protein after

processing (Ngobese and Workneh, 2018). Sweet potatoes contain different amino acids such as threonine, valine, and tryptophan, and previous studies reported improvement of amino acid content with different heat processing methods such as microwaving, autoclaving, drying, boiling, and steaming (Sun et al., 2012). Therefore, it is important to investigate the effect of different deep-frying variables such as frying temperatures, frying times, and sample sizes on total protein content of deep-fried sweet potatoes. In the present study, protein content of raw and deep-fried orange flesh sweet potato was measured using a modified version of the Bradford assay (Bradford, 1976). Protein content of raw orange-fleshed sweet potato was 3.56 mg/g fresh weight basis (FW), whereas deep-fried samples had protein content in the range from 7.56 to 24.82 mg/g dry weight (DW) basis (0.7-2.4 %). Previous studies reported 2-6% protein content (dry weight basis) in orange-fleshed sweet potato cultivars (Alam et al., 2016; Astawana and Widowati, 2011). Similarly, Anwar et al. (2019) observed 3.62% crude protein content in deep fried sweet potato chips.

The effect of selected deep-frying independent variables and their interaction with protein content is presented as three-dimensional (3D) response surface plot (Figure 6.2). A three-dimensional response surface plot indicated a fair relationship between independent variables and protein content as dependent variable for deep-fried sweet potato cubes. Additionally, response surface plot suggested the major changes in direction (increase/decrease) of response variable (protein content) with change in independent variables. In general, 3D response plots were developed by keeping one independent variable constant at center point and using the other two as a function of response variable. For instance, response plots for protein content were generated using effects of frying temperature and sample size, while frying time was kept as a constant variable at the center point as shown in the Figure 6.2. Analysis of variance (ANOVA)

showed a significant impact of frying temperature and sample size on protein content of deep-fried sweet potato cubes, whereas frying time had no significant impact on protein content ($p<0.05$). The highest protein content of deep-fried sweet potato cubes was observed with 155 °C frying temperature, 1.5 cm cube size, and 4.30 min frying time. Therefore, in this study, high frying temperature did not affect the protein content of sweet potato fries. The multiple regression model revealed coefficient of determinations ($R^2 = 0.74$) for protein content, which indicated that 74% of the total variation could be well explained by the developed regression model. Additionally, insignificant lack of fit value (p value=0.8859) also showed good fit of this regression model for the experimental data. Therefore, the multiple regression model developed in this study indicated a good predictor/indicator for understanding the changes in protein content during deep-frying processing (Myers et al., 2016).

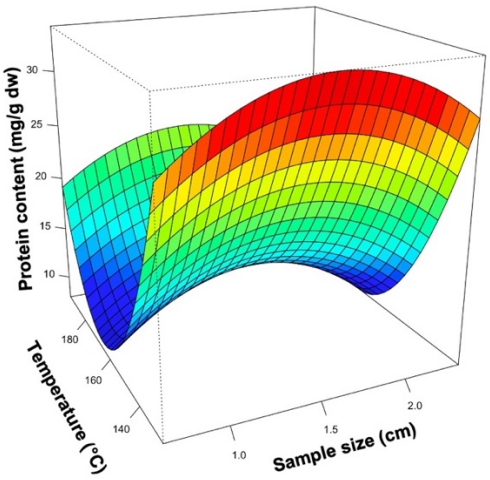

Figure 6.2. Response surface plot representing changes in protein content of deep-fried orange-fleshed sweet potato cubes with different deep-frying variables

6.4.2.2. Total Soluble Phenolic Content

In this study, the total soluble phenolic (TSP) content of deep-fried sweet potato cubes varied between 5.01 and 13.05 mg GAE/g DW (Table 6.1), whereas raw sweet potato showed

TSP content of 1.19 mg GAE/g FW (fresh weight basis). The effect of selected deep-frying independent variables and their interaction with TSP content of deep-fried sweet potato cube is illustrated as three-dimensional (3D) response surface plot (Figure 6.3). In the present study, significant ($p<0.05$) effect of frying temperature on TSP content of sweet potato cubes was observed. Overall, the TSP content of sweet potato increased proportionately with increase in frying temperatures (as shown in 3D response plots Fig. 6.3). Additionally, higher TSP content was also observed with smaller cube size (1 cm), while larger cube size (2 cm fried cube) resulted in significant reduction in TSP content (5.01 mg GAE/g DW) at constant frying temperature and frying time.

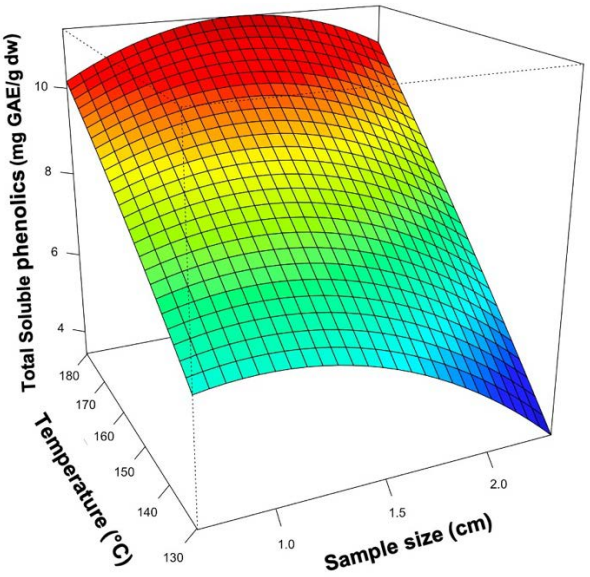

Figure 6.3. Response surface plot representing changes in TSP content of deep-fried orange-fleshed sweet potato cubes with different deep-frying variables

Therefore, results of this study suggested that less exposure of the core part of the larger sweet potato cubes to the thermal processing treatment and related likely less breakage of cell wall matrices probably contributed to the reduction in release of soluble phenolics in fried sweet potato. Previously, Rautenbach et al. (2010) observed similar trend with increase in phenolic content across different sweet potato cultivars following heat treatment. In another study,

different processing methods such as frying, baking, microwave significantly ($p<0.05$) increased phenolic content in cooked sweet potato samples (Ateea et al., 2012). The changes in TSP content observed in this present study were consistent with findings of previous studies (Ateea et al., 2012; Rautenbach et al., 2010), as they found higher phenolic content in deep fried sweet potato samples with high frying temperature. In the present study, multiple regression model for TSP (R^2=0.84) indicated variability of 84 % and 0.1348 insignificant ($p<0.05$) lack of fit value, which suggested a well fitted model to predict TSP content of deep-fried sweet potato cubes.

6.4.2.3. Total Antioxidant Activity

Total antioxidant activity of deep –fried sweet potato cubes was determined by using DPPH and ABTS free radical scavenging assays. The DPPH free radical scavenging assay-based antioxidant activity ranged from 0.51 to 1.46 mM Trolox/g DW among different deep-fried sweet potato samples. Statistical (ANOVA) comparison results showed a significant ($P<0.05$) effect of sample size on DPPH-based antioxidant activity of deep-fried sweet potato. However, the effect of frying temperature and frying time on DPPH-based antioxidant activity of sweet potato cube was statistically insignificant. The antioxidant activity (based on DPPH free radical scavenging assay) (3D response plot of Figure 6.4a) decreased with increase in sweet potato cube size and at high frying temperature. The optimum level of DPPH-based antioxidant activity (1.08 mM Trolox/g DW) was observed with select independent variables, such as frying temperature of 155 °C, frying time of 4 min 30 sec, and sample size of 1.5 cm. Results of antioxidant activity of the current study was different from the findings of previous studies (Ateea et al., 2012; Rautenbach et al., 2010), where antioxidant activity proportionately increased with increased in frying temperature. In the present study, antioxidant activity decreased under high frying temperature, which indicated potential thermal sensitivity and easy

degradable nature of different antioxidant compounds of sweet potatoes (Sharma et al., 2016). A multiple regression model as shown in Table 6.3, provided details of fitted model for DPPH at $p < 0.05$ significant level, with R^2 value of 0.86 (86 % variability in the response variable was explained by the developed model).

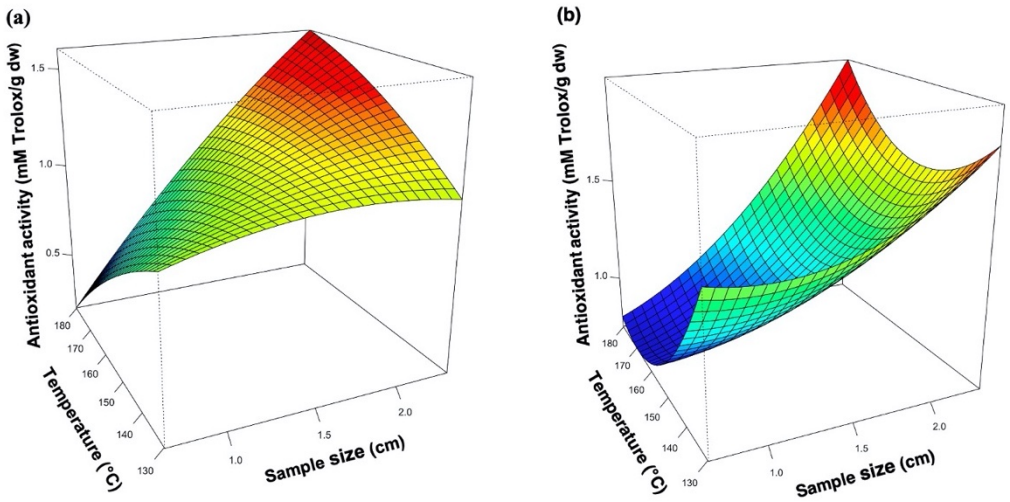

Figure 6.4. Response surface plots representing the results of DPPH-based antioxidant activity (a), and ABTS-based antioxidant activity (b) of deep-fried orange-fleshed sweet potato cubes and overall changes in antioxidant activity with different deep-frying process variables

Additionally, a lack of fit (0.1788) value was found to be insignificant at $p < 0.05$ level, which suggested a well fitted/predicted model for understanding the impact of different deep-frying process variables on antioxidant activity of deep-fried sweet potato cubes.The antioxidant activity based on ABTS free radical scavenging assay was between 0.47 to 1.46 mM Trolox/g DW (Table 6.2). However, statistical analysis of the model revealed a higher variability and significant lack of fit, which resulted in a weak model, unfit for determining the impact of deep-frying variables on ABTS-based antioxidant activity of deep-fried sweet potato cubes (Figure 6.4b).

6.4.2.4. Anti-hyperglycemic Relevant α-Amylase and α-Glucosidase Enzyme Inhibitory Activities

Anti-hyperglycemic relevant α-amylase and α-glucosidase enzyme inhibitory activities were measured using *in vitro* assay models and indicate potential of such fried sweet potato as part of diets to manage post-prandial glucose increase (Kwon et al., 2006). Like antioxidant activity, process optimization based on anti-hyperglycemic functionality of fried food is also essential for designing sweet potato-based healthy snacks. In the present study, a statistically significant effect ($p < 0.05$) of sample size on α-amylase enzyme inhibitory (%) activity of deep-fried sweet potato cubes was observed. Additionally, a significant ($p<0.05$) linear effect of sample size, and its interaction with frying temperature on α-amylase enzyme inhibitory (%) activity of deep-fried sweet potato cubes (p-value of 0.0330) was also found. The *in vitro* enzyme assay results of the present study were in agreement with the findings of a previous thermal processing study (drying and boiling), where high retention of α-amylase enzyme inhibitory activity up to 60 % was observed in deep-fried sweet potatoes (Rekha and Padmaja, 2002). In the present study, higher α-amylase enzyme inhibitory activity was found with smaller sample size (1 cm), and it decreased proportionately with increase in sample size (2 cm) (Figure 6.5a). The results of a multiple regression model provided a R^2 value of 0.61(61% of variation), and insignificant lack of fit (p-value of 0.228), which indicated a good fit of the model for predicting the impact of deep-frying variables on α-amylase enzyme inhibitory activity of deep-fried sweet potato cubes.

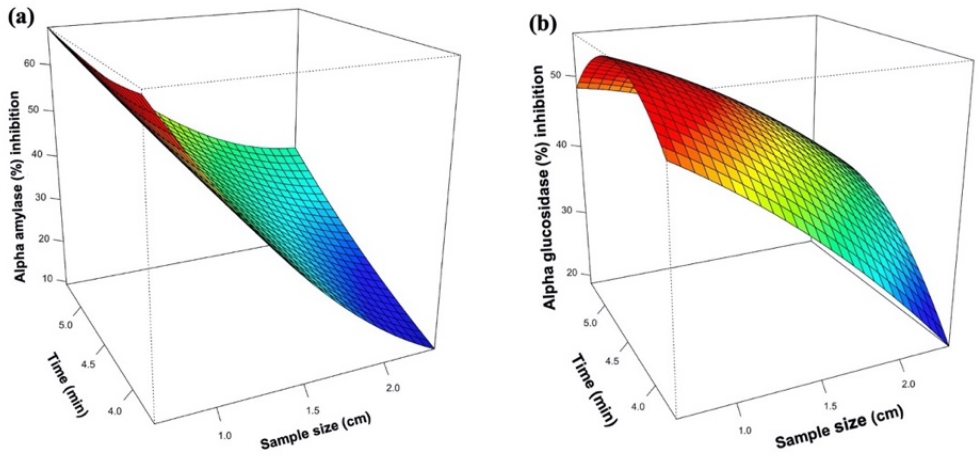

Figure 6.5. Response surface plots indicating changes in α-amylase (a) and α-glucosidase (b) enzyme inhibitory activities of deep-fried orange-fleshed sweet potato cubes with different deep-frying process variables

Statistical analysis (ANOVA) also found a significant ($p<0.05$) effect of sweet potato cube sample size on α-glucosidase enzyme inhibitory activity of deep-fried sweet potato. The linear effect of frying temperatures on α-glucosidase enzyme inhibitory activity was statistically insignificant ($p<0.05$), while impact of interactions between frying temperature and sample size was found to be statistically significant ($p<0.05$) (Figure 6.5b). Optimum α-glucosidase enzyme inhibitory activity (42.14%) was observed with frying temperature of 155 °C, frying time of 4 min 30 sec, and sample size of 1.5 cm. Therefore, high frying temperature did not affect the anti-hyperglycemic relevant functionalities of deep-fried sweet potato tubes. Results of this study suggested that the smaller cube size and high frying temperature might be beneficial for higher retention of phenolic-linked bioactive compounds and for improving associated human health related functionalities of deep-fried sweet potatoes that could be integrated into wider diets for post-prandial glycemic control and management.

6.4.2.5. Color Measurement

Consumer acceptance of fried products are largely influenced by color parameters (L, a*, and b* values of fried foods) and visual appearances of the processed foods. Especially color

difference value is extremely important in the food industry to determine consumer acceptance of developed fried products. In this study, color diiference values of fried samples were measured using Macbeth, color-eye 7000 reference spectrophotometer. Color diiference values of fried sweet potato cubes significantly decreased with increase in frying temperatures (color diiference value=18 at 170°C), which resulted into dark brown color after frying (Figure 6.6 & 6.8). Previously, a similar trend in color diiference values were found in deep-fat fried yellow-fleshed cassava chips (Oyedeji et al., 2017). Substantial increase in color diiference values and development of dark brown color in deep-fried sweet potato cubes might be due to a non-enzymatic browning reaction between reducing sugars and protein content and crust formation at higher temperature frying (Caetano et al., 2018).

At lower frying temperature, the color diiference values of fried sweet potato cubes was significantly lower (Figure 6.6). In this present study, the optimum color diiference values of deep-fried sweet potato cubes was found to be as 43.96. Previously, Sobukola et al. (2008) reported higher acceptability of fried foods with slightly higher color diiference values, where formation of golden-brown crust was observed in the outer surface of the fried foods. Based on the statistical analysis (ANOVA) of color diiference values, it was clear that frying temperature, and sample size had significant ($p<0.05$) impact on color and appearance of the deep-fried sweet potato cubes. However, the degree of regression coefficient (R^2) for color diiference values (Table 6.3) was higher than 0.9 ($R^2=0.9417$) (94.17% of the variation in the data) and significant ($p<0.05$) lack fit value (p value=0.012) indicated that the model is not well suited for predicting the impact of deep frying variables on color of the fried sweet potato samples.

Table 6.2. Response surface analysis result for different experimental runs of deep-fried sweet potato samples

Experimental runs	Frying Temperature (°C)	Frying Time (min)	Sweet potato cube size (cm)	TSP (mg GAE/g DW)	DPPH inhibitory action (mM Trolox/g DW)	ABTS inhibitory action (mM Trolox/g DW)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)	Protein content (mg/g DW)	Color difference value	Weight loss percent
1	140	4	1	6.90	1.13	1.27	26.60	50.79	20.71	26.04	39.13
2	140	4	2	5.01	1.21	1.50	33.43	30.71	10.99	14.49	23.73
3	140	5	1	7.08	1.06	1.13	49.19	50.55	14.24	17.24	43.18
4	140	5	2	5.31	1.23	1.21	40.02	36.43	10.13	19.58	26.56
5	170	4	1	9.06	0.78	0.94	77.52	43.26	23.11	47.76	53.06
6	170	4	2	7.68	1.27	1.48	0.00	37.71	12.82	20.87	27.85
7	170	5	1	8.68	0.66	0.97	40.29	43.24	23.06	31.90	54.55
8	170	5	2	8.56	1.37	1.33	0.00	43.12	12.85	26.92	30.77
9	129.77	4:30	1.5	5.59	0.99	1.59	64.49	36.18	12.49	23.72	26.92
10	180.22	4:30	1.5	13.05	1.01	1.21	33.68	55.76	22.21	41.86	42.86
11	155	3:57	1.5	7.52	1.19	1.40	23.31	35.39	7.56	31.49	33.33
12	155	5:34	1.5	8.62	1.00	1.13	68.21	46.74	11.57	3.03	39.58
13	155	4:30	0.67	8.41	0.51	0.69	72.56	61.24	24.82	7.08	59.13
14	155	4:30	2.34	8.46	1.46	1.87	29.92	30.50	11.98	14.50	25.29
15	155	4:30	1.5	9.01	1.30	1.44	34.66	40.73	9.92	14.03	36.36
16	155	4:30	1.5	8.59	1.09	1.26	26.02	38.21	16.50	1.00	33.96
17	155	4:30	1.5	8.10	1.07	1.29	0.00	44.32	12.30	4.65	36.17
18	155	4:30	1.5	8.80	1.06	1.04	39.11	48.40	21.47	9.42	35.23
19	155	4:30	1.5	8.82	1.02	1.15	40.34	46.12	18.85	1.23	34.45
20	155	4:30	1.5	9.14	1.15	0.87	55.04	36.43	16.66	4.32	33.81
21	155	4:30	1.5	7.57	1.17	1.38	28.54	42.74	11.72	10.43	35.97
22	155	4:30	1.5	9.30	1.07	0.51	36.44	32.32	15.42	9.43	33.65
23	155	4:30	1.5	9.08	1.04	0.47	41.54	38.45	10.98	2.45	34.74

TSP: Total soluble phenolics, DPPH: 2, 2-Dipheny-1—Picrylhydrazyl free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) radical cation assay.

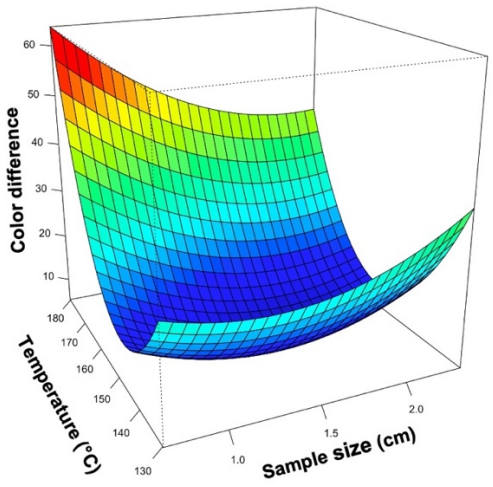

Figure 6.6. Response surface plot showing changes in color difference of deep-fried orange-fleshed sweet potato cubes with different deep-frying process variables

6.4.2.6. Weight Loss Percent

In this deep frying process optimization study to retain bioactive functional benefits, the weight loss percent values increased with increase in frying temperature (56.06 % at 170 °C and 23.79 % at 140 °C), while it decreased with increase in sweet potato cube size (43.18 % with 1 cm cubes, whereas 26.56 % in 2 cm cubes) (Figure 6.7). Increase in weight loss percent with increasing frying temperature suggested significant loss of water under high temperature frying (Manjunathan et al., 2014). Analysis of variance (ANOVA) results showed a significant ($p<0.05$) impact of frying temperature, frying time, and sample size on weight loss percent of deep-fried sweet potato cubes. Additionally, interaction between frying temperature and sample size also had a significant ($p<0.05$) effect on weight loss percent of sweet potato cubes. The multiple regression model (Table 6.3) ($R^2=0.99$), and the lack of fitness (p value=0.62) result indicated a well fitted model for predicting the impact of different process variables on weight loss percent of deep-fried sweet potato cubes. Overall, the reduction of moisture content and weight loss in deep-fried sweet potato cubes have significant relevance for understanding the shelf life and

post-harvest preservation qualities of this processed food and how this translates into preservation of health targeted phenolic bioactives.

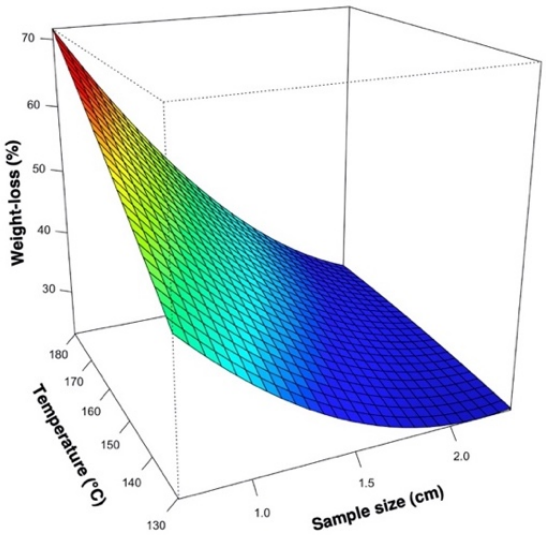

Figure 6.7. Response surface plots of weight-loss per cent of deep-fried orange-fleshed sweet potato cubes at different experimental conditions

Figure 6.8. Effect of deep-frying conditions such as frying temperature, frying time, and sample size on the visual quality of deep-fried orange-fleshed sweet potato cubes

Table 6.3. Analysis of variance (ANOVA) for the regression models of TSP, antioxidant activity, and anti-diabetic properties of deep-fried sweet potato cubes

Model source	Degrees of freedom	Sum of squares	Mean sum of square	F-value	p-value	R^2	Lack of fit
TSP	9	28.61	3.18	7.01	0.0013	0.84	0.1348
DPPH	9	0.86	0.09	9.08	0.0003	0.86	0.1788
ABTS	9	1.25	0.13	1.48	0.2503	0.50	0.9123
α-Amylase inhibitory activity	9	6028.91	669.88	2.29	0.0852	0.61	0.2298
α-Glucosidase inhibitory activity	9	925.55	102.84	3.22	0.0276	0.69	0.2568
Protein	9	417.30	46.37	4.12	0.0107	0.74	0.8859
Color difference	9	2021.28	224.59	23.32	<.0001	0.94	0.0122
Weight loss percent	9	1828.76	203.19	205.89	<.0001	0.99	0.6293

TSP: Total soluble phenolics, DPPH: 2, 2-Dipheny-1—Picrylhydrazyl free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) radical cation assay.

6.5. Conclusions

In the present study, the impact of independent processing variables such as frying temperature, frying time, and sample size on total soluble phenolic content (TSP), antioxidant properties, and type 2 diabetes relevant anti-hyperglycemic functionalities of deep-fried sweet potato cubes was investigated using a novel statistical model. The results showed a proportionate increase in TSP and protein content with increase in frying temperature, while both parameters decreased with increase in sample size. Furthermore, increasing frying temperature had a negative impact on antioxidant activity of deep-fried sweet potato cubes. The response surface methodology (RSM)-based central composite orthogonal (CCOD) model used in this study helped to optimize the select independent variables of deep-frying process in orange-fleshed sweet potato. The optimal deep-frying conditions found in this study were frying temperature of 155 °C, frying time of 4 min 30 sec, and sample size of 1.5 cm, which resulted in higher

retention of protein, total soluble phenolics, and associated antioxidant and type 2 diabetes relevant anti-hyperglycemic functionalities in deep fried sweet potato cubes. Overall, the results of this study suggested that a smaller sample size and high frying temperature is beneficial to design healthy sweet potato based fried foods, which can be rationally integrated in health-focused food solution strategies such as phenolic antioxidant enriched anti-hyperglycemic functionality for overall glycemic control with better diets. However, future studies with different flesh colored sweet potato cultivars and different frying variables such as types of oil and different shapes and cuts of sweet potatoes are required to optimize the bioactives-rich and improved nutritional qualities of deep-fried sweet potato for products and for advancing their value-added health-targeted commercialization.

CHAPTER 7. BAKING PROCESS OPTIMIZATION OF SWEET POTATO BASED ON PHENOLIC BIOACTIVES-LINKED ANTIOXIDANT AND ANTI-HYPERGLYCEMIC PROPERTIES USING RESPONSE SURFACE METHODOLOGY (RSM) MODEL

7.1. Abstract

Baked sweet potato food products are gaining popularity among consumers due to their superior nutritional qualities and sensory characteristics. However, sometimes baking process affect the nutritional qualities and potential health benefits of the processed foods. Therefore, it is important to optimize different processing variables during baking for improving retention and stability of nutritionally relevant bioactive profiles and associated health benefits of baked foods. Based on this need for health-focused process optimization, the aim of this study was to optimize different baking variables in orange-fleshed sweet potato (OFSP) based on phenolic bioactive-linked antioxidant and anti-hyperglycemic properties relevant and targeted for dietary management of early stages of type 2 diabetes. In this study, the statistical model response surface methodology (RSM) was used to investigate the impact of independent baking variables (baking temperature-140 to 180 °C, baking time-15 to 35 min, and size of sweet potato cubes of 1 to 2 cm^3) on total soluble phenolic (TSP) content, protein content, antioxidant activity, and anti-hyperglycemic properties of baked sweet potato (OFSP). The results of this *in vitro* assay model-based study indicated the optimum baking conditions of sweet potato was baking temperature of 160 °C, baking time of 25 min, and sample size of 1.5 cm. Under this optimum baking conditions, higher retention of protein (12.11 mg/g dry weight (DW)), TSP (2.72 mg GAE/g DW), high antioxidant activity (1.40 mM Trolox/g DW DPPH based assay, and 0.59 mM Trolox/g DW-ABTS based assay), and anti-hyperglycemic relevant moderate α-amylase (71.5%), and α -glucosidase (40%) enzyme inhibitory activities were observed in baked sweet

potato. Overall, the results of this study suggested that baked sweet potato with optimized baking conditions can be potential integrated and targeted in health-focused dietary solution strategies, especially to manage chronic oxidative stress and chronic hyperglycemia commonly associated with early stages of type 2 diabetes.

7.2. Introduction

Sweet potato is a popular tuber crop used widely as fresh and processed foods (fried, baked, boiled) across different countries and continents. Due to such wide use and popularity among consumers, many food and nutritionally relevant scientific studies have investigated the potential health benefits of sweet potatoes, especially their dietary benefits against diet-linked non-communicable chronic diseases (NCDs) (Bovell-Benjamin, 2007). Overall, sweet potatoes are considered as medium to low glycemic food, with high antioxidant, anti-hyperglycemic, and anti-hypertensive relevant functional properties (Allen et al., 2012). The diversity of sweet potatoes based on their flesh color, taste, and texture has encouraged food industries to commercially develop several human health relevant processed foods such as baked, fried, boiled, and steamed sweet potato-based food products. Additionally, as sweet potato is highly perishable, post-harvest processing is an effective strategy to improve shelf-life of sweet potato-based foods (Musyoka et al., 2018). Therefore, post-harvest processing of sweet potato tubers are widely used to avoid post-harvest spoilage and subsequent loss during the storage. In African and Asian countries, sweet potato tubers are primarily processed to make several food products such as extruder snack products, aseptic puree, juice, French fries, baked and fried chips, pasta, ice cream, breakfast cereal, and different kinds of traditional fermented foods (fufu) and beverages (Truong et al., 2018).

Sweet potato-based baked foods are generally preferred by the consumers due to their less oil content, crispy caramelized taste, and attractive textures. However, baking process has significant impact on structural and physicochemical properties such as breakdown of cell wall matrices and release of soluble bioactive compounds in baked foods (Dincer et al., 2011, Lai et al., 2013). Previous studies have reported that processing of sweet potatoes such as baking, frying, steaming, and boiling potentially enhance some nutritional and sensory characteristics (USDA, 2019). In this context of improving nutritional qualities, significant ($p<0.05$) increase in the total phenolic content was reported in boiled and baked sweet potatoes, while total starch, and sugar content reduced (Dincer et al., 2011). Similarly, increase in phenolic content, ascorbic acid, and improved antioxidant activities were observed in baked and boiled sweet potatoes when compared to raw sweet potato sample (Dincer et al., 2011). However, contrasting results were observed in other studies, as home processed sweet potatoes via baking and boiling had nearly 7 % to 40 % reduction in phenolic content (Jung et al., 2011). Rabah et al. (2004) reported strong antioxidant activity and potential anti-cancer relevant chemo preventive properties of baked sweet potatoes (*Ipomoea batatas* Lam. Cv. Koganesengan). Furthermore, Allen et al. (2012) reported medium glycemic index in differently processed sweet potatoes, which included baked (64 ± 4.3), microwaved (66 ± 5.7), and steamed (63 ± 3.6) products. Hou et al. (2019) optimized sweet potato roasting process to improve physicochemical properties and found higher phenolic content and free radical scavenging activity in roasted sweet potatoes.

However, most of these previous studies did not target process optimization related to baking based on their nutritional related bioactives relevant for human health benefits linked to phenolic antioxidant associated hyperglycemia management of early stages of type 2 diabetes.Therefore, it is important to optimize different baking variables based on the retention

of nutrients and associated health benefits related to phenolic antioxidant and hyperglycemia functions. Specifically, optimization of processing conditions such as cooking temperature, cooking time, size and shape of sample based on the profile of bioactive compounds and their associated hyperglycemia relevant human health protective functions is essential prior to integrate baked sweet potatoes in such targeted health-focused dietary solution strategies. Therefore, specific objective of this study was to optimize different processing variables such a baking temperature, baking time, and sweet potato sample size based on their impacts on protein content, total soluble phenolic content (TSP), antioxidant property, and anti-hyperglycemic functionalities of baked sweet potatoes using the statistical model (Response Surface Methodology-RSM). Such process optimization is essential for designing sweet potato based baked foods with higher nutritional qualities and targeted human health benefits, especially to manage chronic oxidative stress and hyperglycemia commonly associated with early stages of type 2 diabetes and other NCDs.

7.3. Materials and Methods

7.3.1. Sweet Potato Sample and Baking Process

Organic orange-fleshed sweet potato tubers were purchased from local grocery store (Natural Grocers, Fargo, ND) for baking process optimization. Tubers were wiped with cloth to remove dirt and peeled with a hand peeler. Peeled tubers were cut into cubes with different sizes (1, 1.5, 2 cm) and 120 g of sweet potato cubes was used for each experimental run. Based on preliminary baking experimental results, the response surface methodology (RSM) statistical tool was used to get different combinations of select independent variables such as baking temperature, baking time, and sweet potato cube sample size as shown in Table 7.1. The oven was pre-heated (140-180 °C for each select baking temperature) before baking sweet potato

cubes and baking times were monitored using digital kitchen timer. The selection of each independent variables was based on preliminary optimization results and subsequent statistical analysis using RSM based central composite orthogonal design (CCOD), as presented in Table 7.2. Initially, sweet potato cubes were smeared with 10 mL olive oil (for 120 g sample) prior to keeping it in oven for baking. After each baking experiment, samples were taken out for cooling and stored into labelled low-density polyethylene (LDPE) zip-lock bags for further biochemical analysis.

7.3.2. Hot Water Extraction of Baked Sweet Potato Cubes

Baked sweet potato samples were extracted using hot water for biochemical analysis. Sample extraction was carried out by blending 40 g of sample separately from each baking experimental run in 100 mL of hot distilled water (preheated to 100 °C) for 5 min using a Warring blender. Then blended samples were centrifuged at 8000 rpm for 20 min and centrifugation was repeated for two times. The supernatant was collected and stored in the refrigerator (2-4 °C) for further biochemical analysis.

7.3.3. Chemical Used

Porcine pancreatic alpha–amylase (EC 3.2.1.1), rat intestinal alpha–glucosidase (EC 3.2.1.20), 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Bio-rad, and Trolox, 2, 2- Azobis (2- amidinopropane) dihydrochloride (AAPH)) and other reagents were purchased from Sigma Chemical Co (St. Louis, MO).

7.3.4. Total Soluble Phenolic Content

A modified protocol described by Shetty et al. (1995) was used to determine the total soluble phenolic (TSP) content of baked sweet potato samples. At first, 0.5 mL of hot water extracted sweet potato sample was added to 10 mL glass test tubes and was diluted with 0.5 mL

of distilled water. For measuring blank, 0.5 mL of distilled water was used instead of the sample. Then in each tube, 1 mL of 95 % ethanol, 5 mL of distilled water, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent, and 1 mL of 5% sodium carbonate were added sequentially. The content of the glass tubes was mixed thoroughly by using a vortex mixer and incubated in the dark for 60 min. Then sample absorbance was measured using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY) set at 725 nm. The absorbance values were converted to TSP content and expressed in milligrams gallic acid equivalent (GAE) per gram of dry weight (DW) based on a standard curve that was established using different concentrations of gallic acid in 95% ethanol.

7.3.5. Total Antioxidant Activity

The total antioxidant activity of the baked sweet potato extracts was measured using two different assays: 2, 2-Dipheny-1—Picrylhydrazyl (DPPH) free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay. The DPPH assay as described by Kwon et al. (2006) was used in which 0.25 mL of hot water extracted sweet potato sample was taken in 1.5 mL micro-centrifuge tubes. Then 1.25 mL of 60 mM DPPH (in 95% ethanol) was added to the tubes containing sample extract. The tubes were mixed well using a vortex mixer to ensure proper mixing and then incubated for 4 min. After that mixtures were centrifuged at 13,000 rpm for 1 min to pellet down any of the precipitate. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). Each sample had a corresponding control which contained 0.25 mL of distilled water instead of the sample. For the ABTS assay (Re et al., 1999), the same procedure was followed except in this case 1 mL of ABTS (matured stock adjusted with 95% ethanol) was added to 0.05 mL of baked sweet potato

extracts and the absorbance was measured at 734 nm using a UV-visible spectrophotometer (Genesys 10S UV- VIS spectrophotometer, Thermo Scientific, NY). A control using 95% ethanol instead of sample was also used. Based on the absorbance readings, the percentage of inhibition for both DPPH and ABTS radicals was calculated using following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \times 100 \qquad (7.1)$$

7.3.6. α-Amylase Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the Worthington Enzyme Manual (1993a). The buffer used was 0.1 M sodium phosphate (pH 6.9) with 0.006 M sodium chloride added to it. A volume of 500 µL of each sample extract was added to the test tubes while the control tubes had 500 µL of buffer instead of the sample. Additionally, each sample extract had a corresponding sample blank tube which contained 500 µL of the sample extract. Then 500 µL of porcine pancreatic amylase (0.5 mg/ mL buffer) enzyme solution was added to all the tubes except for the sample blank and incubated at 25 °C for 10 min. After incubation, 500 µL of 1% starch (1 g/100 mL buffer) was added to all the tubes and incubated for another 10 min. The reaction was then stopped by adding 1 mL of 3,5-dinitro salicylic acid and the tubes were placed in a boiling water bath for 10 min. After that, tubes were taken out and cooled at room temperature. The reaction mixture in the tubes was then diluted by adding 10 mL of distilled water to adjust the absorbance of the control to the approximate range of 1.0±0.02 and the absorbance was measured at 540 nm using a UV-VIS 10 Genesys spectrophotometer (Thermo-Fisher. Waltham, MA).

The percentage of inhibition of α-amylase enzyme inhibitory activity of baked sweet potato samples was calculated based on the absorbance readings and using the following formula,

$$\% \text{ Inhibition} = \frac{\text{Abs control} - (\text{Abs sample extract} - \text{Abs sample blank})}{\text{Abs control}} \times 100 \qquad (7.2)$$

7.3.7. α-Glucosidase Enzyme Inhibitory Activity

The assay protocol used was based on the method described in the Worthington Enzyme Manual (1993b) with some modifications were taken from McCue et al. (2005). A volume of 50 µL, 25 µL and 10 µL of sample extracts was pipetted into 96 well microtiter plates and used as undiluted, half diluted, and 1/5th diluted respectively, for determining potential dose depended responses. The half dilution and 1/5th dilutions were made up to a total of 50 µL in volume by adding 25 µL and 40 µL of 0.1 M potassium phosphate buffer (pH 6.9), respectively. Each sample extract had a corresponding control of 50 µL of phosphate buffer. Finally, the volume in all the wells was made up-to 100 µL by the addition of 50 µL of phosphate buffer in each well including the control. Then 100 µL of 0.1M potassium phosphate buffer (pH 6.9) containing α-glucosidase enzyme (1 U/mL) was added to each well and incubated at 25 °C for 10 min. After this 50 µL of 5 mM p-nitrophenyl-α-D- glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were then incubated at 25 °C for 5 min. Absorbance readings were taken before (0 min) and after (5 min) incubation period using a micro plate reader (Thermomax, Molecular device Co., Virginia, USA) set at 405 nm.

The percentage of inhibition of α-glucosidase enzyme inhibitory activity of baked samples was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs control 5min} - \text{Abs control 0min}) - (\text{Abs extract 5min} - \text{Abs extract omin}}{2\text{Abs control 5min} - \text{Abs control 0min}} \times 100 \quad (7.3)$$

7.3.8. Total Protein Content

The total protein content of baked sweet potato cubes was measured using a modified version of the Bradford assay (Bradford, 1976). The protein dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted in 1:4 with distilled water and 5 mL of the diluted dye was added to 50 µL of baked sweet potato sample extracts, mixed thoroughly and incubated in the dark for 5 min. For the blank, 50 µL of distilled water was used instead of the sweet potato sample extracts. All samples were analyzed in triplicates and the absorbance was then measured at 595nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, MA). A standard curve was prepared using bovine serum albumin (BSA) in different concentrations and the protein content was expressed as mg/g dry weight basis.

7.3.9. Color Measurements

CIE (International Commission on Illumination) coordinates (L, a*, and b* values) system was used to analyze color differences of baked sweet potatoes and Macbeth, color-eye 7000 reference spectrophotometer was used to measure the color. The software used was ProPalette 5.0. L values indicate lightness/darkness, a* values represent redness/greenness, and b* values indicate blueness/yellowness of baked samples respectively. Total color difference (ΔE) of baked sweet potatoes was calculated from the following equation (Altunakar et al., 2004)

$$\Delta E = \sqrt{[(L^* - L^*_{standard})^2 + [(a^* - a^*_{standard})^2 + [(b^* - b^*_{standard})^2]} \qquad (7.4)$$

Where, standard values referred to raw sweet potato L, a*, and b* values. Values were recorded in triplicates.

7.3.10. Weight Loss Determination

The weight loss percent of baked sweet potato samples were determined using weighing before and after baking. The weigh loss percent was calculated according to the following formula,

$$\text{Weight loss ratio (\%)} = (W1 - W2) \times 100\%/W1 \quad (7.5)$$

Where,

W1= weight of selected sweet potato cubes before baking.

W2= Weight of selected sweet potato cubes after baking.

7.3.11. Statistical Modeling for Optimization of Sweet Potato Baking

In this study, a CCOD (Box and Wilson, 1951) model was set up to study the impact of different baking processing variables on protein content, phenolic content, antioxidant activity, and type 2 diabetes relevant anti-hyperglycemic property, which were determined using *in vitro* assay models. The range for each processing variables were set up based on the results of preliminary experiments. Three selected independent variables examined under this study were, X1: baking temperature (140 to 180 °C)), X2: baking time (15 to 25 min), and X3: sweet potato cube sample size (1 – 2 cm). The experimental design yielded 23 experimental runs in completely random order as per three variable design of CCOD. The design obtained from initial analysis of the CCOD model were based on eight factorials points, six axial points, and nine replicated center points (Mason et al. 2003). The five levels determined were -1.68, -1, 0, +1and +1.68 as shown in Table 7.1. Additionally, optimal processing conditions were predicted using second-order polynomial function to find potential correlations between selected independent and dependent variables. Analysis of variance (ANOVA) as presented in Table 7.3 was interpreted to fit each individual model, as well as 3D (three-dimensional) surface response plots

were developed to understand potential interaction effect of independent and depend variables on sweet potato baking process.

7.3.12. Data Analysis

Data analysis was carried out assuming a second order polynomial equation for each of the response variables as a function of each independent variables; baking temperature (X1), baking time (X2), and size of sweet potato cubes (X3). The generation of experimental runs and optimization of selected variables was done using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The data were expressed as the mean, and statistically significant difference at $p < 0.05$ was determined. All data were mean of 12 observations (n=12) for each biochemical assay.

Table 7.1. Codes derived from CCOD model for different baking processing combinations of sweet potato cubes

Code	Temperature (°C)	Time (min)	Size-cubes (cm)
-1.681	126.36	8.19	0.659
-1	140	15	1
0	160	25	1.5
1	180	35	2
1.681	193.63	41.81	2.34

7.4. Results and Discussions

7.4.1. Multiple Regression Model

The increasing awareness of consumers about nutritional benefits of foods and subsequent shifts in dietary and nutritional preferences has contributed to higher demands and rapid growth (21% between 2015-2018) of sweet potato-based food products worldwide (Nachay and Bartelme, 2014). The desirable sensory qualities, nutritional profile and associated health benefits are major attributes that contributed to this increasing demand of sweet potatoes and sweet potato-based processed foods. Processed sweet potato-based foods such as baked sweet potato is extremely popular and widely consumed across different countries and continents.

However, the nutritional qualities and associated health benefits of sweet potato-based foods vary widely based on the type of flesh color and cultivars, processing strategies and storage conditions (Oke and Workneh, 2013). Therefore, optimizing different processing strategies and processing conditions based on nutritional profile and human health protective functions of processed sweet potato-based foods has significant relevance, especially for advancing high-value and health-targeted food applications.

In this present study, processing conditions of baking of sweet potatoes were optimized based on phenolic bioactive –linked antioxidant and anti-hyperglycemic functionalities relevant and beneficial for management of early stages of type 2 diabetes (Table 7.2). The response surface methodology (RSM) based CCOD model was used to investigate the impact of the different baking conditions such as baking temperature, baking time, and size of sweet potato cubes on several above targeted human health benefits relevant response variables. The response variables specifically targeted in this study were protein content, phenolic content (TSP), antioxidant activity, anti-hyperglycemic property relevant α-amylase, and α-glucosidase enzyme inhibitory activities, color differences, and weight-loss per cent of baked sweet potato cubes.

The multiple regression model for each response variable is presented in Table 7.3. Most of the regression models for select response variables had coefficient of determination in the range of 74% to 97%, except for TSP content which had low regression coefficient (R^2 value = 0.59). Additionally, insignificant ($p<0.05$) lack of fit values of each response was also determined and presented to explain fitness of the developed RSM model for effectively predicting the effect of independent variables (baking conditions) on human health relevant bioactive qualities of baked sweet potatoes which can be targeted in health-focused food solutions.

7.4.2. The Impacts of Different Baking Conditions on Human Health Relevant Response Variables in Baked Sweet Potato

7.4.2.1. Protein Content

Protein is the most critical macronutrient of plant-based foods with significant anti-diabetic relevance, as diet rich in plant-based protein is considered beneficial for prevention and management of early stages of type 2 diabetes (McMacken and Shah, 2017). Therefore, understanding the effect of different baking process parameters on protein content of baked sweet potato has significant human health relevance, especially for targeting in dietary support strategies to manage early stages of type 2 diabetes and associated complications. In the present study, protein content of the baked orange-fleshed sweet potato cubes varied between 8.40 to 39.48 mg/g dry weight (DW), while raw sweet potato sample had protein content of 3.51 mg/g fresh weight (FW). The effect of select baking process variables and their interaction with protein content is illustrated as three-dimensional (3D) response surface plots (Figure 7.1). The three-dimensional response plot showed decrease in protein content with increase in baking temperatures. Previously, denaturation of protein at higher baking temperature and subsequent enhancement in protein digestibility was reported in the presence of reducing sugars such as maltose, fructose, and lactose by Karmas and Harris et al. (2012).

In the present study, coefficient of determination (R^2 value = 0.76) result for protein content revealed presence of 76% variation in the data evaluated. However, statistical analysis for determining lack of fitness (0.0015), indicated a weak model, which was unfit for determining the impact of baking process variables on protein content of baked sweet potato cubes (Table 7.3). Overall, lower baking temperature between 120-140°C and smaller size of sweet potato cubes resulted in higher protein content in baked sweet potato sample. However, the

health benefits of plant-based foods not only just depend on the protein content, but the interaction of protein with other bioactive compounds such as phenolics also plays a critical role in human health protective functionalities like anti-diabetic and anti-dyslipidemic properties (Seczyk et al., 2019). Therefore, determining phenolic bioactive content of processed foods is equally important to better understand their potential benefits against diet-linked chronic diseases such as early stages of type 2 diabetes.

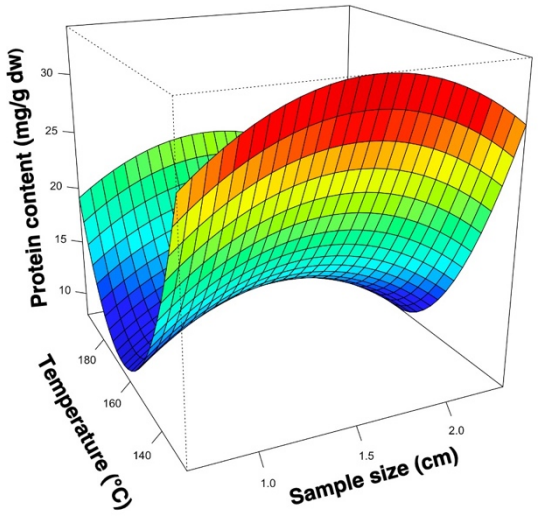

Figure 7.1. Response surface plot representing the impact of baking process variables protein content of baked orange-fleshed sweet potato cubes

7.4.2.2. Total Soluble Phenolic (TSP) Content

In this present study, TSP content of baked sweet potato cubes was determined using Folin-Ciocalteu method described by Shetty et al. (1995). The TSP content of baked sweet potato cubes ranged from 2.26 to 3.08 mg of GAE / 100 g DW. Analysis of variance (ANOVA) results showed significant ($p<0.05$) linear effect of baking time and sample size on TSP content, while effect of baking temperature was not statistically significant (p-value = 0.2460). Based on the ANOVA results, baking time and sample size of the cubes were selected as major independent variables to develop the three-dimensional response surface plot (Figure 7.2), illustrating the

impact of independent baking process variables on TSP content of baked sweet potato cubes. As shown in Figure 7.2, TSP content of baked sweet potato cubes was slightly increased with increase in baking time, and with increase in baking temperature (from 126-140 °C- not presented in 3D response surface plot due to the statistically insignificant effect on TSP content). Similarly, increase in size of sweet potato cubes from 1 to 1.5 cm also resulted in improvement of TSP content in baked sweet potato cubes. Previously, a similar trend with slight improvement in TSP content was reported in baked and boiled (Dincer et al., 2011) as well as in roasted sweet potato samples (Hou et al., 2019). This increase in TSP content with slightly higher baking temperature and longer baking time might be due to the hydrolysis of glycoside bonds and subsequent release of phenolics in cooked samples (Sharma et al., 2015).

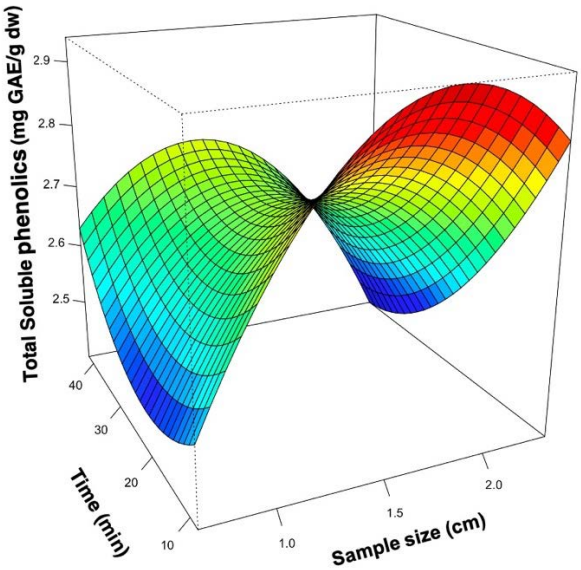

Figure 7.2. Response surface plot illustrating the effect of different baking process variables on TSP content of baked orange-fleshed sweet potato cubes

Similarly, increase in phenolic and flavonoid content was also observed in other cooked vegetables such as broccoli, green peppers, and green beans (Stewart et al., 2000). However, some previous studies on cooked/processed sweet potatoes found reduction or no significant changes in phenolic content after different heat treatments (Padda and Picha, 2008). The multiple

regression model developed in the present study showed lower coefficient of determinations (R^2 = 0.59) for TSP content, which implied only 59% of the total variation. However, insignificant lack of fit value (p value=0.4655) indicated a well-fitted regression model for the TSP content of baked sweet potato cubes. Therefore, the results of the present study indicated that increasing baking time from 15 to 25 min along with baking temperature between 126-160 °C had positive impact on phenolic content of baked sweet potato cubes. This has significant relevance in optimizing baking time and temperature to develop baked sweet potato-based functional foods with high phenolic bioactive content. Additionally, as many human health protective functions such as antioxidant and anti-hyperglycemic properties of plant- based foods positively correlate with their TSP content, this baking process optimization results also provided critical biochemical insights on potential phenolic-linked health benefits of baked sweet potato cubes, especially for type 2 diabetes relevant benefits.

7.4.2.3. Total Antioxidant Activity

Among different human health benefits related functionalities, high antioxidant activity is major biological function of phenolic compounds present in plant-based foods (Kwon et al., 2006). Plant based foods rich in dietary antioxidant like phenolics have significant relevance for dietary and therapeutic applications to manage chronic oxidative stress commonly associated with type 2 diabetes (Shetty and Wahlqvist, 2004; Sarkar and Shetty, 2014). Like phenolic content, antioxidant activity of processed foods also varies with different processing conditions. Therefore, total antioxidant activity of orange-fleshed sweet potato with different baking conditions was measured using two different assays: ABTS, and DPPH free radical scavenging assays. Antioxidant activity (based on ABTS free radical scavenging assay) of baked orange fleshed sweet potato ranged from 0.25 to 0.93 mM Trolox/g DW (Table 7.2). In the present

study, individual independent variables (baking temperature, time, and sample size) as well as their respective interactions (baking temperature × baking time, baking temperature × sample size, and baking time × sample size) had significant ($p<0.05$) linear effect on antioxidant activity based on ABTS assay of baked sweet potato cubes (Figure 7.3a). Increased antioxidant activity was observed with increase in baking temperature until 140-160 °C. The increased antioxidant activity (ABTS) with slight increase in baking temperature might be due to the release of some bioactive compounds with antioxidant potential from breakdown of cell matrices with moderate heat treatment (Hou et al., 2019).

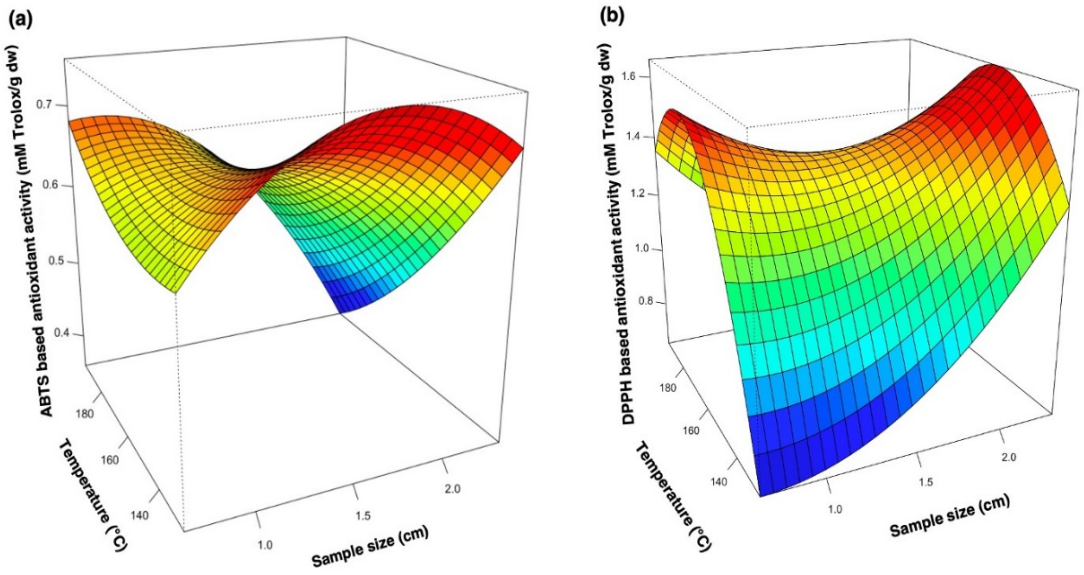

Figure 7.3. Response surface plot representing effect of different baking process variables on antioxidant activity (ABTS-assay based- 7.3 a & DPPH-assay based 7.3 b) of baked orange-fleshed sweet potato cubes

However, with further increase in baking temperature (from 160-180 °C), antioxidant activity slightly decreased, therefore 140-160 °C might be the optimum baking temperature to retain higher antioxidant functions in baked sweet potato cubes. Additionally, sweet potato cubes with bigger size (2 cm) and longer baking time (35 min) resulted into higher antioxidant activity (ABTS-assay), which might be due to the complete exposure of core part of sweet potato cube

to the thermal treatment which likely resulted in the release and mobilization of bioactive compounds with antioxidant potentials. In a previous study, similar ($p<0.05$) increase in total antioxidant activity was reported by Turkmen et al. (2005) in steamed and microwave cooked green peas, beans, and broccoli.

Results of regression model showed (R^2 value = 0.74) 74% variation in the experimental data of antioxidant activity based on ABTS free radical scavenging assay. Furthermore, statistical analysis of the developed model revealed insignificant lack of fitness (0.6355). This result suggested a good fitted statistical model for determining the impact of baking variables on antioxidant activity (ABTS-assay based) in baked sweet potato cubes (Table 7.3).

Antioxidant activity results based on DPPH free radical scavenging assay showed slightly different trend when compared to the ABTS-based assay results. Response Surface Model (RSM) developed from DPPH-assay based antioxidant activity results revealed significant ($p<0.05$) effects of baking temperature on antioxidant activity of baked sweet potato at selected 95% confidence level (Figure 7.3b). However, baking time and size of sweet potato cubes did not have any statistically significant effect on the antioxidant activity (DPPH) of baked sweet potato samples. The linear effect of baking temperature showed increase in antioxidant activity in baked sweet potato cubes with optimum range of baking temperature around 160 °C Therefore, 3D response surface plots were developed based on significant linear relationship ($p<0.05$) between baking temperature and antioxidant activity based on DPPH assay of baked sweet potato cubes. Coefficient of determination (R^2 value = 0.70) from the regression model analysis (Table 7.3) showed 70% variation in the experimental data on DPPH- assay based antioxidant activity. Additionally, statistical analysis of the developed model revealed insignificant lack of fitness (0.3561), which indicated a good fitted model for predicting the

impact of baking process variables on antioxidant activity of baked sweet potato cubes (Table 7.3). Overall, higher retention of antioxidant activity at 140-160 °C baking temperature, which was observed in this present study, has significant relevance for high value food application of baked sweet potato products in dietary interventions to counter chronic oxidative stress and to prevent associated NCDs, such as management of early stages type 2 diabetes. In addition to the antioxidant activity, it is also important to understand the potential anti-hyperglycemic functionalities of plant-based processed foods for their effective integration in type 2 diabetes relevant food solution strategies.

7.4.2.4. Anti-hyperglycemic Relevant α-Amylase and α-Glucosidase Enzyme Inhibitory Activities of Baked Sweet Potato

Inhibition of α-amylase and α-glucosidase, key enzymes involved in carbohydrate digestion, helps to reduce the post-prandial blood glucose load and to maintain glucose homeostasis in diabetic and prediabetic patients (Tundis et al., 2010). Many plant-based foods are good source of natural α-amylase and α-glucosidase inhibitors and therefore can be targeted in safe and inexpensive dietary and therapeutic strategies to manage post-prandial blood glucose, highly beneficial for management of early stages of type 2 diabetes (Apostolidis et al., 2007; Kwon et al., 2006; McCue et al., 2005). In our previous studies, (Chapter 4 & 5), high α-amylase and moderate α-glucosidase enzyme inhibitory activities were observed in sweet potatoes with different flesh color (off-white, orange, and purple). Similar to the phenolic bioactive content and antioxidant activity, anti-hyperglycemic functions such as α-amylase and α-glucosidase enzyme inhibitory activities of sweet potato potentially alter with different food processing strategies and processing conditions. Therefore, investigating the impact of different baking process variables

(independent) on α-amylase and α-glucosidase enzyme inhibitory activities of baked sweet potato has significant merit.

In this present study, α-amylase enzyme inhibitory activity of baked sweet potato cube ranged from 31.51 to 100 % under different baking process conditions. Statistically significant ($p<0.05$) linear effects of independent process variables (baking temperature, baking time, and sweet potato cube sample size) on α-amylase enzyme inhibitory activity was observed in baked sweet potato cubes (Table 7.3 & Figure 7.4). Sweet potato cubes baked at higher temperatures (180°C) had significantly lower α-amylase enzyme inhibitory activity (35.98 %), when compared with lower baking temperature (80.49 % at 140°C). Decreased α-amylase enzyme inhibitory activity at high temperature indicated heat sensitivity of natural α-amylase inhibitors present in orange-fleshed sweet potatoes. Similarly, increase in sweet potato cube size (1 to 2 cm) also had negative impact on α-amylase enzyme inhibitory activity, as it decreased significantly with bigger cube size. Previously, Rekha and Padmaja (2002) reported complete inactivation of α-amylase inhibitor during oven drying (80-100°C) of the chips derived from taro (*Colocasia esculenta*) root. However, same investigators (Rekha and Padmja, 2002) observed 0.8-10% retention of α-amylase inhibition in oven dried sweet potato chips under the same processing condition). In the present study the results of regression co-efficient (R^2 value = 0.93) and statistically insignificant for lack of fitness (0.2905) indicated a good fit model for understanding the effect of baking process variables on α-amylase enzyme inhibitory activity of baked sweet potato cubes. Overall, this result suggested that lower baking temperature and smaller size of the sweet potato cube are ideal baking process condition to retain anti-hyperglycemic function relevant α-amylase enzyme inhibitory activity in baked sweet potato food products. In this study, moderate α-glucosidase enzyme inhibitory activity (31.44 to 48.05 %) was observed in baked

sweet potato cubes under different baking conditions (Table 7.2.). Similar range of α-glucosidase enzyme inhibitory activity was observed in our previous sweet potato cultivar screening (Chapter 4) and beneficial bacteria-based sweet potato fermentation (Chapter 5) studies. Statistically significant ($p<0.05$) linear relationship between baking temperature and α-glucosidase enzyme inhibitory activity was found in this study (Table 7.2 & Figure 7.4b). However, effect of other baking process variables, such as baking time and sample size of sweet potato cubes on α-glucosidase enzyme inhibitory activity of baked sweet potato was statistically non-significant.

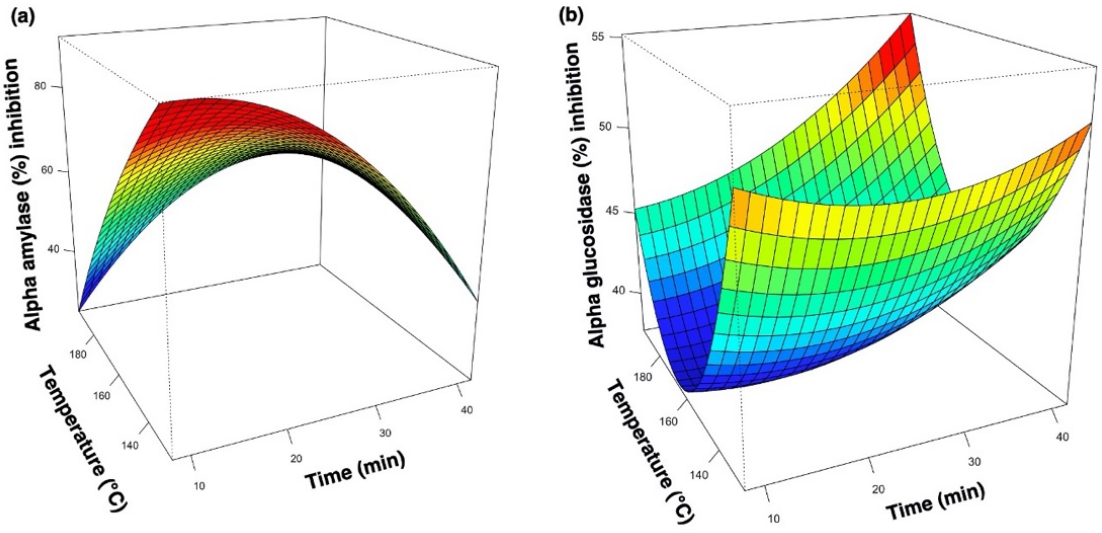

Figure 7.4. Response surface plots representing the effect of different baking process variables on α-amylase (a) and α-glucosidase (b) enzyme inhibitory activities of baked orange-fleshed sweet potato cubes

Overall, the α-glucosidase enzyme inhibitory activity of baked sweet potato slightly decreased with increasing baking temperature, but it was not substantial reduction as observed in α-amylase enzyme inhibitory activity of baked sweet potato. Previously, Ahn et al. (2014), reported reduction of α-glucosidase enzyme inhibitory activity in boiled shoots of goat's beard (*Aruncus dioicus*), an herbaceous perennial plant of *Rosaceae* family. In another study, slight improvement in α-glucosidase enzyme inhibitory activity was observed in pressure cooked

mushroom. Therefore, the effect of food processing conditions on α-glucosidase enzyme inhibitory activity of plant based processed food might depend on the food matrix and forms (free or bound) of natural α-glucosidase enzyme inhibitors in specific food under specific processing condition. The regression model and statistical analysis indicated a well-fitted model, reliable to predict the effect of different baking process variables on α-glucosidase enzyme inhibitory activity of baked sweet potato. Overall, the result of this study suggested that lower baking temperature in the range of 140 °C is optimum for retaining higher anti-hyperglycemic property relevant α-glucosidase enzyme inhibitory activity in baked sweet potato food products. Such biochemically optimized baking process condition is critical for developing anti-diabetic and other human health benefits relevant snacks and foods from oven baked sweet potatoes.

7.4.2.5. Color Difference

Information on human health and nutritional qualities of fresh and processed foods are important to enhance consumer preferences. However, the sensory and aesthetic qualities of processed foods also play critical role in consumer acceptance and preferences, especially for foods that are unknown to certain food culture. Therefore, it is important to determine the color and texture of processed foods, such as baked sweet potatoes. In this present study, effect of three selected independent variables (baking temperature, baking time, and sample size) on color difference of baked sweet potato were evaluated based on standardized L, a*, and b* values and using Macbeth, color-eye 7000 reference spectrophotometer. The color values of baked sweet potatoes obtained from spectrophotometer were used to measure total color differences and by using color of raw sweet potato cubes as reference. The L values of baked samples slightly decreased, while a* and b* values increased after baking of sweet potato cubes. This result on color changes indicated mild Millard reaction during baking process of sweet potatoes,

especially at higher baking temperatures. In the present study statistically significant ($p<0.05$) linear effect of baking temperature, baking time, size of sweet potato cubes and their respective interactions on color differences of baked sweet potato cubes was observed (Figure 7.5 & Table 7.3). The color difference was increased with increase in baking temperature, while decreased with increase in size of sweet potato cubes. Therefore, higher baking temperature and smaller sample size of sweet potato cubes might have resulted into browning reactions. The desirable golden orange color and formation of the crust in the surface of baked sweet potato cubes were observed with140 and160 °C baking temperature (Figure 7.7).

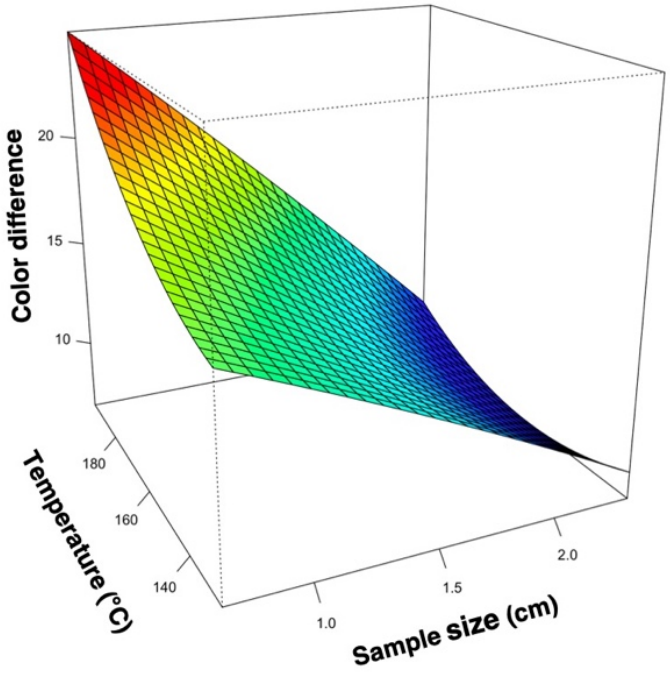

Figure 7.5. Response surface plot illustrating the effect of baking process condition on the colour difference of baked orange-fleshed sweet potato cubes

7.4.2.6. Weight Loss Percent

Determining the weight loss percent of baked sweet potato cubes has significant relevance for post-harvest preservation and their commercial food applications. The weight loss percent values of baked sweet potato cubes were in the range from 7.04 to 38.36 % (7.2). The

response surface plot presented in Figure 7.6 showed increase in weight loss percent with increasing baking temperature (38.36 % at 180°C and 9.72 % at 140 °C at constant baking time and sample size). Increased weight loss percent with increasing baking temperature indicated potential dehydration of sweet potato cubes under higher baking temperature (Manjunathan et al., 2014)). Analysis of variance (ANOVA) results for weight loss percent of baked sweet potato cubes showed statistically ($p<0.05$) significant impact of baking temperature, baking time, and sweet potato cube size on weight-loss percent of baked sweet potato. The multiple regression coefficient and the lack of fitness (p value=0.9027) results also indicated a well fitted model, reliable to understand the effect of different baking process variables on loss of moisture in baked sweet potato cubes. Overall, higher baking temperature might be desirable for long term storage and better preservation of baked sweet potato food products.

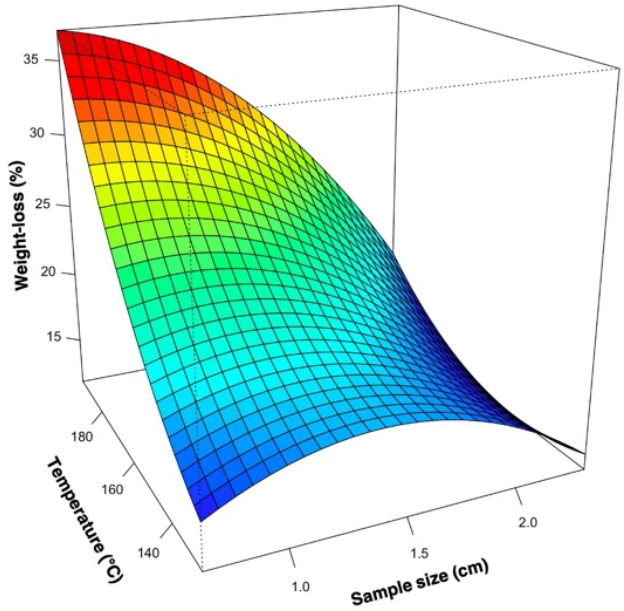

Figure 7.6. Response surface plot indicating the effect of different baking process variables on weight loss per cent of baked orange-fleshed sweet potato cubes

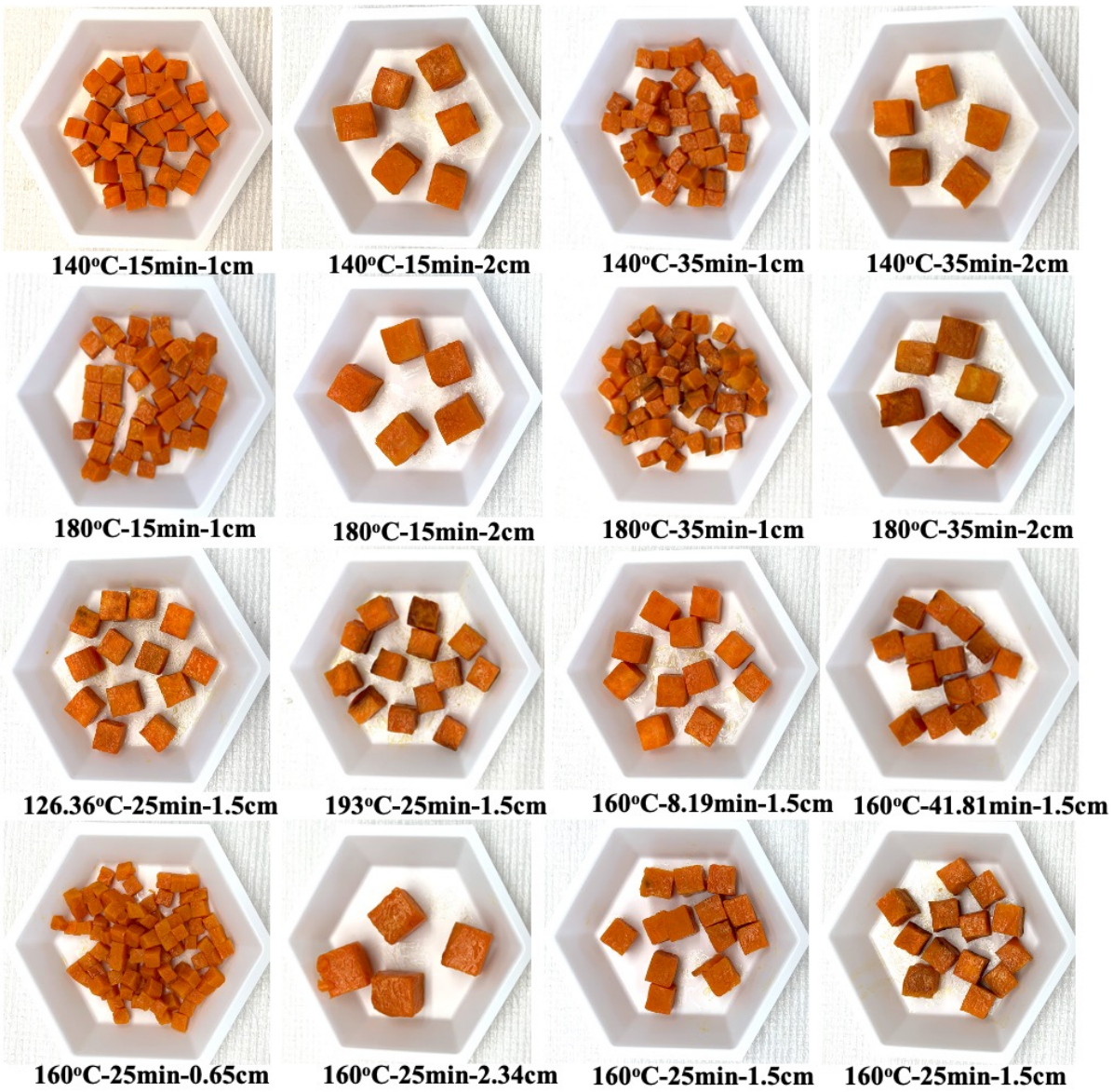

Figure 7.7. Effect of different processing conditions such as baking temperatures (140, 160, 180°C), baking times (15 min, 25 min, 35 min), and size of sweet potato cubes (1 cm, 1.5 cm, 2 cm) on the visual quality of the baked sweet potato cubes

Table 7.2. Response surface analysis of biochemical parameters with different baking process variables of baked sweet potato cubes

Experimental runs	Baking Temperature (°C)	Time (min)	Sweet potato cube size (cm)	TSP (mg GAE/g DW basis)	DPPH inhibition (mM Trolox/g DW basis)	ABTS inhibition (mM Trolox/g DW basis)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)	Protein (mg/g DW basis)	Color difference	Weight loss percent
1	140	15	1	2.82	1.12	0.86	80.49	46.55	19.74	12.01	9.72
2	140	15	2	3.12	1.47	0.57	33.57	47.64	20.49	6.56	8.33
3	140	35	1	2.72	1.36	0.39	61.46	46.40	10.68	18.74	16.18
4	140	35	2	2.84	1.38	0.93	36.39	48.05	10.54	9.92	13.89
5	180	15	1	2.43	1.31	0.53	35.99	44.88	10.11	21.11	17.14
6	180	15	2	2.26	1.14	0.69	31.15	41.11	8.40	8.26	15.28
7	180	35	1	2.81	1.16	0.84	71.90	46.92	10.53	18.34	38.36
8	180	35	2	2.43	0.96	0.64	35.06	45.85	10.55	11.67	24.32
9	126.36	25	1.5	2.48	0.29	0.74	100.00	46.78	39.48	12.97	23.94
10	193.63	25	1.5	2.62	1.17	0.44	56.32	47.86	27.36	17.28	28.57
11	160	8.19	1.5	3.02	0.49	0.43	87.06	34.57	18.02	10.66	7.04
12	160	41.81	1.5	2.51	0.97	0.64	51.15	43.25	11.23	16.38	27.14
13	160	25	0.66	2.34	1.21	0.66	49.56	40.36	13.49	17.58	26.32
14	160	25	2.34	2.53	1.57	0.25	63.98	42.44	13.21	8.74	12.12
15	160	25	1.5	2.67	1.51	0.43	70.92	31.44	17.05	14.12	18.31
16	160	25	1.5	2.81	1.30	0.70	60.32	40.90	11.87	15.15	24.29
17	160	25	1.5	3.02	1.61	0.54	80.32	35.42	14.27	12.47	22.35
18	160	25	1.5	2.54	1.13	0.57	66.43	39.23	14.75	11.99	20.24
19	160	25	1.5	3.08	1.70	0.73	79.05	40.36	12.62	12.63	22.43
20	160	25	1.5	2.46	1.41	0.64	72.54	41.32	12.91	11.09	21.87
21	160	25	1.5	2.53	1.42	0.78	69.97	39.77	13.72	13.96	19.99
22	160	25	1.5	2.40	1.30	0.68	75.37	38.43	11.86	12.43	20.32
23	160	25	1.5	2.97	1.26	0.80	69.65	40.99	16.64	13.21	24.23

TSP: Total soluble phenolics, DPPH: (2, 2-Dipheny-1—Picrylhydrazyl) inhibition), ABTS: [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)].

Table 7.3. Analysis of variance (ANOVA) for the regression models of different response variables

Responses	Degree of freedom	Sum of squares	Mean sum of squares	F-value	p-value	R-square value	Lack of fit (Pr > F)
Protein	9	768.21	85.36	4.45	0.0077	0.76	0.0015
TSP	9	0.56	0.06	0.92	0.5327	0.59	0.4655
DPPH	9	1.01	0.11	3.17	0.0329	0.70	0.3561
ABTS	9	0.46	0.05	3.94	0.015	0.74	0.6355
α-Amylase enzyme inhibitory activity	9	6416.09	712.89	16.38	<.0001	0.93	0.2905
α -Glucosidase enzyme inhibitory activity	9	321.15	35.68	3.38	<.0001	0.70	0.4796
Color difference	9	252.92	28.10	10.71	0.0001	0.88	0.0905
Weight loss percent	9	1078.90	119.88	38.12	<.0001	0.97	0.9027

TSP: Total soluble phenolic, DPPH: (2, 2-Dipheny-1—Picrylhydrazyl) inhibition), ABTS: [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid).

7.5. Conclusions

Consumer perception of baked foods as nutritionally superior and health promoting products is driving its market demands worldwide. However, the nutritional qualities and associated human health benefits of baked foods vary widely based on different processing conditions and their impacts on nutritionally relevant bioactive components. Therefore, the aim of the present study was to investigate the potential influence of baking processing variables (baking temperature, baking time, and sample size) on protein content, phenolic content (TSP), antioxidant activity, anti-hyperglycemic property relevant α-amylase and α-glucosidase enzyme inhibitory activities, color differences, and weight-loss percent of baked sweet potato cubes using response surface methodology(RSM)-based statistical model. The specific objective was to find optimum baking conditions (temperature, time and cube size) which would result in higher retention of human health relevant bioactives such as phenolics and macronutrient such as protein with implications for improved human health protective functions relevant for prevention

and management of early stages of type 2 diabetes. In this process optimization study, protein content, and anti-hyperglycemic relevant α-amylase and α-glucosidase enzyme inhibitory activities decreased with increasing baking temperature. However, increase in TSP content and associated antioxidant activity was observed with increase in baking temperature (140-160°C) and with larger size of sweet potato cubes. Additionally, higher baking temperature also resulted into dark golden color and higher weight-loss percent in baked sweet potato cubes. Overall, results of this study suggested that baking temperature between 140-160°C and baking time of 25 min are ideal process condition for higher retention of phenolic bioactive and associated antioxidant activity. Moderate anti-hyperglycemic property relevant for type 2 diabetes benefits was also observed in baked sweet potato under same baking conditions. The present study provided critical *in vitro* model-based biochemical insights on optimization of baking process variables for developing baked sweet potato-based foods with improved human health protective functions, especially for their integration in dietary and therapeutic intervention against diet-linked chronic diseases such as early stages of type 2 diabetes. However, future baking process optimizations study with different flesh color sweet potatoes and inclusion of additional baking variables are required to develop functional food and food ingredients from baked sweet potatoes with high antioxidant and anti-diabetic functionalities relevant for early stages type 2 diabetes and other NCD-linked health benefits.

CHAPTER 8. STEAMING AND BOILING PROCESS OPTIMIZATION OF ORANGE-FLESHED SWEET POTATO BASED ON PHENOLIC BIOACTIVE-LINKED ANTI-DIABETIC PROPERTIES USING RESPONSE SURFACE METHODOLOGY (RSM) MODEL

8.1. Abstract

Boiling and steaming are two traditional home cooking processes, used widely for vegetable-based food preparations. Additionally, both steaming and boiling are also economical and most convenient processes to prepare tenderized and easy to digest foods from raw vegetables, and therefore can be used in health-targeted food preparations. However, nutrient profile, bioactive content, and associated human health relevant bioactive qualities such as antioxidant property of vegetables alter significantly during boiling and steaming. In general, steamed and boiled sweet potatoes are consumed widely, however, the changes of nutritional and bioactive qualities linked with associated health benefits during these cooking processes are not well understood. Therefore, the aim of this study was to optimize steaming and boiling processing conditions of orange-fleshed sweet potato (OFSP) based on the health relevant bioactive (protein and phenolics) content and associated antioxidant and anti-hyperglycemic functionalities relevant for supportive dietary management of early stages of type 2 diabetes and Gestational diabetes. Optimization of steaming and boiling processing variables was carried out by using response surface methodology (RSM) based central composite orthogonal design (CCOD), a well-accepted and effective statistical model to determine potential relationship between independent (processing conditions) and response variables (nutritional and bioactive qualities) of an experimental design. For both steaming and boiling experiments, the CCOD model resulted into sixteen experimental runs based on two selected independent variables such

as cooking time (steaming: 4 to 16 min and boiling: 5 to 9 min) and sample size of the sweet potato cubes (1, 1.5, and 2 cm). The optimum processing conditions for steaming were cooking time of 10 min and sweet potato cube size of 1.5 cm, which resulted into higher retention of protein [0.16 mg/g fresh weight (FW) basis], total soluble phenolic content (0.44 mg GAE/g FW), high antioxidant activity (1.08 mM Trolox/g FW-DPPH based assay and 1.18 mM Trolox/g FW-ABTS based assay), and moderate anti-hyperglycaemic properties (37.43 and 37.30 % of α-amylase and α-glucosidase enzyme inhibitory activities). Similarly, high protein content (0.19 mg/g FW), total soluble phenolic content (0.41 mg GAE/g fw), high antioxidant activity (0.25 mM Trolox/g FW-DPPH based & 0.42 mM Trolox/g FW ABTS based), and low anti-hyperglycaemic functionalities (3.25 % and 13.24 % α-amylase and α-glucosidase enzyme inhibitory activities) were observed in optimum boiling conditions with cooking time of 7 min and sweet potato cube size of 1.5 cm. Overall, higher antioxidant and anti-hyperglycemic properties were found in steamed sweet potatoes when compared to the boiled samples. Therefore, the results of this study indicated that steaming of sweet potato is an effective cooking strategy for sustaining retention and stability of human health relevant bioactives and associated functionalities, which can be integrated in health-focused dietary interventions for management of diet and lifestyle-linked chronic diseases such as type 2 diabetes and Gestational diabetes.

8.2. Introduction

Sweet potatoes are rich in human health promoting nutritional compounds such as desirable resistant starch, essential micronutrients, vitamins, dietary fiber, and bioactive compounds relevant for wider health-targeted food and nutritional security solutions (Kusano and Abe, 2001). The rich micronutrient (magnesium) and bioactive (phenolics and carotenoid) profile in a desirable starch matrix background of sweet potatoes, especially in orange-fleshed

sweet potato are potentially beneficial to address micronutrient and vitamin (vitamin A) deficiency and imbalanced nutrition-linked non-communicable chronic disease (NCDs) challenges such as type 2 diabetes (Hotz et al., 2012; Jones and Brauw, 2015). Additionally, due to such balanced nutritional profile (macronutrients and micronutrients), sweet potato-based foods can be targeted for maternal and child health solutions emerging from Gestational diabetes during pregnancy through post-birth mother and children nutrition (Dutta, 2015; Ludvik et al., 2002). However, it is important to optimize sweet potato-based processed foods based on their nutritional profile and associated bioactive-linked health benefits prior to integrating in health-focused food solution strategies, especially for improving maternal and child health nutrition and to combat chronic disease challenges.

In previous rapid *in vitro* assay model-based screening and biotransformation studies (data presented in Chapter 4 & Chapter 5), high phenolic bioactive-linked antioxidant, anti-hyperglycemic and anti-hypertensive properties in aqueous extracts of raw orange-fleshed sweet potatoes were observed. However, sweet potatoes are not commonly consumed as raw due to their compact starch granules, which contributes to difficulty of absorbing essential nutrients (Bovell-Benjamin, 2007). Additionally, excess consumption of raw sweet potatoes could lead to severe indigestion resulting into abdominal cramps and diarrhea. Therefore, for human consumptions, sweet potatoes are commonly cooked or processed, which helps the breakdown of compact starch granules facilitating the digestion process and absorption of essential nutrients (Huang et al., 2016). Additionally, cooking also helps the absorption of bioactive compounds of sweet potatoes in the stomach by rupturing the cell walls of plant-based foods, breakdown of hydrogen bonds, and subsequent release of bound phenolics in cooked food matrix (Vosloo, 2005). Previously, improvement of phenolic bioactive content and associated antioxidant

property was observed in cooked-sweet potatoes (Ateea et al., 2012). The high phenolic content and high antioxidant activity were observed in deep-fried sweet potatoes followed by baked, boiled, and microwave cooked sweet potatoes (Ateea et al., 2012). Similarly, 21 to 79 % increase in phenolic content was observed in boiled sweet potatoes (Rautenbach et al., 2010). In another study, Tang et al. (2015) found higher retention of phenolic compounds, anthocyanins, and carotenoids in steamed, roasted, and boiled sweet potato samples. Likewise, Saikia and Mahanta, (2013) observed that among three different cooking methods, steaming was more effective for higher retention of phenolic content and associated antioxidant activity of several vegetables when compared to boiling and roasting methods (Saikia and Mahanta, 2013).

However, many previous studies also found contrasting results, especially negative impacts of cooking on bioactive content and associated functionalities in vegetable-based processed foods (Koh, 2005; Pellegrini et al., 2009). The reduction of bioactive content and degeneration of nutrients after thermal treatments or cooking are mostly due to the drastic changes in cellular matrix and structural integrity of vegetables. Pellegrini et al. (2009) reported reduction in antioxidant activity with deep-frying and boiling cooking processes in select vegetables. Similarly, Hong and Koh (2015) found 50-80 % reduction in anthocyanin content in steamed purple-fleshed sweet potato (PFSP) samples when compared to the raw samples. Additionally, significant reduction in phenolic content was reported in boiled PFSP samples, while no changes in phenolic content was observed in baked and steamed sweet potato samples (Hong and Koh, 2015). These contrasting results in terms of changes in phenolic linked antioxidant activities during various cooking methods, especially for steaming and boiling might be due to differences in cooking process conditions such as cooking temperatures, cooking times, size and shape of sweet potato food samples.

Therefore, it is essential to optimize different cooking process conditions of steaming and boiling of sweet potatoes and was done in this study for orange fleshed type based on human health protective bioactive (protein and phenolics) content and associated antioxidant and anti-hyperglycemic functionalities. This is relevant particularly for health-focused dietary applications against emerging chronic diseases such as type 2 diabetes and Gestational diabetes. Additionally, such cooking process optimization will also help to design orange-fleshed sweet potato-based foods both for adults and children with optimum nutritional and bioactive profiles using most economical and convenient home cooking methods such as steaming and boiling. Higher retention and stability of bioactive nutrients and associated functionalities based on cooking process optimization is also relevant for improving maternal and child nutrition, as steamed and boiled sweet potatoes are easy to digest and widely consumed as part of daily household diet. Based on above rationale, the primary aim of this study was to optimize steaming and boiling cooking process conditions (cooking time and sample size) of orange fleshed sweet potatoes based on their protein content, total soluble phenolic content, antioxidant activity, anti-hyperglycemic property, color differences, and weight-loss percent using Central Composite Orthogonal Design (CCOD) of response surface methodology (RSM) model.

8.3. Materials and Methods

8.3.1. Sweet Potato Sample and Steaming and Boiling Process

Organic orange-fleshed sweet potato (cv. Covington) tubers were purchased from local market (Natural Grocers, Fargo, ND) for steaming and boiling process optimization. Tubers were wiped with cloth to remove adhering dirt/sand and peeled using a hand peeler. Then peeled tubers were cut into desired cubes (1, 1.5, 2 cm) (Table 8.1 & 8.2) and weighed (100 g) separately for each experimental run. Steaming experiments were carried out using a household

steamer purchased from Walmart (Fargo, ND), and stainless steel-based cooking vessel was used for boiling experiment. For steaming experiment, 200 mL of water was added at the bottom of the steamer and 100 g sweet potato cubes were kept in the steaming rack during steam cooking. For boiling experiment, 100 g of sweet potato cubes were added and cooked in pre-boiled water (500 mL) (100 °C). Different cooking times (Table 8.1 & 8.2) based on the preliminary results of the CCOD analysis were used for sweet potato boiling and steaming experiments. Each steaming and boiling experimental runs were performed with fresh batch of water and after each run, utensils were cleaned thoroughly to avoid mixing of leached nutrients and any residual samples from previous experimental run.

8.3.2. Hot Water Extraction of Steamed and Boiled Sweet Potato Cubes

Steamed and boiled sweet potato cube samples were placed over a paper towel to remove excess water and allowed to cool at room temperature. Then individual samples were packed into labelled low-density polyethylene (LDPE) zip-lock bags. The steamed and boiled samples were extracted using a hot water extraction protocol, which was optimized in previous processing experiments (Chapter 6 & 7). Hot water extraction was carried out by blending 40 g of cooked (steamed and boiled) sweet potato cube sample in 100 mL of hot distilled water (preheated to 100 °C) for 5 min using a Warring blender. Then blended samples were centrifuged at 8000 rpm for 20 min and centrifugation was repeated two times. The supernatant was collected and stored in sealed centrifuge tubes and kept in the refrigerator (2-4 °C) for further human health targeted biochemical analysis using *in vitro* assay models.

8.3.3. Chemicals Used

Porcine pancreatic alpha–amylase (EC 3.2.1.1), rat intestinal alpha–glucosidase (EC 3.2.1.20), 2,2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-

picrylhydrazyl (DPPH), Bio-rad, and Trolox, 2, 2- Azobis (2- amidinopropane) dihydrochloride (AAPH)). These chemicals were purchased from Sigma Chemical Co (St. Louis, MO).

8.3.4. Total Protein Content

The total protein content of the steamed and boiled OFSP cubes was measured using a modified version of the Bradford assay (Bradford, 1976). The protein dye reagent (Bio-Rad protein assay kit II, Bio-Rad Laboratory, Hercules, CA) was diluted in 1:4 with distilled water and 5 mL of the diluted dye was added to 50 µL of the sweet potato sample extracts, mixed thoroughly and incubated in the dark for 5 min. For the blank tube, 50 µL of distilled water was added instead of the sweet potato sample. All samples were analyzed in triplicates and the absorbance was measured at 595 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, MA). A standard curve was prepared using bovine serum albumin (BSA) in different concentrations and the protein content was expressed as mg/g fresh weight (FW) basis.

8.3.5. Total Soluble Phenolic Content

The total soluble phenolic (TSP) content of hot water extracts of steamed and boiled OFSP was determined based on the protocol described by Shetty et al. (1995). The assay was carried out by taking 0.5 mL of extracts to the sample in glass test tubes and diluted for 2 times by adding 0.5 mL of distilled water. For measuring blank, 0.5 mL of distilled water was added instead of the sample. Then in each tube, 1 mL of 95 % ethanol, 5 mL of distil water, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent, and 1 mL 5% sodium carbonate were added sequentially. Then all reagents were mixed thoroughly by using a vortex mixer and incubated under dark condition for 60 min. After incubation, samples were taken out and mixed again before reading the absorbance using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer,

Thermo Scientific, NY) set at 725 nm. The absorbance values were converted to total soluble phenolic content (TSP) expressed in milligrams gallic acid equivalent (GAE) per gram FW, based on a standard curve that was established using different concentrations of gallic acid in 95% ethanol.

8.3.6. Total Antioxidant Activity

The total antioxidant activity of the steamed and boiled OFSP extracts was measured using two different assays: 2, 2-Dipheny-1—Picrylhydrazyl (DPPH) free radical scavenging assay, and 2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation assay. For the DPPH assay, as described by Kwon et al. (2006), 0.25 mL sweet potato cooked sample extract was taken into 1.5 mL centrifuge tubes, while 0.25 mL 95 % ethanol was added to the control tube. Then 1.25 mL of 60 mM DPPH (in 95% ethanol) was added to each micro-centrifuge tubes containing sample extract. The tubes were mixed well using a vortex mixer to ensure proper mixing and then incubated for 5 min. The mixture was then centrifuged at 13,000 rpm for 1 min to pellet down any of the precipitate. The absorbance of the supernatant was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10S UV-VIS spectrophotometer, Thermo Scientific, NY). For the ABTS assay (Re et al., 1999) the same procedure was followed except in this case 1 mL of ABTS (in 95% ethanol) was added to 0.05 mL of cooked sample (steamed or boiled) or control (95 % ethanol) and the absorbance was measured at 734 nm using a UV-visible spectrophotometer (Genesys 10S UV- VIS spectrophotometer, Thermo Scientific, NY). Based on the absorbance readings, the percentage of inhibition for both DPPH and ABTS radicals was calculated using the equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance extract}}{\text{Absorbance control}} \text{ x } 100 \qquad (8.1)$$

The percentage of inhibition obtained from the DPPH and ABTS radical scavenging assays was then expressed as mM Trolox equivalents (TE) per gram of sample based on a Trolox standard curve.

8.3.7. α-Amylase Enzyme Inhibitory Activity

The assay protocol used in this study was adapted from the Worthington Enzyme Manual (1993a). The buffer used was 0.1 M sodium phosphate (pH 6.9) with 0.006 M sodium chloride added to it. A volume of 500 µL of each sample extract was added to the test tubes while the control tubes had 500 µL of buffer instead of the sample. Additionally, each sample extract had a corresponding sample blank tube which contained 500 µL of the sample extract. Then 500 µL of porcine pancreatic amylase (0.5 mg/mL buffer) enzyme solution was added to all the tubes except for the sample blank and incubated at 25°C for 10 min. After incubation, 500 µL of 1% starch (1 g/100 mL buffer) was added to all the tubes and incubated for another 10 min. The reaction was then stopped by the addition of 1 mL of 3,5-dinitro salicylic acid (DNS) and the tubes were placed in a boiling water bath for 10 min. After that, tubes were taken out and cooled at room temperature. The reaction mixture in the tubes was then diluted by adding 10 mL of distilled water to adjust the absorbance of the control to 1.0 ± 0.02 and the absorbance was measured at 540 nm using a UV-VIS Genesys spectrophotometer (Genesys UV- visible, Milton Roy Inc, Rochester, NY).

The percentage of inhibition of α amylase enzyme inhibitory activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - (\text{Abs sample extract} - \text{Abs sample blank})}{\text{Abs control}} \text{ x } 100 \qquad (8.2)$$

8.3.8. α-Glucosidase Enzyme Inhibitory Activity

The assay protocol used was based on the method described in the Worthington Enzyme Manual (1993b) with some modifications were taken from McCue et al. (2005). A volume of 50 µL, 25 µL and 10 µL of sample extract was pipetted into 96 well microtiter plates and used as undiluted, half diluted and 1/5th diluted, respectively for determining potential dose depended response. The half dilution and 1/5th dilutions were made up to a total of 50 µL in volume by adding 25 µL and 40 µL of 0.1 M potassium phosphate buffer (pH 6.9), respectively. Each sample extract had a corresponding control of 50 µL of phosphate buffer instead of the sample. Finally, the volume in all the wells was made up-to 100 µL by the addition of 50 µL of phosphate buffer in each well including the control. Then 100 µL of 0.1M potassium phosphate buffer (pH 6.9) containing α-glucosidase enzyme (1 U/mL) was added to each well and incubated at 25 °C for 10 min. After this 50 µL of 5 mM p-nitrophenyl-α-D- glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were then incubated at 25 °C for 5 min. Absorbance readings were taken before (0 min) and after (5 min) incubation period using a microplate reader (Thermomax, Molecular device Co., Virginia, USA) set at 405 nm.

The percentage of inhibition of α-glucosidase enzyme inhibitory activity was calculated based on the absorbance readings and using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs control 5min} - \text{Abs control 0min}) - (\text{Abs extract 5min} - \text{Abs extract omin}}{2\text{Abs control 5min} - \text{Abs control 0min}} \text{ x } 100 \quad (8.3)$$

8.3.9. Color Difference

Color difference was calculated from L, a*, and b* values measured for OFSP steamed and boiled samples (as presented in table 8.3 and 8.4) using Macbeth, color-eye 7000 reference spectrophotometer. The software used was ProPalette 5.0. L values indicate lightness/darkness,

a* values represent redness/greenness, and b* values indicate blueness/yellowness of cooked samples respectively. Total color difference (ΔE) of both steamed and boiled OFSP samples was calculated from the following equation (Altunakar et al., 2004)

$$\Delta E = \sqrt{[(L^* - L^*_{standard})^2 + [(a^* - a^*_{standard})^2 + [(b^* - b^*_{standard})^2]} \quad (8.4)$$

Where, standard values referred to raw sweet potato L, a*, and b* values. Triplicates values were recorded at room temperature and mean values were derived.

8.3.10. Weight Loss and Weight Gain Determination

The weight loss per cent of steamed and weight-gain per cent of boiled OFSP samples were determined using weighing before and after boiling. The weigh-loss per cent was calculated according to the following formula,

$$\text{Weight loss ratio } (\%) = \frac{(W1 - W2)}{W1} \text{x } 100 \quad (8.5)$$

$$\text{Weight gain ratio } (\%) = \frac{(W2 - W1)}{W2} \text{ x } 100 \quad (8.6)$$

Where,

W1= Weight of selected sweet potato cubes before cooking.

W2= Weight of selected sweet potato cubes after cooking.

8.3.11. Statistical Modeling for Optimization of Steaming and Boiling OFSP cubes

In this study, response surface methodology (RSM) based central composite orthogonal design (CCOD) (Box and Wilson, 1951) was used to study the impact of two independent processing variables of steaming and boiling on protein content, TSP content, antioxidant, and anti-hyperglycemic functionalities which were determined using *in vitro* assay models. The range for each processing variables were set up based on preliminary experiments. The two selected independent variables examined under this study were, X1: time, and X2: sample size.

Based on selected independent processing variables, CCOD resulted in 16 experimental runs for steaming as well as for boiling in completely random order. The resulting design included four factorials points, four axial points and eight replicated center points (Mason et al. 2003) and five levels of codes for both steaming and boiling are presented in Table 8.1 and 8.2. In addition, optimal processing conditions were predicted using second-order polynomial function to correlate selected independent and dependent variables. Analysis of variance (Table 8.5 and 8.6) was interpreted to fit the developed models, as well as surface response plots were derived to explain effect of independent variables (processing variables) on selected response variables (nutritional and bioactive qualities).

Table 8.1. Codes derived from CCOD model for different steaming processing combinations of sweet potato cubes

Code	Time (min)	Sample Size (cm)
-1.414	1.516	0.79
-1	4	1.0
0	10	1.5
1	16	2.0
1.414	18.484	2.21

Table 8.2. Codes derived from CCOD model for different boiling processing combinations of sweet potato cubes

Code	Time (min)	Sample Size (cm)
-1.414	4.17	0.707
-1	5	1
0	7	1.5
1	9	2
1.414	9.828	2.207

8.3.12. Data Analysis

Optimization of data analysis was carried out assuming a second order polynomial equation for each of the response variables as a function of independent variables; X1: cooking time and X2: size of sweet potato cubes. The generation of experimental runs and optimization

of selected variables was done using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The data were expressed as the mean, and statistically significant difference at $p < 0.05$ was determined. All data were mean of 12 observations (n=12) for each biochemical assay parameters.

8.4. Results and Discussion

8.4.1. Multiple Regression Modeling

Boiling and steaming are most convenient cooking methods used widely in common household cooking to prepare diverse plant-based foods. Additionally, boiled and steamed vegetables are also preferred in baby food preparation as they are easy to digest and more palatable. However, nutritional content and associated health benefits of vegetables alter significantly during steaming and boiling cooking process (Poelman et al., 2013; 2015). Therefore, optimizing steaming and boiling process conditions of vegetables based on the retention of bioactive compounds and associated human health relevant functionalities has significant dietary and therapeutic benefits. Based on this rationale, the main objective of this present study was to optimize steaming and boiling cooking process conditions of orange-fleshed sweet potato (OFSP) based on phenolic-linked antioxidant and anti-hyperglycemic properties. Such health-targeted cooking process optimization has significant relevance for high-value food applications of sweet potato to address diet-linked chronic disease challenges, such as type 2 diabetes and Gestational diabetes. Additionally, health and nutritionally relevant optimization of boiling and steaming of OFSP will also help to develop conveniently prepared baby foods with optimum nutritional qualities to support hunger related macro and micronutrient deficiencies in developing and developed countries.

In this present study, optimization of steaming and boiling cooking process of OFSP was carried out using a statistical model as summarized in Table 8.3 and 8.4. The response surface methodology (RSM) based CCOD was used to observe the effect of the different processing variables (cooking time and sample size) on the phenolic bioactive linked antioxidant and anti-hyperglycemic functionalities of both steamed and boiled OFSP food samples. The impacts of independent variables on each response variables are presented as three-dimensional (3D) response surface plots. Additionally, effect of different processing conditions on the visual appearance of steamed and boiled OFSP samples are also presented (Figure 8.7 & 8.8). Furthermore, statistical analysis of the multiple regression model explaining the variations of the data for each biochemical parameter are presented in Table 8.5 and 8.6.

8.4.2. The Impacts of Steaming and Boiling Parameters on Selected Response Variables

8.4.2.1. Protein Content

Protein is an important nutritional component, widely considered as major dietary antidote against diet and lifestyle linked NCDs, especially critical for managing type 2 diabetes and Gestational diabetes. Reduced risk of type 2 diabetes with higher intake of vegetable-based protein was observed in several epidemiological studies (Chiu et al., 2014; Satija et al., 2016; Tonstad et al., 2013). Furthermore, processed vegetables rich in dietary protein is also relevant for addressing hunger related macro and micronutrient deficiency and associated health complications of both children and adults (Schonfeldt and Hall, 2012). Therefore, optimizing cooking process conditions based on protein content of cooked vegetables, such as sweet potato has significant relevance for nutritional insecurity and diet-based public health solutions.

During processing and cooking of sweet potatoes, enzymatic hydrolysis can lead to the formation of protein hydrolysates, which are relevant for diverse health related functionalities

such as, antidiabetic, antihypertensive, and antimicrobial properties (Zhang and Mu, 2016). In this present study, protein content of steamed and boiled OFSP samples (Table 8.3 & 8.4) was determined using Bradford assay (Bradford, 1976). Overall, protein content of the cooked sweet potato sample was found in the range of 0.12 to 0.97 mg/g FW for steamed OFSP samples, and 0.12 to 0.22 mg/g FW for boiled OFSP samples. Additionally, protein content of OFSP reduced significantly in steamed and boiled samples when compared to the raw OFSP sample (3.55 mg/g FW). However, changes in protein content in cooked OFSP sample was based on cooking process conditions such as cooking time and size of sweet potato cubes. The impact of independent processing variables such as cooking time and size of the sweet potato cubes on protein content of steamed (Figure 8.1 a) and boiled (8.1b) sweet potato are presented as 3D response surface plots.

In this present study, cooking time had statistically significant ($p<0.05$) impact on protein content of steamed sweet potato, while effect of sweet potato cube size was statistically insignificant ($p<0.05$). Higher protein content was observed with shorter cooking time (steaming of 2 cm cubes for 5 min steaming had 0.24 mg/g FW of protein) in the steamed OFSP sample (Table 8.3). However, results of the boiling study showed statistically significant ($p<0.05$) effect of sweet potato cube sizes on protein content, while impact of cooking time was not statistically significant. Overall, at shortest cooking time (extreme lower axial point of CCOD in the present study shown in Table 8.1 & 8.2) good retention of protein content among steamed (0.97 mg/g FW at 1 min 51 sec steaming time) and boiled OFSP samples (0.175 mg/g FW at 4 min 17 sec boiling time) was observed. Previously, Yuan et al. (2009) reported higher retention of protein (2.6 mg/g FW) among steamed broccoli compared to boiled and stir-fried samples at constant cooking time (5 min). In the present study, as shown in 3D response surface plots (Figure 8.1a &

8.1b), protein content gradually decreased with increasing cooking time in both steamed and boiled OFSP samples. Higher cooking time in both steaming and boiling had negative impact on protein content, which indicated a potential inactivation of protein during steaming and boiling cooking process. However, protein content increased with increase in size of sweet potato cubes in boiling experiment. The optimum sweet potato cube size for boiling experiment, which resulted into higher protein content was 2 cm, while for steaming process it was 1.5 cm. Overall, the results of this study provided critical insights about the role of cooking time and size of sweet potato samples on protein content of steamed and boiled OFSP. The shorter cooking time and bigger size of sweet potato cubes are optimum for retaining higher amount of protein in cooked sweet potato sample. These results have significant relevance for developing convenient sweet potato based cooked foods with higher protein content, which can be targeted for addressing both chronic disease challenges such as type 2 diabetes and gestational diabetes, as well as to support hunger related nutritional deficiency of children and adults.

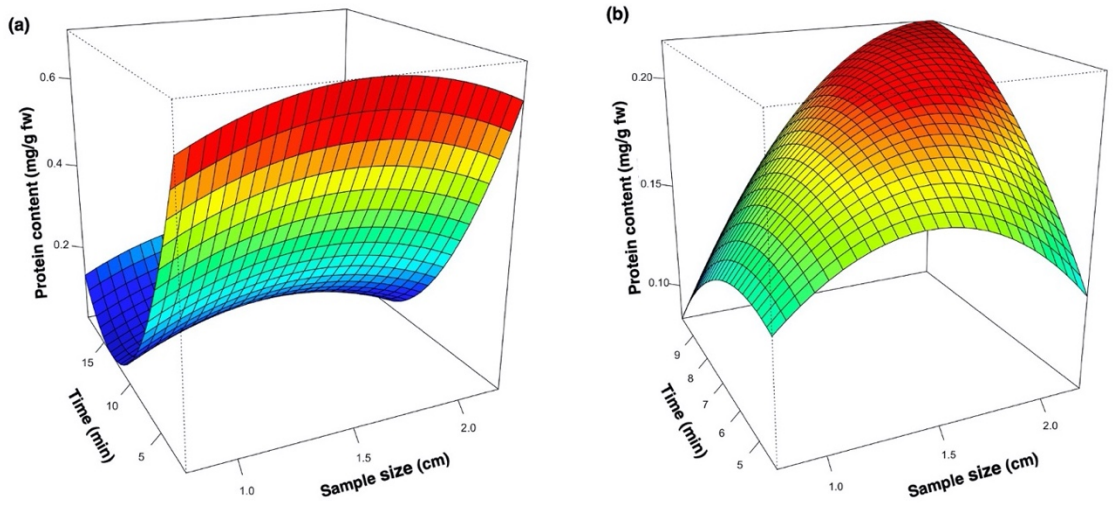

Figure 8.1. Response surface plots illustrating the impact of cooking time and size of sweet potato sample on protein content of steamed (a) and boiled (b) OFSP cubes

The coefficients of determination (R^2 value) of multiple regression model was 0.86 and 0.75 for steaming and boiling process respectively. Additionally, insignificance ($p<0.05$) of lack of fit values for steaming (0.3099) and boiling (0.5365) indicated a well-fitted model suitable for determining the relationship between cooking process variables and protein content of steamed and boiled orange-fleshed sweet potatoes. However, in addition to the protein content it is also important to understand the effect of cooking process variables on other human health relevant bioactive profile such as phenolic compounds of cooked orange-fleshed sweet potatoes, especially for high-value and health-focused food applications targeting NCD-linked health benefits.

8.4.2.2. Total Soluble Phenolics (TSP)

Biologically active phenolic compounds alter significantly during processing and cooking of vegetables, which substantially affect human health related nutritional qualities of cooked foods (Natella et al., 2010; Palermo et al., 2014). Previously, significant reduction of phenolic content was observed in boiled vegetables such as broccoli (Zhang and Hamauzu, 2004), pepper, peas (Turkmen et al., 2005), and sweet potatoes (Min et al., 2012; Tang et al., 2015). However, changes in phenolic content during cooking potentially depends on composition of phenolic compounds, structural stability of the food matrix and different cooking parameters (Palermo et al., 2014). Therefore, in this present study, total soluble phenolics (TSP) content of the cooked (steamed and boiled) orange-fleshed sweet potato samples with different cooking times and sample cube sizes was determined using modified Folin-Ciocalteu method previously described by Shetty et al. (1995). Overall, TSP content of steamed OFSP samples was higher and in the range from 0.44 to 0.60 mg GAE/g FW (Table 8.3), while it was between 0.32 to 0.45 mg GAE/g FW (Table 8.4) in boiled sample. The impact of selected independent variables (cooking

time and sweet potato sample cube size) on TSP content of steamed and boiled OFSP samples are presented as 3D response surface plots (Figure 8.2a & 8.2b).

Higher TSP content was observed with smaller sample cube size (1 cm) and at shorter cooking time (5 min) in steamed sweet potato sample. However, for boiling experiment, TSP content increased with increase in sweet potato cube size. These results indicated a potentially higher thermal degradation and leaching of phenolics into boiling water for smaller cube size (1 cm) sweet potato samples. Overall, TSP content was higher at shorter cooking for both steamed and boiled sweet potato sample. Previously, Padda and Picha (2008) reported 37 % reduction in TSP content in boiled sweet potato samples when compared to the raw samples. Similarly, detrimental effect of boiling on TSP content of sweet potatoes, carrots, and cauliflower was reported by Buratti et al. (2020). In the present study, analysis of variance (ANOVA) results showed statistically significant ($p<0.05$) linear effect of steaming time and interaction effect of steaming time × sample size on TSP content of steamed sweet potatoes. Whereas, for boiling experiment, only statistically significant linear effect of sample cube size ($p<0.05$) on TSP content of boiled sweet potato was observed.

Furthermore, the multiple regression analysis (R^2 = 0.98 and 0.91) and insignificant lack of fit values (p value=0.6715 and 0.3500) showed a well-fitted model, which is suitable for describing the effect of steaming and boiling process variables on TSP content of cooked sweet potato (Table 8.5). The results of this study indicated that steaming with shorter cooking time was the preferable cooking method for higher retention of phenolic compounds in cooked sweet potato sample. However, smaller sweet potato cube size is ideal for steam cooking, while bigger cube size is optimum for boiling, which resulted in higher TSP content. Therefore, steam cooking can be advanced to develop sweet potato-based healthy and convenient foods with

higher nutritional profile and associated bioactive functionalities for health-focused food solutions, such as dietary prevention and management of chronic diseases (NCDs). It is also important to understand that whether changes in phenolic content during cooking have any significant impact on human health relevant functionalities like antioxidant and anti-hyperglycemic properties in cooked sweet potato-based foods.

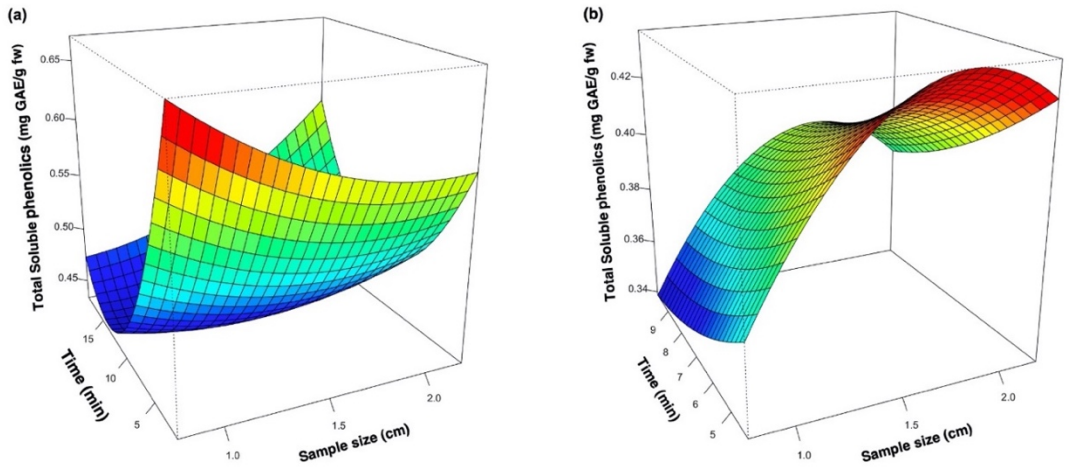

Figure 8.2. Response surface plots representing the effect of cooking process variables on TSP content of steamed (a) and boiled (b) OFSP cubes

8.4.2.3. Total Antioxidant Activity

Dietary antioxidants of plant-based foods have wider human health relevance, as they provide protection against chronic oxidative stress related diseases and promote vascular health, which is critical for management of type 2 diabetes, Gestational diabetes and cardiovascular disease (Akbar et al., 2011; Parast and Paknahad, 2017). Furthermore, dietary antioxidants are also important for managing chronic infection and inflammation commonly associated with hunger-linked malnutrition (Al-Gubory, 2017; Katona and Katona-Apte, 2008). Therefore, higher intake of foods rich in dietary antioxidants is widely recommended to improve health and to combat both infectious and non-infectious diseases (Pham-Huy et al., 2008). As thermal treatments or cooking alter bioactive content and composition of plant-based foods, they also

significantly affect the overall antioxidant property of the cooked foods. In the present study, total antioxidant activity of steamed and boiled OFSP samples was measured using two different assays: DPPH, and ABTS free radical scavenging assays. The total antioxidant activity (DPPH-free radical assay) of steamed OFSP samples ranged between 0.14 and 0.32 mM Trolox/g FW, while for boiled OFSP sample it was between 0.13 and 0.33 mM Trolox/g FW (Table 8.3 and 8.4). The impact of select cooking process variables, cooking time and sample cube size on DPPH free radical scavenging-based antioxidant activity of steamed and boiled OFSP samples are presented as 3D surface plots (Figure 8.3a & 8.4a). Higher antioxidant (DPPH-assay based) activity (0.31 and 0.28 mM Trolox/g FW) was observed in smaller sample size cubes (1 cm) of steamed OFSP samples. Additionally, highest antioxidant activity (DPPH-based) of steamed OFSP sample was found with 10 min of cooking time. Therefore, in steam cooking, longer cooking time (up to 10 min) did not affect the antioxidant activity of OFSP sample. However, in boiled samples bigger sample cube size (1.5-2 cm) and cooking time of 7 min resulted into higher antioxidant activity (Figure 8.4a). Both in steamed and boiled OFSP sample, sample cube size had similar impact on TSP content and antioxidant activity (DPPH-based), which indicated phenolic-linked antioxidant activity in cooked OFSP sample. Overall, the results of regression co-efficient (R^2 value = 0.80) and statistically significant for lack of fitness (0.004) suggested a weak model for understanding the effect of steaming process variables on DPPH based antioxidant activity. However, same regression model analysis and lack of fitness data showed well-fitted model for describing impact of boiling process variables on antioxidant activity (DPPH-based) of boiled OFSP food samples.

Total antioxidant activity based on ABTS- free radical scavenging assay was in the range of 0.30 to 0.56 mM Trolox/g FW in steamed and 0.29 to 0.55 mM Trolox/g FW in boiled OFSP

cubes (Table 8.3 & 8.4). Statistical analysis (ANOVA) revealed insignificant ($p<0.05$) effect of linear terms and significant effect of quadratic term (time × time and sample size × sample size) on ABTS based antioxidant activity of steamed OFSP cubes. The multiple regression model showed R^2 value of 0.82 (Table 8.5), which revealed the presence of 82 % of variation in the data set. Additionally, insignificant lack of fitness ($p = 0.65$) analysis indicated a well-fitted model to understand the effect of steaming variables on antioxidant activity (ABTS-based) of steamed OFSP food sample. However, in the present study, the effect of boiling process variables on ABTS based antioxidant activity was not statistically significant ($p<0.05$). Additionally, significant lack of fitness (p-value 0.0009) analysis revealed that the model is not well-fit to determine the relationship between boiling conditions with antioxidant activity (ABTS-based) of OFSP food samples ($p < 0.05$). Significant reduction of total antioxidant activity (ABTS-based) was observed in boiled OFSP with increase in cooking time. Previously, Sikora et al. (2008) observed reduction of antioxidant activity (ABTS-based) in several boiled vegetables such as kale, broccoli, Brussels sprout, and cauliflower. Similarly, Tian et al. (2016) observed reduction of antioxidant activity in boiled purple-fleshed sweet potato.

Overall, higher mean antioxidant activity of steamed and boiled sweet potato sample was observed with ABTS-based free radical cation assay, when compared to the results of DPPH-free radical scavenging assay. The differences in antioxidant activity results between two assays are based on their different sensitivity and affinity towards hydrophilic and hydrophobic moieties of the specific food matrix (Floegel et al., 2011). Previously, Ateea et al. (2012) also found higher antioxidant activity with ABTS based assay in cooked and processed (boiling, baking, frying, and microwave cooking) vegetables. One of the key findings of the present study is the higher retention of antioxidant activity in steamed OFSP, even after 10-15 min cooking. Such higher

retention and stability of antioxidant activity in steamed OFSP has wider human health and food-solution relevance. Therefore, steam cooking might be the ideal and most convenient food processing strategy, that can be targeted to improve retention of human heath protective dietary antioxidants in orange-fleshed sweet potatoes.

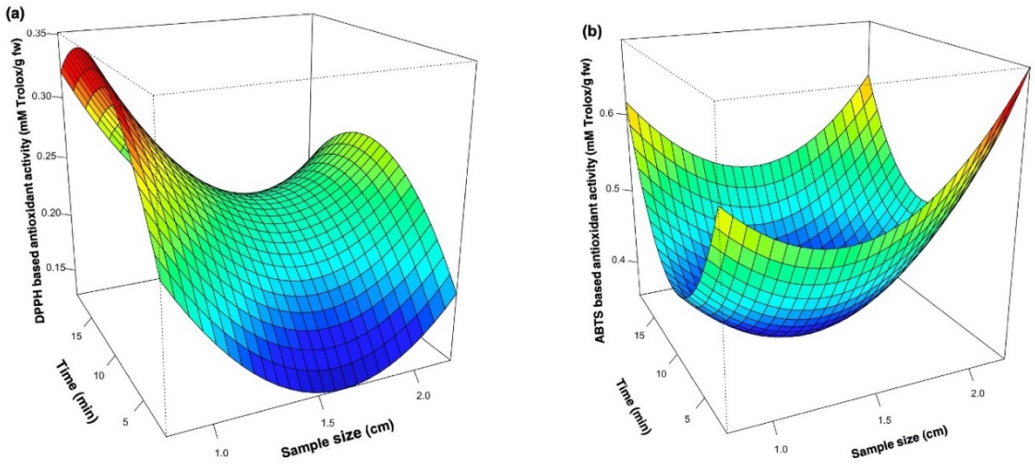

Figure 8.3. Response surface plots representing the effect of steaming process variables on antioxidant activity of steamed OFSP based on DPPH (a), and ABTS-based (b) assays

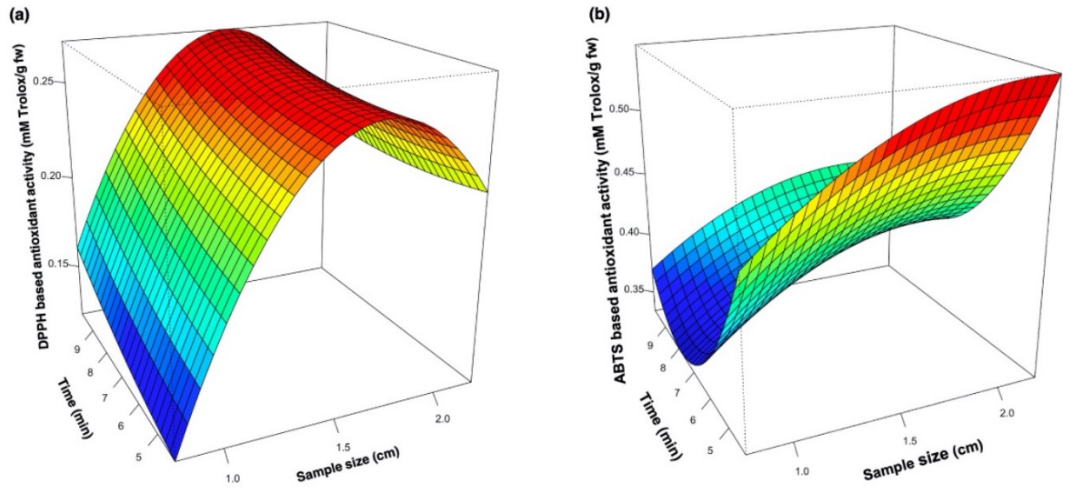

Figure 8.4. Response surface plots representing the effect of boiling process variables on antioxidant activity of boiled OFSP based on DPPH (a), and ABTS-based (b) assays

The steam cooked OFSP can be integrated in dietary support strategies to combat chronic oxidative stress linked NCDs, such as type 2 diabetes and Gestational diabetes. Furthermore, the higher antioxidant activity of steamed OFSP also have relevance in providing cellular protection

against common infections, and this cooking strategy can be targeted for designing easy to digest and health relevant sweet potato-based foods for infants, toddler, and young children as well as for the adults.

8.4.2.4. α-Amylase and α-Glucosidase Enzyme Inhibitory Activities

Managing post-prandial blood glucose level and combating chronic hyperglycemia are critical for patients with type 2 diabetes and Gestational diabetes (Bimson et al., 2017; Ceriello et al., 2004). Therefore, many therapeutic interventions for diabetes management target drugs and foods with anti-hyperglycemic functionalities. In this context, inhibition of key enzymes involved in glucose metabolism, such as α-amylase and α-glucosidase, slowdown the breakdowns of complex carbohydrates into glucose and its absorption in the bloodstream, which subsequently help to maintain the glucose homeostasis (Tundis et al., 2010). In our previous *in vitro* assay model-based studies (Chapter 4 & 5), we observed moderate α-amylase and α-glucosidase enzyme inhibitory activities in orange-fleshed sweet potatoes. However, significant changes in inhibitory activities of these enzymes with thermal processing (Chapter 6 & 7) was also found. Therefore, in this present study effect of steaming and boiling process variables on α-amylase and α-glucosidase enzyme inhibitory activities was investigated. The steaming and boiling process optimization based on anti-hyperglycemic property relevant α-amylase and α-glucosidase enzyme inhibitory activities is important for safe and effective dietary application of steamed and boiled OFSP targeting type 2 diabetes and Gestational diabetes benefits.

In this present study, steamed OFSP had significantly higher α-amylase enzyme inhibitory activity in the range of 10.54 to 93.52 % inhibition (Table 8.3) when compared to the boiled OFSP food sample (0.97 to 19.55 % inhibition) (Table 8.4). For steamed sample, shorter cooking time (less than 5 min) and smaller size of the sweet potato cubes (1 cm) resulted in

higher α-amylase enzyme inhibitory activity. However, for boiled OFSP, higher α-amylase enzyme inhibitory activity was observed with larger size (2 cm) of the sweet potato cubes and with longer cooking time (7-9 min). The differences in α-amylase enzyme inhibitory activity between steaming and boiling cooking processes might be due to the direct contact of aqueous thermal treatment and potential degradation of anti-hyperglycemic property relevant bioactives in boiled OFSP food sample. Similar trend regarding the effect of cooking time and sample cube size on protein content, TSP content, and antioxidant activity (DPPH based) was also observed in boiled OFSP food sample. Previously, Yuan et al. (2009) and Padda and Picha (2008) found significant leaching off phytochemicals and associated functionalities during boiling of sweet potatoes. Statistically significant ($p<0.05$) effect of cooking time on α-amylase enzyme inhibitory activity of steamed OFSP was observed, while effect of sample size was not significant. However, for boiled OFSP samples, statistically significant ($p<0.05$) effect of sample cube size on α-amylase enzyme inhibitory activity was observed, while effect of cooking time was statistically insignificant. The multiple regression analysis and insignificant lack of fit value (0.0944) indicated that the developed model is well-fitted to explain the effect of boiling cooking conditions on α-amylase enzyme inhibitory activity.The higher α-amylase enzyme inhibitory activity of steamed OFSP food sample has significant relevance for its potential anti-diabetic management related dietary applications. Additionally, the present study also provided critical insights on optimizing steaming process conditions (shorter cooking time and smaller sample size) of OFSP for retaining higher anti-hyperglycemic functionalities, which is relevant for potential dietary interventions against type 2 diabetes and Gestational diabetes.

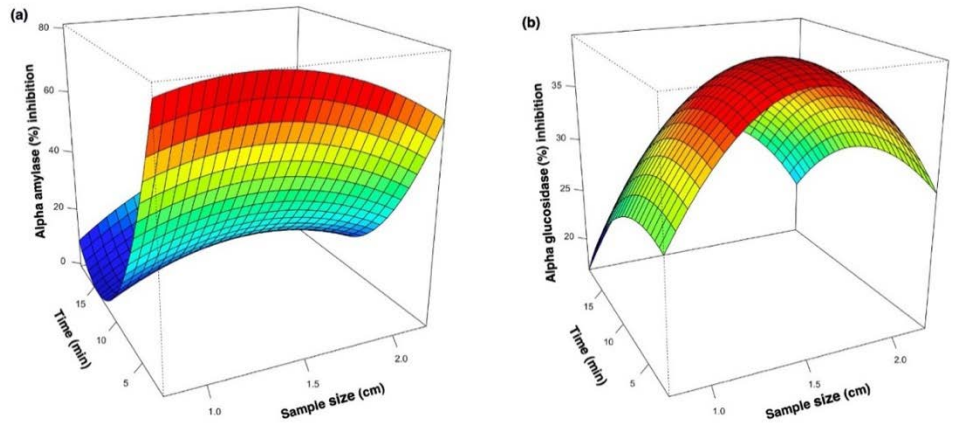

Figure 8.5. Response surface plots illustrating the effect of steaming process variables on α-amylase (a) and α-glucosidase (b) enzyme inhibitory activities of steamed OFSP cubes

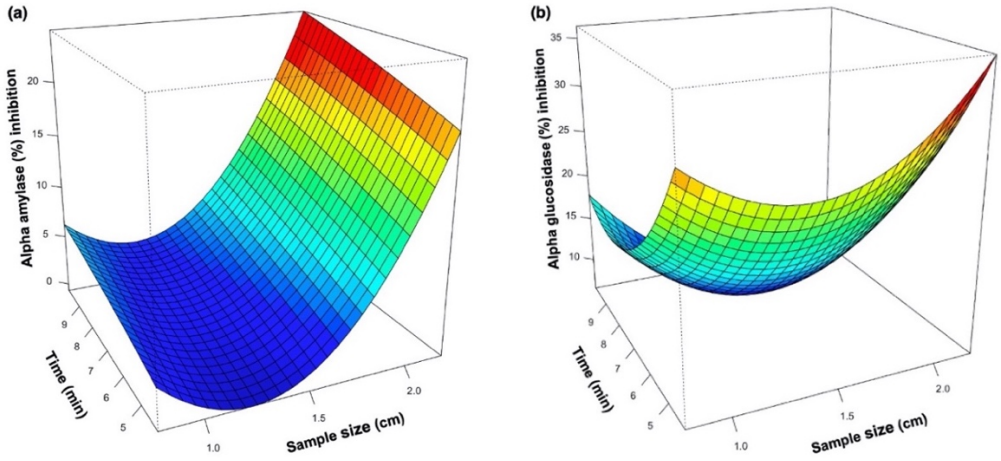

Figure 8.6. Response surface plots illustrating the effect of boiling process variables on α-amylase (a) and α-glucosidase (b) enzyme inhibitory activities of boiled OFSP cubes

Like the results of our previous studies (Chapter 4 &5), the mean α-glucosidase enzyme inhibitory activity found in this process optimization study was lower than the mean α-amylase enzyme inhibitory activity in OFSP sample. Interestingly, the adverse effect of steaming on α-amylase enzyme inhibitory activity was more severe when compared to the effect of same cooking conditions on α-glucosidase enzyme inhibitory activity of steamed OFSP sample. In steamed OFSP samples, α-glucosidase enzyme inhibitory activity was in the range of 23.21 to 47.83 %, while it was between 0.47 and 31.29 % in boiled sweet potato sample. Overall, higher α-glucosidase enzyme inhibitory activity was observed with medium sweet potato cubes (1.5 cm)

and shorter cooking time (5 min) for steaming experiment. However, α-glucosidase enzyme inhibitory activity did not reduce significantly with increasing steam cooking time. Therefore, the results of the present study indicated that steaming is potentially more effective cooking method to retain higher anti-hyperglycemic functionalities in OFSP. Results of the boiling experiment revealed that larger size (2 cm) of the sweet potato cubes and shorter cooking time (5 min) resulted into higher α-glucosidase enzyme inhibitory activity in boiled OFSP food samples. The magnitude for reduction of α-glucosidase and α-amylase enzyme inhibitory activities after boiling of OFSP food sample was similar. Therefore, boiling cooking process had more detrimental impact on anti-hyperglycemic relevant functionalities of OFSP. Previously, Oboh et al. (2013) found reduction in α-glucosidase and α-amylase enzyme inhibitory activities after blanching of *Amaranthus cruentus* leaf. Similarly, Ahn et al. (2014) observed reduction in α-glucosidase enzyme inhibitory activity after boiling of Korean vegetable (*Aruncus dioicus*). The results of present and previous studies clearly indicated the boiling cooking process had adverse effect on anti-hyperglycemic functionalities of vegetables such as sweet potatoes. Therefore, steaming or baking are more preferable cooking processes for OFSP to retain higher anti-hyperglycemic functionalities relevant for management of early stages of type 2 diabetes and Gestational diabetes.

Table 8.3. Response surface analysis result for different experimental runs for steamed OFSP samples

Experimental runs	Time (min)	Sweet potato cube size (cm)	TSP (mg GAE/g FW)	DPPH inhibitory action (mM Trolox/g FW)	ABTS inhibitory action (mM Trolox/g FW)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)	Protein (mg/g FW)	Color difference (ΔE)	Weight loss percent
1	4	1	0.56	0.31	0.46	49.88	37.72	0.21	23.43	1.23
2	4	2	0.54	0.19	0.56	28.62	36.86	0.24	8.31	1.10
3	16	1	0.45	0.28	0.47	20.79	28.29	0.13	20.37	-1.19
4	16	2	0.53	0.14	0.52	18.43	29.68	0.15	16.37	7.14
5	1.516	1.5	0.60	0.07	0.53	93.52	35.53	0.98	12.09	1.19
6	18.484	1.5	0.49	0.25	0.50	21.10	28.93	0.12	14.48	2.41
7	10	0.793	0.46	0.29	0.46	-8.34	23.21	0.16	22.16	-5.88
8	10	2.207	0.46	0.32	0.45	23.92	28.97	0.16	12.59	1.12
9	10	1.5	0.44	0.23	0.43	13.81	24.68	0.17	19.59	0.00
10	10	1.5	0.44	0.20	0.33	34.84	47.83	0.13	16.09	-4.65
11	10	1.5	0.44	0.24	0.30	15.56	36.46	0.16	16.77	0.67
12	10	1.5	0.44	0.22	0.32	18.34	29.43	0.15	18.76	0.76
13	10	1.5	0.44	0.22	0.38	25.34	32.34	0.16	17.43	0.98
14	10	1.5	0.45	0.22	0.38	19.43	31.43	0.15	18.66	-1.23
15	10	1.5	0.44	0.23	0.30	15.32	43.55	0.17	19.05	-1.54
16	10	1.5	0.46	0.22	0.38	10.54	41.35	0.17	17.28	0.99

TSP: Total soluble phenolics, DPPH: (2, 2-Dipheny-1—Picrylhydrazyl) inhibition), ABTS: [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)].

Table 8.4. Response surface analysis result for different experimental runs for boiled OFSP samples

Experimental runs	Time (min)	Sweet potato cube size (cm)	TSP (mg GAE/g FW)	DPPH inhibitory action (mM Trolox/g FW)	ABTS inhibitory action (mM Trolox/g FW)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)	Protein (mg/g FW)	Color difference (ΔE)	Weight gain percent
1	5	1	0.43	0.20	0.48	3.73	31.30	0.16	16.44	2.39
2	5	2	0.45	0.22	0.55	12.66	27.20	0.16	10.43	8.89
3	9	1	0.37	0.17	0.41	9.38	22.05	0.15	17.43	11.83
4	9	2	0.39	0.18	0.46	19.56	12.00	0.22	12.57	15.56
5	4.172	1.5	0.39	0.24	0.47	0.00	19.77	0.18	15.66	4.67
6	9.828	1.5	0.40	0.33	0.38	0.00	0.47	0.17	15.00	3.95
7	7	0.7928	0.32	0.13	0.29	0.00	5.80	0.12	14.65	4.88
8	7	2.207	0.39	0.27	0.36	20.91	16.21	0.19	11.67	4.49
9	7	1.5	0.40	0.22	0.44	0.00	14.52	0.17	10.54	3.88
10	7	1.5	0.42	0.28	0.41	7.67	10.35	0.22	16.43	2.00
11	7	1.5	0.42	0.32	0.40	6.29	14.58	0.22	17.99	4.35
12	7	1.5	0.40	0.26	0.46	0.97	10.79	0.18	18.66	3.92
13	7	1.5	0.40	0.23	0.41	0.00	9.98	0.19	14.32	3.82
14	7	1.5	0.40	0.26	0.41	5.32	13.24	0.19	18.98	4.10
15	7	1.5	0.40	0.23	0.41	6.34	9.82	0.20	19.00	3.86
16	7	1.5	0.40	0.23	0.42	0.00	10.59	0.19	15.32	4.22

TSP: Total soluble phenolics, DPPH: (2, 2-Dipheny-1—Picrylhydrazyl) inhibition), ABTS: [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)].

Table 8.5. Analysis of variance (ANOVA) for the regression models of TSP, antioxidant capacity, and anti-hyperglycemic properties of steamed orange-fleshed sweet potato cubes

Responses	Degree of freedom	Sum of squares	Mean sum of squares	F-value	p-value	R-square value (%)	Lack of fit
Protein content	5	0.01	0.01	10.17	0.0026	86.40	0.3099
TSP content	5	0.03	0.01	123.52	<.0001	98.72	0.6715
DPPH based antioxidant activity	5	0.04	0.01	7.46	0.0050	80.58	0.004
ABTS based antioxidant activity	5	0.08	0.02	9.48	0.0015	82.59	0.6505
Alpha amylase inhibitory action	5	5333.04	1066.60	7.72	0.0033	79.44	0.0304
Alpha glucosidase inhibitory action	5	338.87	67.78	1.67	0.2282	45.55	0.8279
Color difference	5	201.69	40.33	18.92	<.0001	90.44	0.1608
Weight loss percent	5	87.71	17.54	4.56	0.0199	69.53	0.4268

TSP: Total soluble phenolics, DPPH: (2, 2-Dipheny-1—Picrylhydrazyl) inhibition), ABTS: [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)].

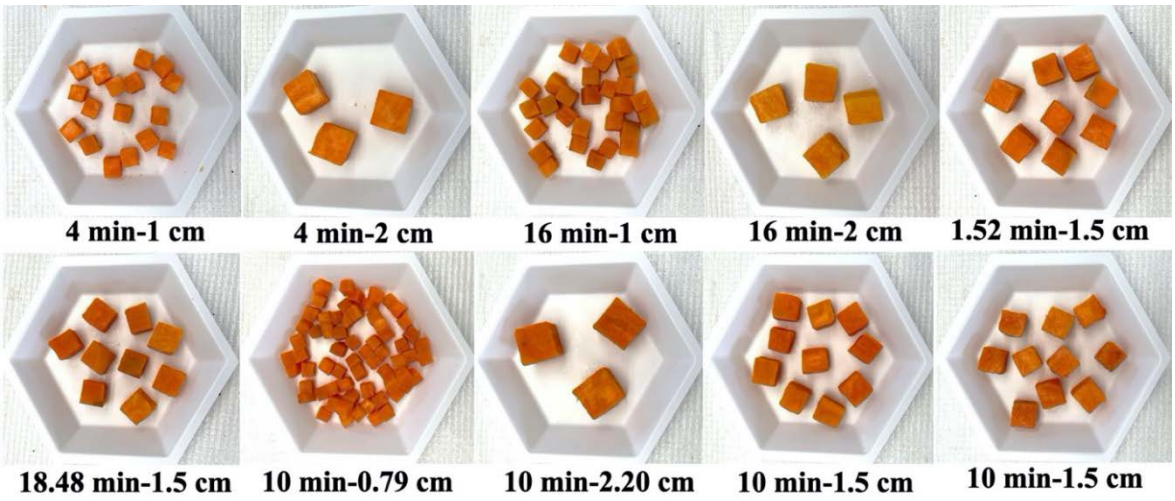

Figure 8.7. Effect of different processing conditions such as steaming time (4 min, 10 min 16 sec, 5 min), and size of sweet potato cubes (1 cm, 1.5 cm, 2 cm) on the appearance and visual quality of steamed orange-fleshed sweet potato sample

Table 8.6. Analysis of variance (ANOVA) for the regression models of TSP, antioxidant capacity, and anti-diabetic properties of boiled orange-fleshed sweet potato cubes

Responses	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	Pr < p	R-square value (%)	Lack of fit
Protein content	5	0.01	0.001	6.15	0.00	75.47	0.5365
TSP content	5	0.01	0.02	17.95	0.00	91.81	0.3500
DPPH based antioxidant activity	5	0.01	0.01	1.77	0.20	48.97	0.0732
ABTS based antioxidant activity	5	0.02	0.01	2.34	0.11	53.92	0.0009
Alpha amylase inhibitory action	5	523.73	104.74	5.59	0.01	73.65	0.0944
Alpha glucosidase inhibitory action	5	551.98	110.39	1.82	0.19	47.69	0.0314
Color difference	5	203.06	40.61	5.21	0.01	72.28	0.4235
Weight gain percent	5	78.32	15.66	20.40	0.00	92.73	0.0782

TSP: Total soluble phenolics, DPPH: (2, 2-Dipheny-1—Picrylhydrazyl) inhibition), ABTS: [2, 2-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)].

Figure 8.8. Effect of different processing conditions such as boiling time (4 min, 10 min 16 sec, 5 min), and size of sweet potato cubes (1 cm, 1.5 cm, 2 cm) on the appearance and visual quality of steamed orange-fleshed sweet potato sample

8.4.2.5. Color Difference

Color measurements determine the visual qualities of processed or cooked food product, which is an important attribute that significantly influences consumer preferences. Therefore, total color difference (ΔE) of both steamed and boiled OFSP samples was measured using Macbeth, color-eye 7000 reference spectrophotometer. Effect of selected cooking process variables on the quality of steamed and boiled OFSP samples are presented in Figure 8.7 and 8.8. In the present study, sample cube size had statistically significant ($p<0.05$) effect on color difference of cooked sweet potato sample. Under both cooking processes (steaming and boiling), higher color differences was found with smaller sweet potato cube size (1 cm cubes) (Figure 8.9a & 8.9b). The greater surface area and exposure of smaller sample size to direct thermal treatments could have resulted in higher color differences in smaller OFSP samples.

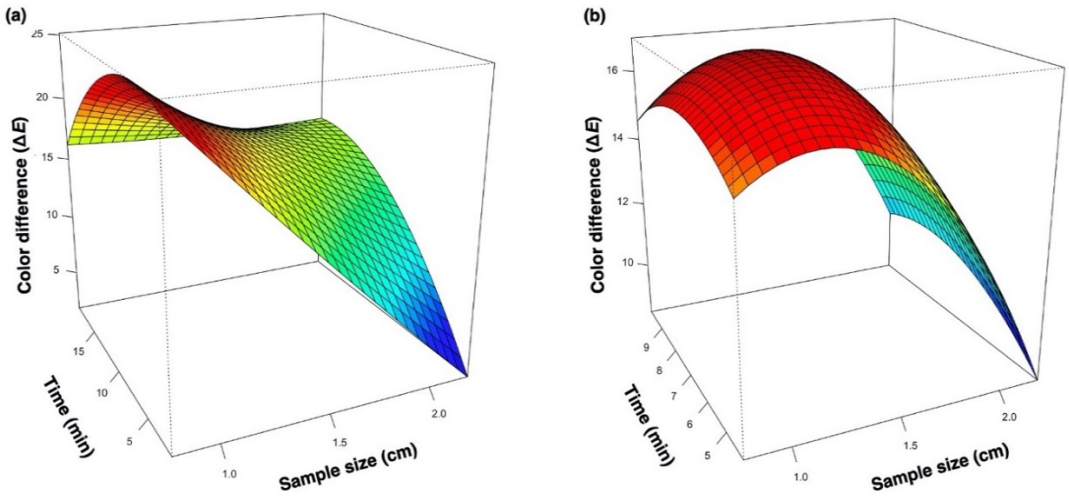

Figure 8.9. Response surface plots illustrating the effect of cooking process variables on color difference of steamed (a) and boiled (b) OFSP cubes

Overall, cooking time did not have significant effect on color differences of sweet potato cubes. Additionally, the color difference values were slightly higher for steaming experiment than the boiling experiment.

The developed multiple regression models and insignificant lack of fit (0.1608 and 0.4235) data indicated a well-fitted model for determining the effect of steaming and boiling cooking variables on color differences of cooked OFSP cubes. The color difference results of this study revealed that steamed and boiled OFSP sample retain the original color and may not affect consumers preference significantly.

8.4.2.6. Weight Loss and Weight Gain Percent of Steamed and Boiled OFSP Samples

During steaming weight loss (%) was observed in steamed OFSP samples, while boiling resulted into weight gain in cooked OFSP cubes (Table 8.3 & 8.4). In boiling experiment, the weight gain was due to the absorption of water in cooked OFSP cubes. In this experiment, statistically significant effect of steaming process conditions on weight loss percent of steamed OFSP food sample was observed. For instance, higher weight-loss percent was found in bigger size of sweet potato cube and with longer cooking time of 16 min (Figure 8.10a). The multiple regression analysis (Table 8.5) and the lack of fitness (p value = 0.4268) data indicated a well-fitted model for indicating weight loss percent in steamed sweet potato. The results suggested that longer cooking time and larger sweet potato cube size is ideal for improving shelf life (less water content) and stored preservation of steamed OFSP sample.

However, weight gain percent in boiled OFSP samples indicated absorption of water during cooking process. Higher weight gain percent was found in larger cube size (2 cm cubes) and with longer cooking time (9 min) (Figure 8.10b). Therefore, longer cooking and sweet potato cubes with bigger size absorbed more water during boiling. This higher absorption of water in boiling experiment might have resulted in lower bioactive content (protein and phenolic) and reduction of associated antioxidant and anti-hyperglycemic functionalities. Therefore, it is important to further optimize the boiling process conditions based on different human health

relevant nutritional attributes, prior to targeting boiled sweet potato in dietary interventions relevant for management of diet and lifestyle-linked chronic diseases, such as type 2 diabetes and Gestational diabetes.

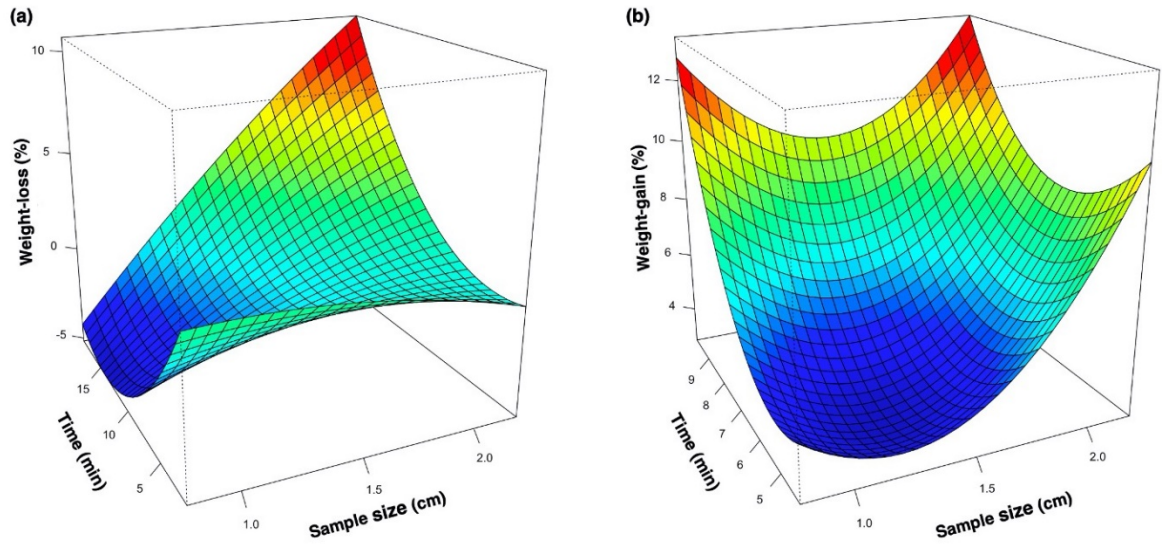

Figure 8.10. Response surface plots of illustrating the effect of different cooking process variables on weight loss percent of steamed (a) and weight-gain percent of boiled (b) OFSP cube samples

8.5. Conclusions

Sweet potatoes are commonly boiled or steamed for regular household intake worldwide. However, both processing conditions alter human health related nutritional and bioactive qualities of cooked sweet potato-based foods. Therefore, health-focused process optimization of steaming and boiling processes of sweet potato is important for their potential high value food applications, especially for integration in dietary solutions against chronic diseases such as type 2 diabetes and Gestational diabetes. Such cooking process optimization based on nutritional and bioactive qualities also has relevance for application of sweet potato-based foods to promote maternal and child health nutrition. Therefore, the study optimized cooking times and size of OFSP cubes for steaming and boiling cooking processes, based on protein content, phenolic content, antioxidant activity, and anti-hyperglycemic properties of cooked sweet potato food

samples. Overall, higher protein content, TSP content, antioxidant activity, and antihyperglycemic functionality relevant α-amylase and α-glucosidase enzyme inhibitory activities were observed in steamed OFSP when compared to the boiled sample. Additionally, higher bioactive content and associated functionalities was observed in steamed OFSP with shorter cooking time (5-7 min) and smaller size of the sweet potato cubes (1.5 cm). Significant reduction of phenolic-linked antioxidant and anti-hyperglycemic properties was observed after boiling of OFSP cubes. Such reduction of human health relevant response variables in boiled OFSP samples might be due to the destruction of intracellular structure from direct contact of thermal treatment and subsequent leaching off nutrients into the water during boiling process. Higher retention of protein, TSP, antioxidant activity, and anti-hyperglycemic property in steamed OFSP sample with optimum cooking process conditions has significant relevance for its potential application in dietary support strategies to manage type 2 diabetes and Gestational diabetes. Therefore, this present study provided critical biochemical insights about optimum cooking (steaming and boiling) conditions for developing convenient, easy to digest, inexpensive, and nutritious foods from orange-fleshed sweet potatoes relevant for health-focused food solutions targeting NCD-benefits. However, further optimization studies with different flesh color and different sweet potato cultivars and with other cooking process variables are required to confirm the findings of this study and to advance cooked sweet potato-based foods for wider value-added health applications and global nutritional insecurity solutions.

CHAPTER 9. ENHANCING RAPID WOUND HEALING RESPONSE IN BRUISED AND CUT POTATO TUBER USING NATURAL BIOPROCESSED ELICITORS

9.1. Abstract

Bruising and wounding of harvested potato tubers is a serious post-harvest challenge, which causes significant economic loss to the potato industry. Therefore, improving wound healing (WH) responses of cut and bruised tubers is essential to counter bruising damage and to control subsequent infections, maintaining higher quality tubers, and for ensuring better seedling sprouting, growth, and overall productivity. In this context, it is important to advance safe and inexpensive strategies, which are metabolically-driven to improve WH responses in cut and bruised tubers after bruising damage. Therefore, the main objective of this study was to investigate the efficacy of natural bioprocessed elicitors, such as water-soluble chitosan oligosaccharide (COS as 0.125 g L^{-1}) and bioprocessed cranberry pomace (Nutri-Cran as 0.125 g L^{-1}) for improving WH responses in cut potato tubers. Additionally, the efficacy of elicitor treatments was determined by comparing with fluoridone (2.5 g L^{-1}) and MES (2-(N-morpholino) ethanesulfonic acid buffer as control) treatments. Certified seed potato tubers of two cultivars (Russet Burbank and Russet Norkotah) were obtained and a disc model system was used to study the WH response of cut tubers. Furthermore, the role of protective redox-linked and anabolic pentose phosphate pathway (PPP) for improving WH responses and suberization processes in cut potato tubers after 4 days of elicitor treatments were also investigated. In this study, enhanced WH response in cut potato tubers with improved accumulation of suberin biopolymers (suberized cells) was observed in COS (0.125 g L^{-1}) elicitor treated cut discs through microscopic examination. Additionally, improved PPP-linked redox regulation (high glucose phosphate dehydrogenase activity) and higher phenolic content was also observed in

COS treated tubers. Therefore, the results of this study indicated that natural bioprocessed elicitors such as water-soluble COS can be rationally targeted to improve WH responses of cut and bruised potato tubers and to mitigate associated post-harvest losses.

Keywords: Antioxidant enzyme, bio-elicitors, pentose phosphate pathway, phenolics, suberization, wound-healing

9.2. Introduction

Potato (*Solanum tuberosum* L.) is the leading non-cereal food crop in the world and consumed widely either as cooked or processed foods. In the United States, potato ranks first among the vegetable crops with the total market value of $3.77 billion (Agricultural Market Resource Center, 2018). For commercial production, potato is propagated vegetatively from certified seed tubers. Maintaining higher quality tubers is important for both its' use as seed tuber and for regular marketing as food source. Potato tubers can be damaged during harvesting, post-harvest handling, packing, and transporting, which leads to exposure of wounded bare tissues to different kind of stresses (Singh et al., 2020). These unintended wounding not only causes significant damages to bruised tubers, but also triggers a series of decaying processes involving significant metabolic and biochemical changes such as loss of texture (skin), water loss, and increasing susceptibility to microbial spoilage (Grudzinska and Barbas, 2017, Hollingshead et al., 2020). Therefore, one of the most serious challenges facing the potato industry is bruising and wounding of cut seed and harvested tubers, which causes significant economic loss of around>$320 M/yr in the US. Based on this need, it is essential to advance sustainable solution strategies for improving wound-healing (WH) responses of cut and bruised potato tubers to address this serious post-harvest tuber quality issue and to mitigate associated economic losses.

Additionally, rapid WH regulation in cut and bruised tubers is also important and relevant for maintaining better nutritional quality of potatoes.

In general, potato tissues respond by initiating a series of temporal and more permanent endogenous responses following wounding of cut and bruised potato tubers. Such WH response and suberization processes involve closing layer formation, wound periderm formation, synthesis of suberin polyphenolics (SPP), and suberin polyaliphatics (SPA) (Lulai, 2007; Lulai and Neubauer, 2014). Biosynthesis and accumulation of suberin biopolymers involve two distinct stages with specific biological functions such as i) closing layer formation and ii) wound periderm formation (Lulai et al., 2016a, b). In the first stage of closing layer formation, the existing parenchyma cells at the wound surface become suberized, forming a functional suberin layer, which provide barrier against fungal and bacterial pathogens and restrict water loss (Lulai et al., 2016). Previously, increased concentration of auxin (indole acetic acid-IAA) and precursors of cytokinin were observed during WH process of cut potato tissues (Lulai et al., 2016). Additionally, WH responses in part are closely linked to phenylpropanoid pathway leading the biosynthesis of suberin polyphenolics (Jiang et al., 2019; Wang et al., 2020). Both, upregulation of phenylpropanoid pathway and enhanced biosynthesis of IAA and cytokinin response are directly related to redox-linked pentose phosphate pathway (PPP) and associated anabolic responses (Sarkar and Shetty, 2014; Shetty and Wahlqvist, 2004). Furthermore, right balance of redox protective phenolics from a reduced oxidative state dependent on PPP regulation and subsequent improvement of antioxidant enzyme responses can potentially help to counter catabolic response induced oxidative breakdowns (Shetty, 1997; Shetty and Wahlqvist, 2004) this may be relevant in wounded tissues. These protective redox dependent metabolic reactions modulated by dehydrogenases could also enhance WH responses through critical

modulation of defense related pathways, such as PPP, and would likely maintain balance between catabolic and anabolic pathway responses as suggested previously for general plant phenolics-linked redox responses (Shetty and Wahlqvist, 2004).

Therefore, based on the above rationale for stimulation of biosynthesis of redox-protective polyphenols through upregulation of PPP and downstream phenylpropanoid pathway and subsequent polymerization of polyphenols is potentially critical for rapid WH responses including, suberization processes and closing layer formation in cut and bruised tubers. The biosynthesis of these secondary metabolites, such as redox-protective polyphenols, also have relevance in defining nutritional qualities and associated human health benefits in potato tubers (Saleem et al. 2011; Sarkar and Shetty, 2014). Therefore, improvement of biosynthesis of protective polyphenols in cut and bruised tubers, through upregulation of critical redox-linked PPP and associated anabolic responses, has dual benefits of improving rapid WH response and enhancing human health relevant nutritional qualities**.**

In this context, metabolically-driven innovation are required to enhance WH responses of cut and bruised potato tubers, while also improving post-harvest preservation and nutritional qualities. However, a sustainable strategy to improve WH responses must also be safe, inexpensive, and easily in application, so that growers and other stakeholders can implement efficiently to protect cut and bruised potato tuber from infection and overall quality deterioration. Overall, such innovation in improving WH response of cut sand bruised tuber will also help to counter low retail price and production saturation and will ensure better economic security to potato growers of the US and worldwide.

The primary objective of this study was to advance metabolically-driven elicitation strategies to stimulate redox-linked PPP regulation and associated anabolic responses for

improving rapid WH responses of cut and bruised potato tubers. Two bioprocessedand edible elicitors, water soluble chitosan oligosaccharide (COS) and bioprocessed cranberry pomace (Nutri-Cran), were targeted in this study to enhance rapid wound-healing responses in cut potato tissues. In general, chitosan oligosaccharides are potent antioxidants and its application in plant tissues induces series of plants defense reactions including increased in production of glucanhydrolases, biosynthesis of phenolic compounds and synthesis of specific phytoalexins with antifungal activity (Muzzarelli et al., 2012). Additionally, minimization of respiration rate and reduction of water loss through semipermeable coating of chitosan was observed in fresh fruits and vegetables (Bautista-Banos et al., 2006). Furthermore, it was reported that chitosan post-harvest coatings as well as a pre-harvest chitosan spray in fruits reduced ethylene production, delayed ripening, allowed retention of firmness and controlled decay (Meng et al., 2008; No et al., 2007; Reddy et al., 1999)**.** Like COS, bioprocessed cranberry pomace (Nutri-Cran) is also a rich source of phenolic compounds with diverse biological functions including high antioxidant activity and antimicrobial property (Vattem et al., 2004; 2005; Zheng and Shetty, 2000). Therefore, based on these promising oxidative-stress protective properties, water soluble COS and Nutri-Cran were targeted to improve rapid WH responses of cut and bruised potato tubers through stimulation of redox-linked PPP regulation and associated anabolic responses including suberization process. Overall, the broad objective of this study was to provide a safe and effective tool to growers and potato industry for improving WH responses of cut and bruised tubers and to counter significant wound related post-harvest losses.

9.3. Materials and Methods

9.3.1. Potato Cultivars

Certified mini-tuber seeds of two potato cultivars, Russet Burbank and Russet Norkotah, were used to study the WH response of cut potato tubers. Tubers of cv. Russet Burbank were provided by Valley Tissue Culture (Halstad, MN, USA), while Russet Norkotah tubers were collected from the Seed Quality Laboratory of North Dakota State Seed Department (Fargo, ND, USA).

9.3.2. Tuber Storage and Preparation

Mini-tubers (10-15 g for Russet Burbank and 5-8 g for Russet Norkotah) of both potato cultivars were stored in the dark at 4°C (~95% relative humidity) before conducting the experiment. The cold storage of tubers helped to retard deterioration and sprouting. For each experimental run, tubers were removed from the cold storage, cleaned gently with water, and extra water on the surface was soaked with paper towel. Then cleaned tubers were equilibrated in the dark at 20°C (~95% relative humidity) for three days.

9.3.3. Wounding Treatment

Potato tubers were wounded using tuber disc model system as described by Lulai et al. (2016). In this wounding model, cylinders of tissue were laterally excised from each tuber with a vertical cork borer and discs (11 mm diameter and 3 mm thick) of parenchyma tissue were cut from the cylinder. The discs were treated immediately with natural elicitors (COS, and Nutri-Cran), fluriodone (FLD), and MES (2-(N-morpholino) ethanesulfonic acid buffer).

9.3.4. Bioprocessed Elicitors and Chemical Treatment

Two bioprocessed natural elicitors were used in the current study –soluble chitosan oligosaccharide from crustacean shells, containing ascorbic acid sidechains (COS; Kong Poong

Bio, Jeju, South Korea) and bioprocessed cranberry pomace (Nutri-Cran; Decas Botanical Synergies, Wareham, MA, USA). The elicitors were dissolved in 10 mM MES (2-(N-morpholino) ethanesulfonic acid buffer, pH 5.7) @ 0.125 g/L rate. Additionally, a xenobiotic compound fluridone (FLD), which inhibits formation of carotenoid, precursor of ABA biosynthesis was also used. For fluridone treatment, 0.032 g of FLD was initially dissolved in 10 mL dimethyl sulfoxide (DMSO) and further diluted (0.1 mM) with MES buffer. For control, cut potato discs were only treated with MES buffer.

9.3.5. Bioprocessed Elicitor Treatments

Freshly cut discs (24 discs for each treatment) were placed in respective treatment solutions (250 mL) and incubated in a rotary shaker (~66 cycles min^{-1}) for 60 min. During incubation in shaker, treatment solutions were changed in every 20 min (total 3 times). Then all treatment solutions were drained and cut discs (5 for each biological replicate and total 4 biological replicates) were immediately taken into 15 mL plastic test tubes and stored in -80°C for 0 day MES (control) treatment. Cut discs of the remaining treatments were allowed to wound-heal in the dark at 20°C (~95% relative humidity) for four days.

9.3.6. Suberization Rating and Microscopy

After 4 days incubation, 4 discs from each treatment were placed in 20 mL Farmer's fixative (absolute ethanol/ acetic acid in 3:1 v/v ratio) for determination of suberization ratings. Accumulation of suberin polyphenolics (SPP) was investigated microscopically in triplicate from 20 µm thick section of cut potato discs. The microscope used for SPP rating and digital imaging was Zeiss Axioscope 50 microscope configured for epifluoroscent illumination (Lulai and Corsini, 1998). Digital images of closing layer cell wall of the wounded tissues were obtained with a Zeiss color AxioCam camera (Carl Zeiss Inc. Thirnwood, NY, USA). Rating of

suberization was carried out using a method described by Lulai and Corsini (1998), where 0= none, 5= complete around the 1 st cell layer, and 7= complete around 1st and 2nd cell layers.

9.3.7. Tissue Grinding for Biochemical Analysis

After 4 days of incubation cut potato discs were immediately frozen in liquid nitrogen and were stored at -80°C before grinding and extraction. Samples from each treatment and each biological replicate were ground (30 sec) into a fine powder under liquid nitrogen temperature using TissueLyser. Fine grounded powder was then transferred to 2 mL Eppendorf centrifuge tubes and immediately kept in liquid nitrogen and stored in -80°C.

9.3.8. Sample Extraction for the Determination of Total Soluble Phenolic Content and Antioxidant Activity

Frozen potato tissues (100 mg FW) were transferred to glass vials containing 95% ethanol (5 mL) and stored at -10°C for 48 h. The samples were homogenized using a tissue tearor (BioSpec Products Inc., Bartlesville, OK) and the homogenate was centrifuged (13,000 rpm; 5 min). The supernatant was used to determine the total soluble phenolic content and antioxidant activity of the potato tissues *in vitro*.

9.3.9. Total Soluble Phenolic Content

The total soluble phenolic content in the ethanol extracts of potato tissue was measured using the Folin-Ciocalteu (FC) method as described by Shetty *et al*. (1995). The extract (1 mL) was combined with 95% ethanol (1 mL) and distilled water (5 mL) in a test tube. To this mixture, FC reagent (0.5 mL; 50% v/ v) and Na_2CO_3 (1 mL; 5% v/v) were added, mixed thoroughly with a vortex shaker and incubated in the dark for 60 min. Absorbance of the resultant solutions were measured spectrophotometrically at 725nm (Genesys UV-visible, Milton Roy Inc., Rochester, NY). A standard curve was prepared using various concentrations of gallic

acid (10 - 300µg/mL) in 95% ethanol. Absorbance values were converted to total soluble phenolic concentration and expressed as milligram equivalents of gallic acid equivalent per gram FW of potato tissue sample.

9.3.10. Total Antioxidant Activity (ABTS Free Radical Scavenging Capacity Assay)

Antioxidant activity was measured using the ABTS [2,2′ –azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay (Pellegrini *et al*., 2002). A stock solution containing ABTS radical cations was prepared by mixing 7 mM ABTS solution (5 mL) with 140 mM K_2S_2O4 solution (88 mL). This mixture was stored in a foil wrapped container, in the dark at 4°C for 12–16 h before use. Prior to performing the assay, the stock solution was diluted with 95% ethanol, to prepare a working ABTS solution with an absorbance of 0.70 ± 0.02 units at 734 nm. The ABTS working solution (1 mL) was added to the ethanol extract of potato tissue (50 µL) in a plastic vial and mixed thoroughly. The mixture was incubated at room temperature for 2.5 minutes and absorbance was measured at 734 nm. The antioxidant activity of the extracts was expressed as percentage (%) inhibition of ABTS radical formation and was calculated per the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \text{ x } 100 \qquad (9.1)$$

9.3.11. Sample Extraction for the Determination of Enzyme Activities *In Vitro*

At each time point, 250 mg (fresh weight; FW) of potato tissue was excised, flash frozen in liquid nitrogen, and stored at -80˚C for biochemical analysis. Prior to conducting the biochemical assays, potato tissue samples were macerated using a chilled pestle and mortar in an ice bath, with 5 mL of pre-chilled enzyme extraction buffer (0.5% polyvinylpyrrolidone (PVP), 3 mM EDTA, and 0.1 M potassium phosphate buffer; pH 7.5). The tissue homogenates were

centrifuged at 13,500 rpm for 10 min and immediately transferred to an ice bath. The supernatant was used for further biochemical analysis.

9.3.12. Total Protein Content

The Bradford protein binding assay was used to measure the amount of total protein present in the sample extracts (Bradford, 1976). To 50 µL of extract, 5 mL of dye reagent (diluted with water in the ratio 1:4) was added. The mixture was thoroughly mixed on a vortex shaker, incubated in a dark cabinet for 5 min and its absorbance was measured at 595 nm, against a blank (50 µL distilled water in 5 mL dye reagent).

A standard curve was prepared using standard solutions of bovine serum albumin, dissolved in distilled water, based on which the protein content of the sample extracts was calculated. These values were used in subsequent calculations to determine the activities of various enzymes involved in ROS mitigation mechanisms and the PAPPP regulation.

9.3.13. Glucose-6-Phosphate Dehydrogenase (G6PDH) Assay

A modified method originally described by Deutsch (1983) was used to determine G6PDH activity. The enzyme reaction mixture contained 5.88 µmol ß-NADP, 88.5 µmol MgCl2, 53.7 µmol glucose-6-phosphate, and 0.77 mmol maleimide was prepared. This solution was used to set the baseline (zero) of the spectrophotometer at 340 nm. Potato tissue extract (100 µL) was added to the reaction mixture (1 mL) and the shift in absorbance over 5 min was used to quantify G6PDH activity in the samples based on the extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}).

9.3.14. Succinate Dehydrogenase (SDH) Assay

To assay the activity of succinate dehydrogenase a modified method described by Bregman (1987) was used. A reaction mixture containing 0.4 M potassium phosphate buffer (1.0

mL; pH 7.2), 0.15 M sodium succinate (40 µL; pH 7.0), 0.2 M sodium azide (40 µL), and 2,6-dichlorophenolindophenol (DCPIP; 10 µL; 6.0 mg/mL) was prepared. The baseline of the spectrophotometer at 600 nm was established using the enzyme extraction buffer solution. Potato tissue extract (0.2 mL) was added to the reaction mixture (1 mL) and the rate of change of absorbance per min was measured to quantify SDH activity in the sample, based on the extinction coefficient of DCPIP (19.1 mM^{-1} cm^{-1}).

9.3.15. Proline Dehydrogenase (PDH) Assay

A modified method described by Costilow and Cooper (1978) was used to assay the activity of proline dehydrogenase. The enzyme reaction mixture contained 100 mM sodium carbonate buffer (pH 10.3), 20 mM L-proline solution and 10 mM NAD was used. Potato tissue extract (200 µL) was added to the reaction mixture (1 mL) and the increase in absorbance was measured at 340 nm over an interval of 3 min at 32 °C was measured. One unit of PDH activity was defined as amount required to cause a shift of 0.01 absorbance units per min at 340 nm (1.0 cm light path).

9.3.16. Determination of Proline Content

High performance liquid chromatography (HPLC) analysis was performed using an Agilent 1200 liquid chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a diode array detector (DAD 1100). The analytical column was a reverse phase Eclipse C18, 250 nm 4.6 mm with a packing material of 5 µm particle size. The samples were eluted out in an isocratic manner with a mobile phase consisting of 20 mM potassium phosphate (pH 2.5 by phosphoric acid) at a flow rate of 1 mL/min and detected at 210 nm. A known quantity of L-Proline dissolved in 20 mM potassium phosphate solution was used to calibrate the standard

curve. The amount of proline in the potato tissue sample was reported as milligram of proline per gram FW.

9.3.17. Guaiacol Peroxidase (GPX) Assay

A modified method described by Laloue *et al*. (1997) was used to assay the activity of guaiacol peroxidase. The enzyme reaction mixture containing 0.1 M potassium phosphate buffer (pH 6.8), 56 mM guaiacol solution, and 50 mM hydrogen peroxide was used. Potato tissue extract (10 µL) was combined with the reaction mixture (990 µL) and the shift in absorbance over 3 min was measured. The rate of change in absorbance per min was used to quantify the enzyme in the mixture, based on the extinction coefficient of the oxidized product tetraguaiacol (26.6 mM^{-1} cm^{-1}).

9.3.18. Superoxide Dismutase (SOD) Assay

The activity of SOD was measured by monitoring the reduction of nitroblue tetrazolium (NBT) by potato tissue extracts at 560 nm (Oberley and Spitz, 1984). The reaction mixture consisted of 13.8 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1.33 mM diethylenetetraaminepentaacetic acid (DETEPAC); 2.45 mM NBT (0.5 mL); 1.8 mm xanthine (1.7 mL) and catalase (40 IU/mL). Phosphate buffer (100 µL) and xanthine oxidase (100 µL) were added to 0.8 mL of reagent mixture. The change in absorbance at 560 nm was measured over 1 min and the concentration of xanthine oxidase was adjusted to obtain a linear curve with a slope of 0.024 - 0.026 units of absorbance per min. Further to this, phosphate buffer was replaced with potato tissue extract and the shift in absorbance over 1 min was calculated. One unit of SOD activity was defined as the amount of protein that inhibited NBT reduction to 50% of the maximum.

9.3.19. Catalase (CAT) Assay

A method originally described by Beers and Sizer (1952) was used to assay the activity of catalase. A reaction mixture was prepared by combining distilled water and 0.059 M hydrogen peroxide (Merck's Superoxol or equivalent grade, Merck Co. & Inc., Whitehouse Station, NJ) in 0.05 M potassium phosphate buffer solution (pH 7.0). This mixture was incubated in a spectrophotometer for 4–5 min to achieve temperature equilibration and to establish a baseline. Further to this, potato tissue extract (0.1 mL) was added to the reaction mixture and the disappearance of peroxide due to catalase activity was monitored by measuring the shift in absorbance at 240 nm in 1 min. The change in absorbance from the initial linear portion of the curve was calculated. One unit of catalase activity was defined as amount that decomposes one micromole of H_2O_2.

9.3.20. Experimental Design and Statistical Analysis

The experiment was carried out with 4 replicates and entire experiments were repeated at least 2 times for each potato cultivar (n=8). Analysis of variance (ANOVA) was performed using SAS software (version 9.4; SAS Institute, Cary, NC). Statistical differences between elicitor treatments and among two potato cultivars were determined using Tukey's least mean square test at a confidence level of 95%.

9.4. Results and Discussions

9.4.1. Microscopic Examination of Suberin Layer Formation and Ratings

Overall, responding to wounding and bruising, plant tissues initiate a series of protective biochemical and metabolic counter measures, which are critical for the essential cellular structural adjustments during healing process (Savatin et al., 2014). The endogenous wound healing (WH) responses provide a natural barrier and protection to damaged tissues against

biotic and abiotic stresses and prevent from further deterioration and spoilage (Minibayeva et al., 2015). Like any other plant tissues, potato tubers are also susceptible to wounding-related damage, especially during harvest, post-harvest handling, transportation, and storage (Buskila et al., 2011). Potato tuber WH responses are closely associated with the suberization of two different cell type, closing layer formation with suberization of wound-exposed parenchyma cells and wound periderm formation through cell division of the meristematically active phellogen layer and formation of multiple layers of organized phellem cells (Lulai et al., 2016a). Therefore, strategies to enhance rapid WH responses of bruised and cut potato tubers will improve these suberization processes leading to the formation of protective barrier in the wound-exposed site. The synthesis and integration of suberin polyphenolics (SPP) and suberin polyaliphatics (SPA) is important for this suberization process of wounded potato tissues (Lulai et al., 2014).

Based on this rationale, we have targeted metabolically-relevant natural bioprocessed elicitors, such as water-soluble COS and bioprocessed cranberry pomace-NutriCran, to enhance suberization processes by improving synthesis and integration of suberin polyphenolics (SPP) in the wounded layer of cut potato tubers. The biochemical rationale for targeting these elicitors is based on their potential to stimulate redox-linked endogenous defense pathways, such as PPP and phenylpropanoid pathway (Shetty and Wahlqvist, 2004), which are directly related to biosynthesis of secondary metabolites such as suberin polyphenolics (SPP). Such natural elicitor –induced upregulation of the anabolic PPP potentially provides redox protection and supports necessary cell and tissue structural adjustments through up-regulation of antioxidant enzyme-linked protective responses and polymerization of soluble phenolics in wounded tissues and cells (Shetty and Wahlqvist, 2004; Sarkar and Shetty, 2014). Additionally, the PPP-linked anabolic responses and metabolic flux are associated with regulation and biological functions of plant

hormones, such as auxin and cytokinin, which are critical for regulation of wound periderm development and suberization processes (Lulai et al., 2016).

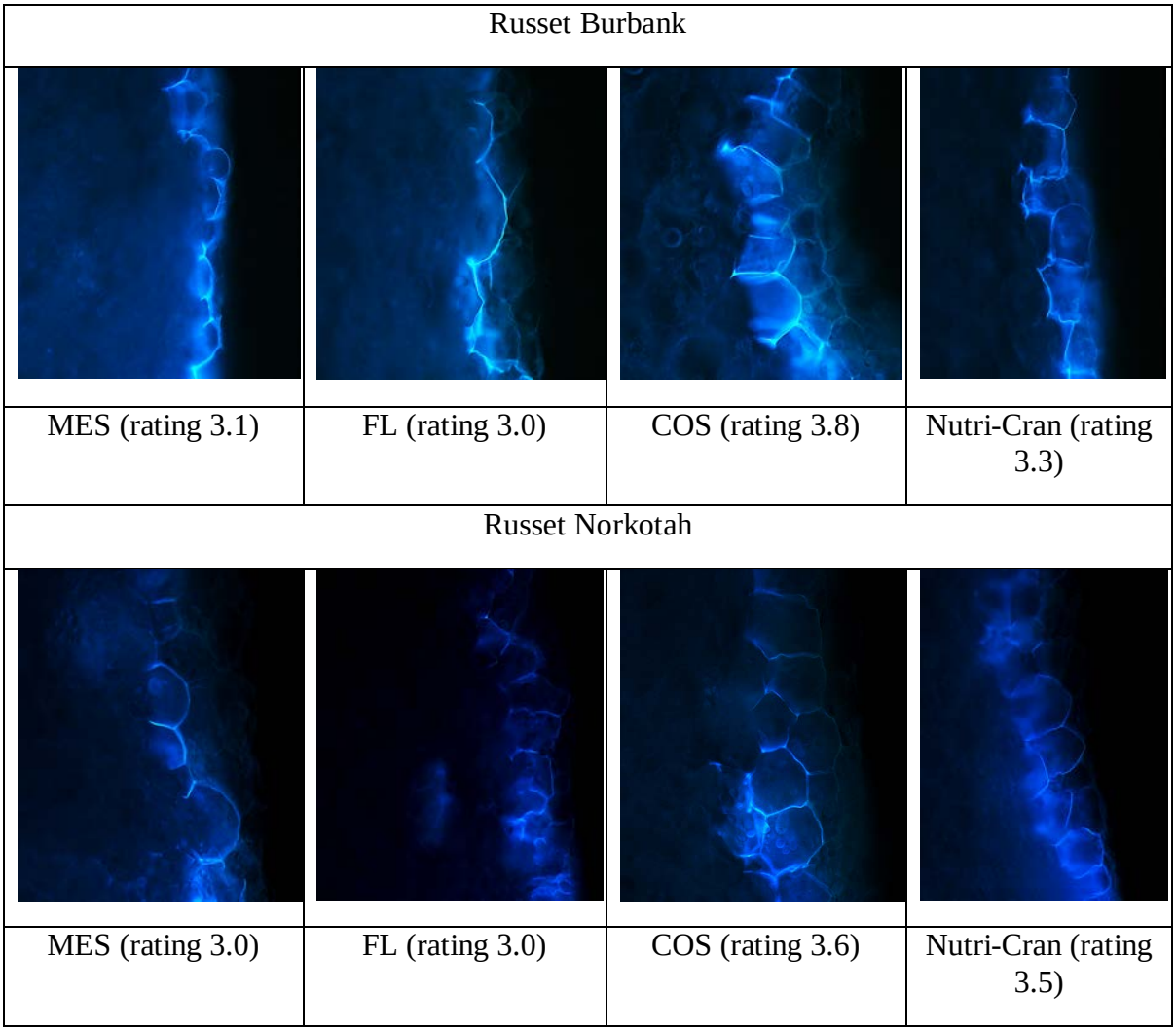

Figure 9.1. Formation of suberin polyphenolic (SPP) and suberization rating in cut potato discs of two cultivars (Russet Burbank and Russet Norkotah) with bioprocessed elicitors (COS and Nutri-Cran), fluridone (FL), and MES (control) treatments after 4 days of wound-healing

In this present study, formation and accumulation of SPP in the wound-exposed layer of cut potato tubers after 4 days of wounding was investigated using fluorescence microscopy (Lulai et al., 2006). Suberization rating based on the formation of SPP in outer cell layer (0=none; 5= complete around the 1st cell layer; and 7= around the 1st and 2nd cell layers) of cut discs of two potato cultivars (Russet Burbank and Russet Norkotah) was determined using the

standardized protocol developed by Lulai and Corsini (1998). In this study, the suberization rating obtained after 4 days of wounding varied between 3.0 to 3.8 based on the formation of SPP in the outer layer of the parenchyma cells (Figure 9.1). Previously, Lulai et al. (2016a) reported SPP rating of 3 to 4.4 in cut discs of Russet Burbank after 3 and 5 days of wounding. Overall, the highest suberization rating (3.8) with formation of SPP in around 60% completed 1st cell layer was observed in COS treated discs of Russet Burbank. For both potato cultivars, Russet Burbank and Russet Norkotah, COS elicitor treatment (0.125 g /L) resulted into highest SPP ratings (3.8 and 3.6 respectively), which is significantly higher when compared to other treatments (MES-control and fluridone) after 4 days of wounding. Additionally, positive impact of Nutri-Cran elicitor treatment on SPP formation (rating 3.6) was observed in cut disc of Russet Norkotah. Therefore, the SPP rating results and microscopic analysis inthis study indicated that bioprocessed elicitors such as water-soluble COS and Nutri-Cran can be rationally targeted to enhance SPP formation and subsequently improving suberization processes in cut and bruised potato tubers. However, the positive impact of these natural elicitors on wounded potato tissues is not just restricted to formation of SPP, but also through supporting critical metabolic and biochemical adjustments during WH processes. Previously, reduction of scab infection (*Streptomyces scabies*) in potato tubers was observed with chitin soil amendment treatment (Vruggink, 1970). Additionally, improvement of WH responses of plant cells through binding of various materials was also found with COS coating treatment (Hirano et al., 1999). Therefore, the application of COS in wounded tubers can have diverse benefits including improving suberization processes, up-regulation of endogenous defense responses, preventing water loss, and protection against bacterial and fungal pathogenic infections. All of these protective

mechanisms are critical to improve WH response and to maintain higher quality potato tubers during post-harvest stages.

9.4.2. Total Soluble Phenolic Content and Total Antioxidant Activity

Enhanced biosynthesis of secondary metabolites (phenolics) is a natural endogenous defense response of plants to counter stress-induced physiological and metabolic breakdowns, including wound-induced damages of the plant tissues (Jacobo-Velazquez et al., 2015; Sharma et al. 2019; Shetty and Wahlqvist 2004). The stress protective role of plant phenolics is not restricted to their antioxidant activity and maintaining cellular redox homeostasis, but also involves structural and physiological adjustments of plant tissues that are essential for WH responses (Becerra-Moreno et al., 2015). In the current study, statistically significant ($p \leq 0.05$) effects of natural elicitor treatment (COS-0.125 g/L) on improving total soluble phenolic content of cut potato tubers were observed 4 days after wounding (Figure 9.2 A).

Previously, synthesis of phenolic compounds such as chlorogenic acid isomers was observed in wounded potato tubers (Torres-Contreras et al., 2014). Similarly, increased phenylalanine ammonia-lyase (PAL) activity, a key enzyme in the phenolics biosynthesis of pathway was observed in wounded tissues of potato tubers (Lulai et al., 2008). In another study, enhanced soluble phenolic content was found in potato tubers after wound-induced damages (Wegener et al., 2014). Therefore, accumulation of phenolics, especially suberin polyphenolics, is critical for WH responses and for suberization processes in cut and bruised tubers. However, the higher accumulation of soluble phenolics can also lead to enzymatic browning due to cell wall breakage and release of polyphenol oxidase (PPO) enzyme (Liu et al., 2018). In our preliminary elicitor dose optimization study, enhanced browning of potato tissues was observed with higher concentration of COS elicitor treatment.

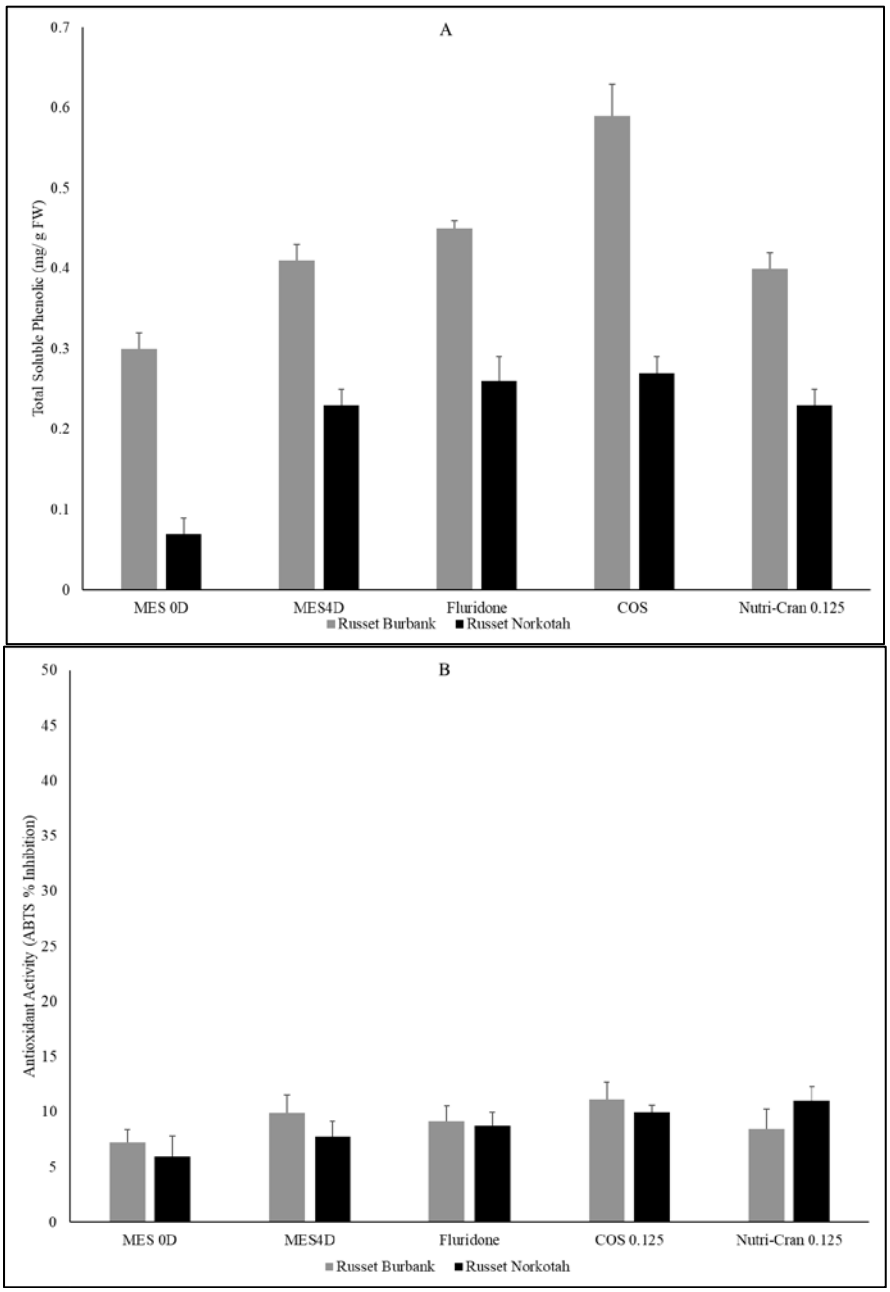

Figure 9.2. Total soluble phenolic content (mg GAE/g FW) (A) and total antioxidant activity (ABTS-based % Inhibition) (B) of cut potato tissues of two cultivars (Russet Burbank and Russet Norkotah) with bioprocessed elicitors (COS and Nutri-Cran), fluridone (FL), and MES (control) treatments after 4 days of wound-healing

Therefore, accumulation of certain phenolic compounds with redox-protective functions and enhanced polymerization of phenolics for cellular structural adjustments is more relevant for improving suberization and WH responses in cut potato tubers. In the present study, higher phenolic content correlated positively with higher SPP rating in COS elicitor treated cut potato

tissues. Additionally, higher ($p \leq 0.05$) phenolic content was observed in cut tissues of cv. Russet Burbank when compared to cv. Russet Norkotah. The results of this study indicated that improving biosynthesis of phenolics with redox protective function is an effective strategy to improve suberization and WH responses in cut and bruised potato tubers. Therefore, a bioprocessed elicitor, such as water-soluble COS in optimum concentration can be rationally targeted to stimulate the biosynthesis of protective phenolic compounds and subsequently to improve rapid WH responses of potato tubers. The enhanced phenolic content of potato tubers with natural elicitor treatment has dual benefits as it is also relevant for improving post-harvest preservation and nutritional qualities (Sarkar and Shetty, 2014).

A most common biological function of plant phenolics is their high antioxidant property, critical for cellular redox regulation. The antioxidant activity of plant phenolics is also relevant for countering wounding stress-induced oxidative breakdowns of plant tissues. Stimulation of phenolic-linked antioxidant activity was previously observed in purple-fleshed potatoes following wounding stress (Reyes and Cisneros-Zevallos, 2003). However, the changes of antioxidant activity following wounding may depend on type of plant tissues (Reyes et al., 2007). In the present study, total antioxidant activity of cut potato tissues was determined using ABTS free radical scavenging assay. Overall, very low antioxidant activity was observed in cut potato tissues (Figure 9.2 B). Additionally, slight improvement in total antioxidant activity was observed after 4 days of wounding treatment. Though, highest antioxidant activity was observed in COS treated cut potato tissues of cv. Russet Burbank, there were no statistically significant differences in antioxidant activity between elicitor and control treatments (control, fluridone, and natural elicitors). Therefore, the polymerization of phenolics during suberization processes and changes of activity of localized antioxidant enzymes in different cellular compartments might

have greater impact on total antioxidant activity and overall redox regulation in wounded potato tubers. Such stress-protective antioxidant enzyme responses are also closely related to redox-linked PPP regulation in stress-induced plant cells (Jacob –Velaquez et al., 2015; Shetty and Wahlqvist, 2004).

9.4.3. Glucose-6-Phosphate Dehydrogenase (G6PDH) and Succinate Dehydrogenase (SDH) Activities

Redox-linked and anabolic pentose phosphate pathway (PPP) regulation provide protective cellular function under abiotic and biotic stresses including wounding stress (Shetty, 2004; Kruger et al., 2011; Lu et al. 2016). Maintaining reducing environment through activity of dehydrogenases such as glucose-6-phopshate dehydrogenase (G6PDH), which also regenerate NADPH is critical to counter wound-induced oxidative stress and associated damages (Shetty and Wahlqvist, 2004). Therefore, critical metabolic PPP regulation and associated anabolic responses, including stimulation of secondary metabolite (phenolics) biosynthesis and potential tissue and cellular structural adjustments for stress protection are part of an effective strategy to improve stress-induced responses in plant cells. Based on this rationale, activity of G6PDH, a key enzyme responsible for the first-rate limiting step of PPP, of cut potato tissues was determined following wounding (4 days) and bioprocessed elicitor treatments. In the present study, G6PDH activity of cut potato tissues increased significantly 4 days after wounding (Figure 9.3 A). Additionally, COS elicitor treatment resulted into highest G6PDH activity in wounded tissues of both potato cultivars.

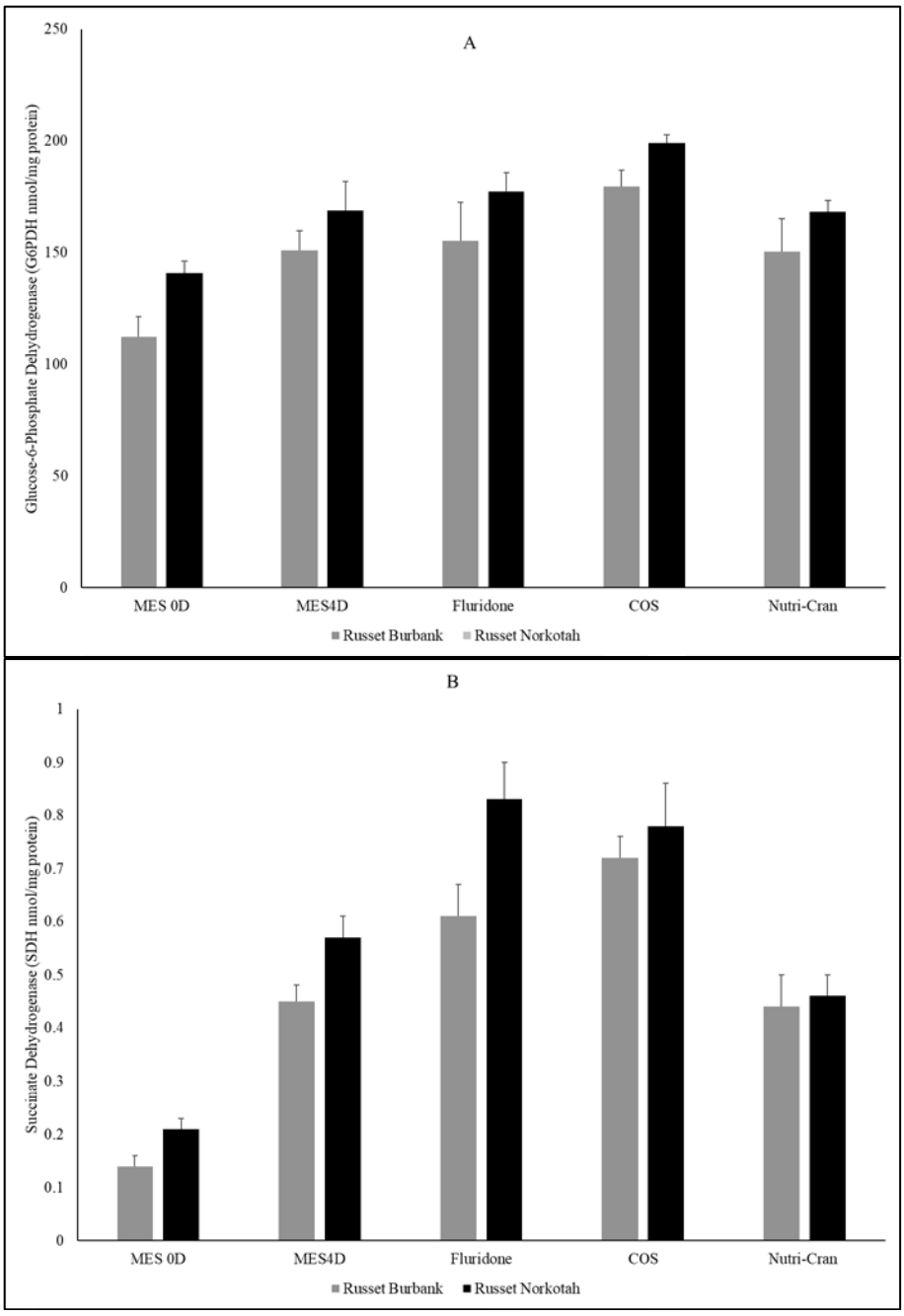

Figure 9.3. Glucose-6-phosphate dehydrogenase (G6PDH-nmol/mg of protein) (A) and succinate dehydrogenase (SDH-nmol/mg of protein) (B) activities of cut potato tissues of two cultivars (Russet Burbank and Russet Norkotah) with bioprocessed elicitors (COS and Nutri-Cran), fluridone (FL), and MES (control) treatments after 4 days of wound-healing

Overall, the results of G6PDH positively correlated with high TSP content and high SPP ratings in COS elicitor treated cut potato tissues. The results of this study suggested that up-regulation of redox-linked and anabolic PPP regulation is critical to improve WH responses in cut and bruised potato tubers. Previously, Verleur (1969) observed high G6PDH activity in potato tubers following wounding damages. Similarly, higher G6PDH activity was also found in wounded root tissues of carrot (Minibayeva et al., 2009). Stimulation of PPP regulation can lead to the improved biosynthesis of redox protective phenolics and suberin polyphenols and can be closely coupled with enhanced antioxidant enzyme responses, which are important for maintaining redox-homeostasis in wounded plant tissues. Advancing a metabolically-driven bio-elicitation strategy (COS) to improve PPP regulation and subsequently enhancing biosynthesis of protective phenolics has significant relevance for improving WH responses of cut and bruised potato tubers.

In addition to PPP, glycolysis of respiration process also provide carbon source (pyruvate) for phenolic biosynthesis in plants. Previously, increased respiration and accumulation of respiratory substrates was observed in wounded discs of potato tubers (Teramoto et al., 2000). In the present study, activity of succinate dehydrogenase (SDH), which oxidizes succinate to fumarate as part of tricarboxylate cycle (TCA)/ Kreb's cycle was also determined in cut potato tissues following elicitor treatment. The SDH activity of cut potato tissues increased significantly 4 days after wounding (Figure 9.3B). Additionally, higher SDH activity was observed in cut tissues of cv. Russet Norkotah when compared to cv. Russet Burbank. Like the results of TSP, G6PDH, and SPP ratings, COS elicitor treatment resulted into higher SDH activity in cv. Russet Burbank. However, in Russet Norkotah, higher SDH activity was observed with FLD, and COS treatments. The results of this study indicated the equal

distribution of carbon flow through PPP and respiration cycle during suberization and wound-healing processes relevant for supporting diverse anabolic needs and maintaining essential cellular functions, including energy generation (ATP) through mitochondrial oxidative phosphorylation. Additionally, as COS elicitor treatment up-regulated both PPP and glycolysis, the enhanced carbon source for biosynthesis of protective phenolic compounds in wounded tissues is relevant for improving WH responses of cut and bruised potato tubers.

9.4.4. Proline Dehydrogenase (PDH) Activity and Total Proline Content

Proline is the most abundant amino acid in the cytoplasm of plant cells and plays critical role in overall endogenous defense responses under abiotic stresses. Previously, formation of systemin, a proline-rich polypeptide was observed in wounded tissues of *Solanaceous* plants such as tomato and potato (Vasyukova et al., 2011). However, the potential active metabolic role of proline and its coupling with PPP regulation (Shetty and Wahlqvist, 2004) in wounded potato tissues was not investigated previously. The active metabolic role of proline in stress induced plant cells and linked to phenolic biosynthesis pathway was first proposed by Shetty (1997). In the proposed model, proline synthesis in the cytosol is coupled with PPP through $NADP^+$/NADPH regulation, and up-regulation of proline associated pentose phosphate pathway (PAPPP) can drive carbon flux towards defense related anabolic responses under abiotic and biotic stresses, particularly phenolic biosynthesis (Hare and Cress, 1997; Shetty, 1997, Shetty and Wahlqvist, 2004, Sarkar et al. 2009). Additionally, proline can act as a reducing equivalent by replacing NADPH in mitochondrial oxidative phosphorylation, enabling greater efficiency in energy synthesis (ATP) by bypassing energy intensive and NADH dependent respiration under stress conditions (Shetty and Wahlqvist, 2004).

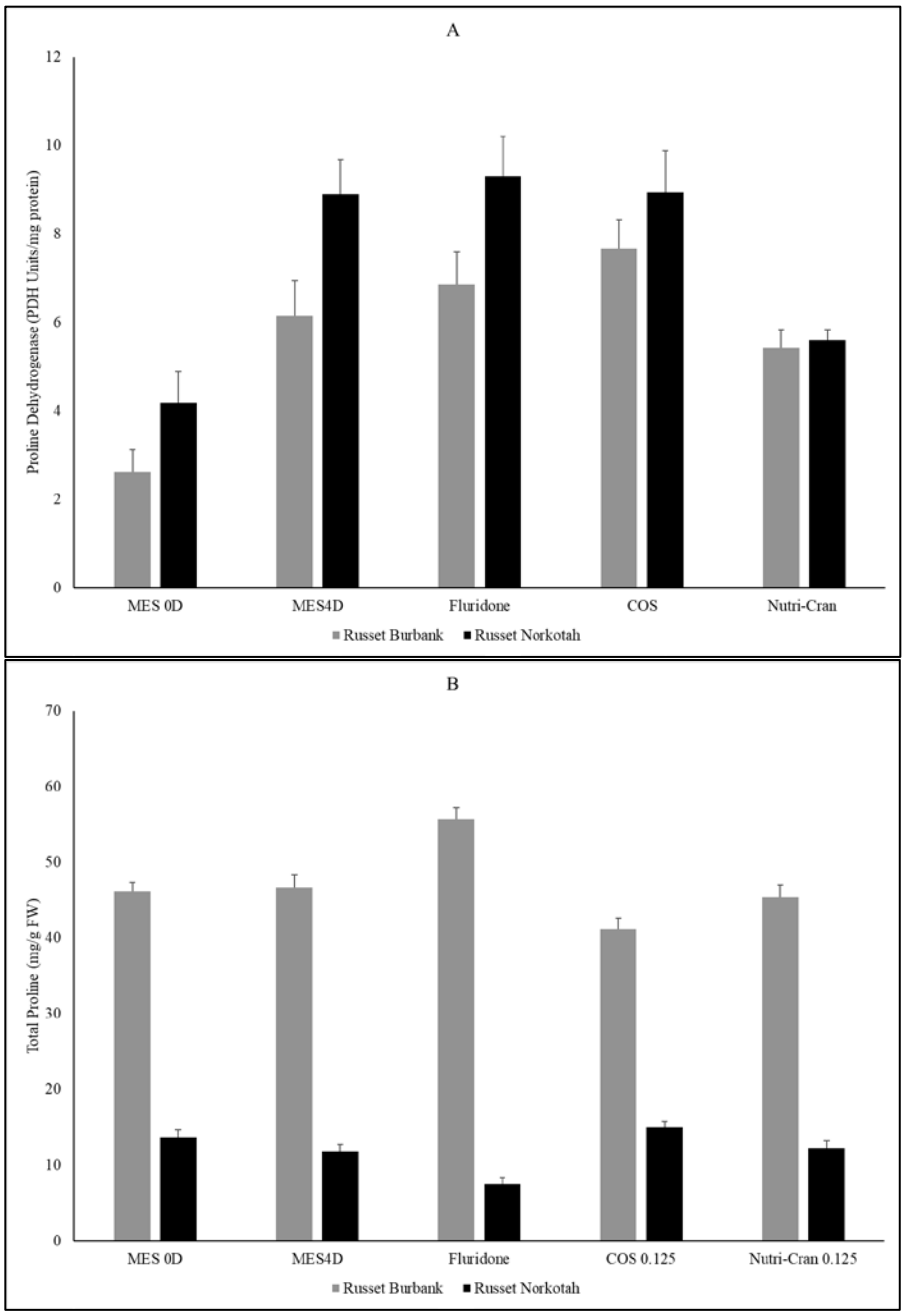

Figure 9.4. Proline dehydrogenase (PDH) activity (Units/mg of protein) (A) and total proline content (mg/g FW) (B) of cut potato tissues of two cultivars (Russet Burbank and Russet Norkotah) with bioprocessed elicitors (COS and Nutri-Cran), fluridone (FL), and MES (control) treatments after 4 days of wound-healing

Reduction in respiration rate and stimulation of redox-linked PPP regulation coupled with active metabolic role of proline can potentially support reductant intensive anabolic requirements during WH responses of cut and bruised potato tubers. Therefore, to understand the potential

active metabolic role of proline in WH responses, activity of proline dehydrogenase (PDH), a key enzyme of proline metabolism, and total proline content of cut potato tissues were investigated. In this present study, significant increase in PDH activity was observed following 4 days of wound healing (Figure 9.4 A). Higher PDH activity of cut potato tissues also positively correlated with high G6PDH activity, suggesting a potential coupling of proline synthesis with PPP regulation to support required anabolic responses during WH process. In cut tissues of cv. Russet Burbank, higher PDH activity was also found with COS elicitor treatment, while for cv. Russet Norkotah, it was statistically at par with MES (control) and fluridone (FLD) treatments. Interestingly, the total proline content of cut potato tissues either reduced (Russet Norkotah) or remained at same level (Russet Burbank) 4 days after wound healing (Figure 9.4 B). The contrasting results based on different trends in PDH activity (increase) and total proline content (decrease) indicated a potential utilization of proline in mitochondrial oxidative phosphorylation during WH responses, especially in cut tissues of cv. Russet Norkotah. Previously, such active metabolic role of proline for improving abiotic stress resilience was observed in different plant systems (Kwon et al., 2009; Sarkar et al., 2009 a, 2009b). Therefore, the results of this present study indicated potential active metabolic role of proline as a reducing equivalent to replace NADH in mitochondrial oxidative phosphorylation coupled with up-regulation of NADPH-requiring PPP, which is an energy efficient defense response, essential for supporting critical biochemical and tissue structural adjustments during WH responses of cut and bruised potato tubers. In this context, natural elicitors, such as COS can be targeted to improve energy efficient and redox-linked pathway (PPP) regulation for supporting necessary anabolic needs and for improving antioxidant enzyme responses during WH processes of cut and bruised potato tubers. This metabolically-driven elicitation strategy targeting dual benefits of phenolic compounds is

also relevant for improving shelf-life and health related nutritional qualities of potato tubers at post-harvest stages.

9.4.5. Antioxidant Enzyme (GPX, SOD, and CAT) Activities

The phenolic biosynthesis through phenylpropanoid pathway regulation and the redox-protective role of phenolic compounds is also associated with antioxidant enzyme responses in plant cells (Shetty and Wahlqvist, 2004). Individual antioxidant enzymes that are widely distributed in different cellular compartments of plant cells provide diverse protective functions, including protection against oxidative stress-induced metabolic breakdowns and associated tissue damages (Caverzan et al., 2012). Therefore, improving antioxidant enzyme responses is essential to counter wounding stress induced cellular damages of potato tubers. Additionally, improved antioxidant enzyme response is also relevant for minimizing further damages from other biotic and abiotic stresses following wounding of potato tubers (Wegner and Jansen, 2013). In this study, activities of three important antioxidant enzymes such as guaiacol peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD) of cut tissues of potato tubers were determined to understand the potential role of these enzymes in WH responses of cut and bruised potato tubers. Overall, higher activities of GPX, and CAT was observed in cut tissues of both potato cultivars after 4 days of wound healing (Table 9.1). On the contrary, the SOD enzyme activity was significantly low in wound-induced cut potato tissues and did not alter even with natural elicitor treatments.

Guaiacol peroxidase (GPX) is a Class-III peroxidase and generally contributes to maintaining a lower concentration of H_2O_2, which in high concentration causes significant cellular damages. Additionally, Class III peroxidases are also involved in diverse cellular protective functions like cell wall cross-linking, cell wall loosening, lignin and suberin

formation, auxin metabolism, and in defense response against biotic and abiotic stresses (van Doorn and Ketsa, 2014). The biosynthesis and formation of suberin polyphenolics is dependent on peroxidase mediated oxidative coupling process (Bernards et al., 2004). Therefore, improved GPX activity has significant relevance in polymerization and lignification of phenolics and formation of suberin in closing layer and wound periderm layer formation of cut and bruised potato tubers. Previously, enhanced peroxidase activity was observed in wounded and bacteria (*Pectobacterium atrosepticum* cause soft rot) inoculated potato tubers (Ngadze et al., 2012). In the present study, significantly higher GPX activity was observed with COS elicitor treatments, especially in cut discs of cv. Russet Norkotah. The higher GPX activity in COS treated cut potato tissues also positively correlated with high SPP rating, high phenolic content, and higher G6PDH (relevant for up-regulation of PPP) activity. Therefore, results of this study suggested that upregulation of PPP, enhanced biosynthesis of protective polyphenolic compounds, and improved GPX activity are interconnected and critical for metabolic regulation that potentially support suberization process in wound-induced cut potato tubers. Furthermore, elicitation with water soluble COS is an effective and metabolically-driven strategy that can stimulate PPP and phenolic-linked protective cellular functions relevant for improving rapid WH responses in cut and bruised potato tubers.

In addition to GPX activity, increased CAT activity was also observed in cut potato tissues by 4 days of wound healing and elicitor treatments (Table 9.1) Scavenging hydrogen peroxide (H_2O_2) is extremely important to prevent cellular damages and for maintaining redox homeostasis under stress condition such as wounding-stress. Like the results of GPX activity, higher CAT activity was also observed in COS treated cut potato tissues. Previously, enhanced CAT activity and improved wound-healing efficiency was observed in potato tubers and

sugarbeet roots (Blaji et al., 2007; Fugate et al., 2016). The improved CAT activity might be relevant for deposition of polyphenolic domain of suberin in wounded tissues (Fugate et al., 2016).

Table 9.1. Guaiacol peroxidase (GPX), superoxide dismutase (SOD), and catalase (CAT) enzyme activities of cut potato tissues of two cultivars (Russet Burbank and Russet Norkotah) with bioprocessed elicitors (COS and Nutri-Cran), fluridone (FL), and MES (control) treatments after 4 days of wound-healing

Treatments	Cultivars	GPX (nmol/mg of protein)	SOD (units/mg of protein)	CAT (units/mg of protein)
	Russet Burbank	8.14 ±0.9	0.06±0.02	10.21±0.9
MES 0day	Russet Norkotah	5.84 ±0.5	0.02±0.01	16.87±1.6
	Russet Burbank	76.0 ±4.4	0.01±0.02	20.29±3.6
MES 4day	Russet Norkotah	151.9 ±10.78	0.01±0.00	29.76±3.4
	Russet Burbank	75.4 ±3.8	0.02±0.02	28.56±2.4
Fluridone (FL)	Russet Norkotah	108.1 ±3.2	0.01±0.01	32.47±2.6
	Russet Burbank	82.1 ±6.5	0.04±0.00	32.71±2.3
COS	Russet Norkotah	170.4 ±9.1	0.01±0.00	41.47±2.6
	Russet Burbank	82.9 ±5.1	0.03±0.01	22.85±1.2
Nutri-Cran	Russet Norkotah	149.3 ±9.7	0.01±0.01	26.70±1.4

± Standard error; n=8

Therefore, results of our study, which revealed enhanced CAT and GPX activity in cut potato tissues indicated critical protective functions of these antioxidant enzymes and their potential role in the suberization process of wound-induced potato tubers. Furthermore, this study also provided a scientific rationale to target novel bio-elicitation strategy, such as COS natural elicitor treatments for improving antioxidant enzyme responses and their associated stress protective functions supporting rapid WH responses of cut and bruised potato tubers.

9.5. Conclusions

The wound healing (WH) responses of plant tissues are a complex process, which involve and integrates different defense related metabolic pathway regulations and associated protective responses. Therefore, understanding critical protective metabolic regulations involved in the WH

processes is important for countering wound-induced damages of economically important plant organs such as potato tubers. In this study, the potential role of redox-linked pentose phosphate pathway (PPP) regulation to improve biosynthesis of protective phenolic compounds and coupled antioxidant enzyme responses relevant for supporting suberization and WH response of cut and bruised potato tubers was investigated. Additionally, a metabolically-driven elicitation strategy with natural bioprocessed elicitors such as water-soluble chitosan oligosaccharide (COS) and bioprocessed cranberry pomace (Nutri-Cran) was targeted to improve rapid WH responses in cut tissues of two potato cultivars, Russet Burbank and Russet Norkotah. Overall, stimulation of redox-linked PPP regulation (high G6PDH activity), increased respiration (SDH activity), enhanced biosynthesis of phenolics, and improved antioxidant enzyme responses (GPX, CAT) were observed in cut potato tissues after 4 days of wound healing. This enhanced PPP-linked metabolic regulation also positively correlated with formation of suberin polyphenolics (SPP) in closing layer of parenchyma cells of exposed potato tissues, which indicated potential protective role of anabolic PPP in suberization process. Additionally, the improvement of PPP-linked protective function and SPP formation was significantly higher with bioprocessed COS elicitor treatment. Results of this study suggested that water soluble COS can be targeted as safe and effective strategy to improve WH responses in cut and bruised potato tubers. Furthermore, based on the dual functional role of phenolic compounds for improving post-harvest quality and human health benefits, this novel elicitation strategy can also be targeted to improve shelf-life and nutritional qualities of potato tubers.

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