DEVELOPING FUNCTIONAL FOOD PRODUCTS THROUGH NOVEL PROCESSING,

INGREDIENT, AND SHELF STABILITY EVALUATION

by

MARK COREY

(Under the Direction of William L. Kerr)

ABSTRACT

The purposes of this dissertation research were: 1) to develop new functional food products for consumers, 2) to evaluate processing or storage stability, antioxidant activity, and health properties of polyphenolic compounds in functional products, and 3) to determine the effects of processing from emerging technologies on properties of functional products.

Novel functional food products were developed, which included a beverage made from fruit juice and high-procyanidin sorghum bran for the metabolic syndrome or diabetic markets, and model green tea (GT)-fortified apple products designated for low- and intermediate-moisture food systems.

The stability of phytochemicals and antioxidant activity was evaluated in GT-fortified apple product over storage in low- and intermediate-moisture systems. Phytochemical degradation was modeled using a pseudo-first-order kinetic model ($\ln C = A \cdot e^{-kt}$). GT catechins including catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG) showed varying levels of stability with $k = 0$ to 0.070, indicating degradation over storage. Analysis of water mobility by differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) supported analytical determinations

that increasing moisture content related to increases in magnitude of k. The onset glass transition temperature (T_{go}) of GT-fortified apple products decreased from 13°C to -34°C from a_w 0.11-0.56, respectively. Low-field ^1H NMR analysis of GT-fortified apple products conducted by free induction decay (FID) showed increasing relaxation times from 60 to 1,000 μs from a_w 0.11-0.75, respectively. Antioxidant activity determinations including total polyphenolic content by the Folin-Ciocalteu method, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH) scavenging activity, and ferric reducing/antioxidant power (FRAP) assay conducted on extracts from high-procyandin sorghum beverage and GT-fortified apple products demonstrated value-added potential of functional ingredient incorporation to products. As an *in vitro* marker for diabetic systems, extracts from high-procyanidin sorghum beverage and GT-fortified apple products showed efficacy in inhibiting glycation of bovine serum albumin.

High-pressure throttling was investigated as an emerging technology used for the processing of functional beverages. Apple juice inoculated with *Zygosaccharomyces bailii*, common spoilage yeast in fruit juices, processed by high-pressure throttling was found to have 7-log reductions in vegetative cells from processing of juice.

INDEX WORDS: Functional foods, High pressure processing, *Zygosaccharomyces bailii*, Glycation, Sorghum, Procyanidin, High-procyanidin sorghum bran, Green tea, Apple, Polyphenolic compounds, Low moisture foods, Intermediate moisture foods, NMR, DSC, Water activity

DEVELOPING FUNCTIONAL FOOD PRODUCTS THROUGH NOVEL PROCESSING,

INGREDIENT, AND SHELF STABILITY EVALUATION

by

MARK COREY

B.S., University of Maine, 2004

M.S., The Pennsylvania State University, 2006

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2009

DEVELOPING FUNCTIONAL FOOD PRODUCTS THROUGH NOVEL PROCESSING,

INGREDIENT, AND SHELF STABILITY EVALUATION

by

MARK COREY

Major Professor:	William L. Kerr
Committee:	Phillip Greenspan
	Jake Mulligan
	Ronald Pegg
	Louise Wicker

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2009

DEDICATION

This dissertation is dedicated to my family for always providing love, support and

encouragement, and God for providing the guiding light to succeed.

ACKNOWLEDGEMENTS

I would like to thank Dr. William Kerr and my committee members for their guidance in helping complete this dissertation, Dr.ssa Vera Lavelli for welcoming me into her laboratory in Italy, and all those who have given their time, energy, thoughts, ideas, or financial support to help me along the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Consumers are becomingly increasingly interested and aware of the role that diet has in maintaining and promoting health and preventing disease. They are demanding premium, convenient food products that also satisfy their needs for health, while providing nutrients beyond basic nutrition. The research community has identified and is still investigating a vast array of chemical compounds found in nature and the diet that have demonstrated positive consequences toward human health. These chemicals are not essential for body metabolism, but contribute to overall health and wellness through their ability to help the body maintain immunity and provide protection from toxins.

Loss of phytochemical content becomes more significant as foods are processed, and fortification such as through functional foods becomes necessary (Henry and Heppell, 2002). The concept of functional foods has only recently gained mainstream popularity. It links consumers' needs for consuming health-promoting food products that provide more than basic nutrition with the goal of aiding in the prevention or maintenance of certain disease states or increasing physiologic performance.

An emerging class of novel functional foods is appearing in the marketplace. This type of product involves adding food ingredients rich in bioactive compounds to common food products, but where the new ingredient has not previously been added to the food product. For example, this could include adding green tea extract to apple products. Little is known how the bioactive compounds will interact and how this will affect their chemical stability and nutritional

significance. Additional information is needed on emerging technologies that can process these products.

The primary goals of this dissertation research were:

1. To increase the availability and type of functional foods

2. To evaluate the processing or storage stability, antioxidant activity, and health properties of polyphenolic compounds in functional products.

To satisfy these objectives, the research studies presented in this dissertation focused on first identifying functional ingredients that may provide unique or complementary health properties to consumers. The ingredients selected were high procyanidin sorghum bran and green tea extract, which were both rich sources of polyphenolic compounds. These bioactive ingredients were then paired with basic, staple foods such as white grape or apple juices and apple fruit products. The purpose was to increase the nutritional content of the basic foods, providing value-added benefits to consumers. Particular attention was also placed on pairing the functional ingredient with a basic food item to provide complementary health benefits. This included increasing the polyphenolic contents of antioxidants, or enhancing specific health benefits associated with consuming the foods, such as possibly reducing physiologic effects associated with type II diabetes or metabolic syndrome.

The novel functional blends selected were high procyanidin sorghum bran added to fruit juice and green tea extract added to apples. Issues concerning processing or storage stability of the products were then identified. This included first evaluating the effectiveness of using continuous-flow high-pressure throttling as a novel processing method for producing functional beverages. This was evaluated by measuring microbial reductions produced from a yeast strain

commonly associated with fruit juice spoilage. The potential of high-procyanidin sorghum bran as a functional ingredient in fruit juices was then investigated through analysis of antioxidant and physicochemical analyses. This was followed by the investigations of the stability and bioactive properties of green tea extract added to apple products in low- and intermediate-moisture food systems. In the last study, the stability of green tea and apple phytochemicals was related to moisture content and water mobility in the low- and intermediate-moisture green tea-fortified apple products.

LITERATURE REVIEW

1.1 Functional Foods

Only recently, a global trend in the food product marketplace has emerged that embraces the idea of helping to prevent or alleviate certain medical conditions, or maintain optimal health through the consumption of nutritiously enhanced food products. These food products may also improve performance, such as for physical activity or mental performance (Wildman, 2001). The food products are considered functional foods because they impart enhanced nutrition and, according to the U.S. Institute of Medicine, include "any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (ADA, 1995). In addition, functional foods are "…a food, either natural or formulated, which will enhance physiological performance or prevent or treat diseases and disorders. Functional foods include those items developed for health purposes as well as for physical performance" (Wildman, 2001).

The exact definition of *functional food* is lacking, but the term generally refers to food products that have been modified beyond their natural state to provide additional nutrition. The functional food components added to food products for enhanced nutrition can be macronutrients which may exert specific physiologic effects, essential micronutrients if the intake is greater than the recommended dietary intake, a non-essential dietary component having nutritive value, or components such as phytochemicals that have no nutritive value (Roberfroid, 2000). When formulating functional foods, two methods are commonly employed. In the first scenario, the functional bioactive components are usually added at levels that are higher than what is naturally present in the food product; in the other instance, the food is fortified with a specific chemical component that is not naturally present in the product. Fortifying foods with bioactive components is intended to maintain, improve, or enhance the nutritional quality of the food for a group, community, or a population (Henry and Heppell, 2002). Besides being added to food, the functional food components can often be purchased and consumed separately as dietary supplements.

The U.S. Food & Drug Administration (FDA) does not have authority to establish a regulatory category for functional foods. The products must be sold as conventional foods, dietary supplements, medical foods, or infant formulas (Ross, 2000). The product label may state how use of the product may help maintain a certain level of bodily health if it is "…truthful, not misleading, and derives from the nutritional value…" of the product, and does not imply a certain disease state (section 403(r)(1)(B) of the FFDCA) . No claims may profess that taking the product will help in the "…diagnosis, cure, mitigation, treatment, or prevention of disease" (section 201 (g)(1)(B) of the FFDCA [Title 21 United States Code (21 USC) section 321(g)(1)(B)]), otherwise the product would be considered a drug. The FDA (US FDA/CFSAN)

allows different types of health claims to appear on food product labels. The claims are closely

mandated and are often based on claim specificity. Some categories are structure/function

claims (SFC) (e.g. calcium builds strong bones) or qualified-health claims (QHC). For example,

> "Limited and not conclusive scientific evidence suggests that eating about 2 tablespoons
> (23 grams) of olive oil daily may reduce the risk of coronary heart disease due to the
> monounsaturated fat in olive oil. To achieve this possible benefit, olive oil is to replace a
> similar amount of saturated fat and not increase the total number of calories you eat in a
> day. One serving of this product contains [x] grams of olive oil" (FDA, 2004).

Other health claims may comply with significant scientific agreement (SSA). For example,

> "Regular exercise and a healthy diet with enough calcium helps teen and young adult
> white and Asian women maintain good bone health and may reduce their high risk of
> osteoporosis later in life" (21 CFR 101.72).

Both SFC and QHC relate how the consumption of a certain health-promoting compound will

reduce the risk of disease or other conditions, but an SFC does not specifically name a disease

(FDA/CFSAN). After the passage of the Dietary Supplement and Health Education Act

(DSHEA) in 1994, if a food manufacturer wants to make a specific health claim for a product

that is not approved by the FDA, then the product can be sold as a dietary supplement (Farr,

1997). The product label must state that the food is not intended for disease maintenance and

that the FDA has not evaluated the product (21CFR321). Thus, regulations governing functional

food marketing can become quite complex.

Navigating through the functional foods and nutraceuticals arena is fraught with

numerous legal and ethical hurdles to overcome. In the U.S., consumers demand products that

can help in the maintenance or prevention of various health conditions. They also desire simple

solutions, such as dietary supplements to combat specific physiologic states or foods to increase performance. Best of all for consumers, these foods are available without a prescription. The consumers' needs are met through the production and sale of functional foods and nutraceutical compounds that provide nutrition beyond what is normally derived from the staple diet. Products need to be designed based on what consumers demand in the marketplace. On the other hand, studying functional components based on what effect they have on the body and what possible health benefits are derived from their consumption would allow for greater continuity in research among countries and cultures (Roberfroid, 2000). Sometimes though, disparities exist between what consumers think is important versus what industry and health professionals view as most relevant (Kleef and others 2002).

Designing functional food and nutraceutical research around both what consumers demand and with what has scientific merit and novelty requires a careful balance. For example, consumers often desire dietary supplements that may consist of isolated dietary components, which are sold as extracts, pills, or capsules. In the U.S., where dietary supplements are not regulated by the FDA in terms of health claims and dosages, their potency, bioavailability, and efficacy are often uncertain. Consumers taking dietary supplements often lack knowledge for proper dosage of the supplement and potential interactions that could occur with existing medications they are taking.

Because of these various problems, supplementing existing food products with nutraceutical compounds at significantly lower dosage forms is more desirable. This would result in nutritionally enhancing the food supply (Pascal, 1996), and would be safer and more efficacious in reaching a much larger segment of the population than just those who currently consume dietary supplements. This notion reiterates the original intent Japan had in creating and

6

regulating the functional foods industry, which was a means to improve the health of its citizens (Farr, 1997). A broader goal of this research was to realize new practical solutions to bridge consumers' demands for consuming functional foods products with proper scientific validation. This was accomplished by investigating how producing the food products they desire using current and novel technologies would affect the enhanced level of nutrition found in functional food products.

1.2 High Pressure Processing

As the demand for functional food products increases, researching and developing new processing technologies is important in order to deliver maximal nutritional benefits and product quality. High-pressure processing (HPP) is a gentler alternative processing-method than thermal processing. It prevents the adverse consequences of thermal processing from occurring. This occurs by preserving the fresh character of raw, whole product in the food or beverage and preventing cooked or other heat-induced products from forming, or protecting thermally-labile compounds (Schmidt and others 2005). This occurs as significantly less thermal energy evolves during HPP, thus the product is not heated to the extent that often occurs during thermal processing.

High hydrostatic pressure methods have been used in the production of beer (Castellari and others 2000), milk (Drake and others 1997; Mussa and Ramaswamy, 1997), emulsions (Dickinson and James, 1998), salmon (Lakshmanan and others 2007), cheese (Juan and others 2007), and hot dogs (Ruiz-Capillas and others 2007). A benefit of HPP is the color retention of fresh product compared to darker colors that sometimes occur as a result from thermal processing (Krebbers and others 2003; Sanchez-Moreno and others 2006). HPP also helps retain

the nutritional content of the product. It helps prevent antioxidant or vitamin degradation, which is due to protective mechanisms exhibited by chemical compounds in response to high pressure. It achieves these results while still maintaining food safety by producing product that is free of pathogens and spoilage microorganisms (Polydera and others 2005).

During HPP, pressures of 100-1,000 MPa (Clark, 2006) are typically created and maintained for a short time, typically for under 40 min, and are combined with moderate heat from 20-95ºC. HPP systems use hydrostatic or throttling pressure. Most scientific literature report results of high pressure treatment on food products using hydrostatic pressure. With HPP, food is often pre-packaged in flexible or semi-rigid bags or plastic containers and placed into a sealed chamber that is then pressurized (Clark, 2006). In a continuous-flow high-pressure throttling device (CFHPT), a continuous stream of liquid is compressed through a throttling valve, similar to a homogenizer. In the department of Food Science and Technology at the University of Georgia, the throttling pressure system available can consistently deliver pressures at 300 MPa. Some minimal heat is often generated in liquids among both HPP-types as a result of adiabatic heating on the order of 3ºC for every 100 MPa (Butz and others 1997). This occurs as a result of the compression of the food and is a function of its composition. For example, air found in a food matrix can compress significantly and contribute to temperature rise (Earnshaw, 1996). Some units also can regulate temperature, and thus many processes combine pressure, temperature, and holding time to exert processing effects.

The CFHPT system is especially suited for beverage production. It allows for easier conversion to a semi-continuous process without the limitations imposed by the more conventional hydrostatic pressure. This is due to how no pre-packaging of materials is necessary prior to processing, and the unit is more adaptable for installation of connection fittings for

8

pumping product. Having the ease of pumping liquid product in between the processing unit and holding tanks is especially desirable for industrial processors. In addition, very little scientific literature using CFHPT in food processing has been found.

The high pressure exerted on liquid media results in the production of significant shear forces that effectively denature some cellular proteins (Sanchez-Moreno and others 2005), while ionizing water and acid molecules that are purported to be lethal to microbial cells (Earnshaw, 1996). HPP is generally detrimental to vegetative cells at pressures greater than 100 MPa, whereas spores are resistant to even extreme pressures greater than 1000 MPa. Some spores will germinate in response to pressure treatment (Earnshaw, 1996), typically of 50-300 MPa. The spores are most resistant to pressure treatment at neutral pH. They can then be destroyed by another subsequent thermal or pressure treatment, but sterilization of product is not possible (Smelt, 1998). The lysing of cellular membranes may even increase the concentration of some non-nutritive bioactive compounds, such as lycopene (Sanchez-Moreno et al., 2005). This would occur as a result of the more complete release of contents from cellular organelles. The high pressures generated may also have a minor effect on the apparent viscosity of liquid product (Polydera et al., 2005).

1.3 Microbiological Considerations of Acidified Beverages

Microbiological contaminants, especially sporeforming microorganisms, are of significant concern when utilizing HPP technologies. Ready-to-drink (RTD) beverages composed of fruit juices or tea are generally low or high acid. The pH of fruit juices typically ranges from 2.4-4.2 (Hatcher and others 2001) and that of RTD tea classified as a still or soft drink is similar, typically ranging between pH 2.5-4.0 (DiGiacomo and Gallagher, 2001).

Pathogens are generally not a significant concern in this type of beverage due to the acidic pH. Some microorganisms including aciduric bacteria, yeasts and molds remain a problem. They often originate from the surface skin or peel of the fruit, such as in pears or apples (Spotts and others 2006). Yeasts and molds are able to grow across a range of temperatures from 5-35ºC, thus even product storage conditions must be considered (Beuchat and Cousin, 2001).

Some acidophilic sporeforming microorganisms including *Alicyclobacillus* and *Sulfobacillus* are able to survive thermal treatment and grow at acidic pH and temperatures greater than 20ºC (Chen and others 2006). Their growth in pasteurized apple juice can produce a phenolic odor and taste (Hatcher et al., 2001) and produce turbidity or form precipitates (Chen et al., 2006). Spores are even capable of withstanding pasteurization. In high-pressure processing, having spores present can present a problem where the high pressure can induce germination (Smelt, 1998).

Acidification or pasteurization are common processing technologies employed for inhibiting sporulation in beverages. The natural constituents of green tea extract are also capable of inhibiting growth of certain types of bacteria, such as growth originating from oral, intestinal, or food origin. The green tea polyphenols also have shown antimicrobial action toward *Bacillus stearothermophilus* (Sakanaka and others 2000).

Yeasts such as *Candida*, *Saccharomyces* and *Torulopsis* spp. can cause spoilage in fruit juices if not properly controlled (Hatcher et al., 2001). *Zygosaccharomyces bailii* is an especially preservative-resistant, potent spoilage organism in high acid beverages. It can produce off-odors, off-tastes, sedimentation, and exert increased pressure on packaging (DiGiacomo and Gallagher, 2001). The yeast *Geotrichum candidum* is associated with milk and dairy products (Lopandic and others 2006), but its presence on processing equipment can be a

10

sign of unsanitary manufacturing equipment. Another potential problem can involve molds such as the heat-resistant *Neosartorya fisheri* which is often present on raw fruit and can grow at refrigeration temperatures less than 5ºC (Hatcher et al., 2001). Molds can produce extracellular enzymes such as pectin methylesterases, which diminish cloud in juices (Nussinovitch and Rosen, 1989). Some of the molds can be toxigenic, such as *Byssochlamys* spp. (e.g. *B. fulva* and *B. nivea*), which can synthesize the mycotoxin patulin (Puel and others 2007). Its presence is a sign of poor quality of raw apples used in juice expression. Their concentrations are usually very low from 1 to 10 CFU / 100 g or mL of sample, thus sample concentration may be necessary for their detection (Beuchat and Pitt, 2001). The ascospores produced by some heat-resistant molds will sometimes germinate when exposed to heat (Beuchat and Pitt, 2001), but their dynamics are very similar to bacterial spores.

The known effects of continuous-flow high-pressure throttling on reduction of microorganisms are largely unknown. In hydrostatic HPP, the natural microflora of tomato paste was not significantly reduced with HPP treatment of 300 or 500 MPa at 20ºC for 2 min, but was significantly reduced with processing pressures at 700 MPa (Krebbers et al., 2003). The microbial reduction induced by pasteurization of milk were comparable to the same product processed by HPP such as for total plate count and coliforms (Drake et al., 1997). In a challenge study, orange juice processed with pressures greater than 250 MPa showed complete inactivation of high initial inoculation loads of *Lactobacillus plantarum* (7-log reduction) and *Saccharomyces cerevisiae* (5-log reduction) (Campos and Cristianini, 2007). After processing at 600 MPa, orange juice had no detectable microbes, and counts remained below log 2 concentrations through 12 weeks of storage (Bull and others 2004). Similar results were also observed in guava juice (Yen and Lin, 1999).

The throttling valve of the CFHPT system at the University of Georgia, which is similar

in design to the valve seat of a homogenizer, may exert variable effects on the survival or growth

of microorganisms present in unprocessed liquid product. Very little literature is available

reporting on the effects of CFHPT on microbial survival in food products (Toledo and Moorman,

2000). In order to evaluate the effectiveness of a processing system such as the CFHPT on food

safety, microbiological sampling needs to occur at several points along the processing system.

This is because the processing and bottling or packaging equipment is the most likely causative

agent responsible for contaminating still or soft drink beverages (DiGiacomo and Gallagher,

2001).   Some of the steps may include sampling at the water source, juice or other ingredient

sources, sweetener solution, filling, empty packaging bottles, finished product, and the

processing equipment and environment (DiGiacomo and Gallagher, 2001). This should include

sampling product throughout refrigerated storage at less than 5ºC.

1.4 *Zygosaccharomyces bailii*

The organism *Zygosaccharomyces bailii* is an osmotolerant, spoilage yeast that is able to

grow in acidic to neutral conditions at pH 2.5-6.0 and environments high in sugars (Fujita and

Kubo, 2005), such as cough syrup (Charnock and others 2005) or fruit and vegetable juices

(Raso and others 1998). It is also capable of forming haploid ascospores that are generally more

resistant to processing variables of pressure, heat, and holding time than vegetative cells (Raso et

al., 1998). Chemical preservatives such as potassium sorbate are effective at inhibiting growth

of *Z. bailii* (Gliemmo and others 2006), but the organism can also metabolize organic acids such

as sorbates and benzoates due to a stress response conferred from a weak acid environment

(Piper and others 2001). When tested in buffer containing citric acid (pH 3.0 to 4.5), *Z. bailii*

showed inhibition at increased pH while levels of the acid remained constant. Glycerol

production increased, whereas ATP and ethanol production remained constant (Nielsen and

Arneborg, 2007). The inactivation of spores and vegetative cells of *Z. bailii* has been studied

using high hydrostatic pressure (Palou and others 1997; Raso et al., 1998; Reyns and others

2000; Zook and others 1999). Reyns et al. (2000) found that greater reduction in counts occurred

when processing sterilized acidic juices or media at 45°C compared to 20°C. At 20°C,

reductions only occurred at processing pressures greater than 220 MPa, whereas at 45°C,

reductions occurred even at 120 MPa. Varying the pH from 3 to 6 did not affect cell counts at

20°C, whereas at 45°C greater reductions occurred at pH 3, although statistical analysis was not

provided. HPP of ascospores of *Saccharomyces cerevisiae* in orange and apple juice showed no

differences in variations of pH (3.5-5.0) on organism lethality or survival (Zook et al., 1999).

Enumeration media used to evaluate spore production and vegetative cell growth of

osmotolerant yeasts such as *Z. bailii* and *S. cerevisiae* varied considerably. Sporulation medias

included yeast extract with potassium acetate (Zook et al., 1999) or agar containing sodium

acetate and potassium chloride (Raso et al., 1998). Enumeration media to conduct viable cell

count included potato dextrose agar (Palou et al., 1997), YPD agar (yeast extract, peptone, and

glucose) (Nielsen and Arneborg, 2007), Sabouraud's dextrose agar medium (bactopeptone,

dextrose, bacto-agar) (Fujita and Kubo, 2005), GYE medium (oxytetracycline glucose yeast

extract agar base without addition of oxytetracycline) (Reyns et al., 2000), or malt wickerham

agar (Perrier-Cornet and others 2005). Selecting the appropriate growth and enumeration media

from a survey of what is available allows for planning for particular experimental needs, such as

being able to grow and enumerate for vegetative cells and spores.

A critical step in developing functional foods for the marketplace involves conducting microbiological challenge or enumeration studies. This is important for validating the processing effectiveness of a technology, so as to produce food products that meet specifications for safety and quality. It is also important to develop functional food products that meet consumers' needs. For functional foods, this involves identifying significant health problems that affect consumers, and then designing functional foods that may aid in meeting those particular health needs.

1.5 Developing Functional Food Products for Health Maintenance

Functional food products are designed to meet the unique health needs of consumers. Products designed to meet a direct consumer need such as for metabolic syndrome or diabetes may also have additional benefits for health-conscious consumers (Verhagen and others 2004). These products may also contain ingredients from multiple bioactive sources, which may exert complementary health benefits. For example, this could involve combining the flavonoid-rich extracts from high-procyanidin sorghum bran to inhibit the glycation of protein, with hot-water extract from cinnamon for aiding in the maintenance of healthy blood glucose levels. Polyphenolic compounds from the sorghum bran and cinnamon would confer complementary health benefits that may provide anti-metabolic syndrome or anti-diabetic benefits, while also increasing the availability of health-promoting products for the average consumer.

The incidences of metabolic syndrome, atherosclerosis, pre-diabetes and Type II diabetes are increasing in Western society due to physical inactivity and following a hyper-caloric and pro-inflammatory diet (Dehnavi and others 2008; Ford and Li, 2008; Luca and Olefsky, 2008). A pro-inflammatory diet includes foods that are often refined or processed, is deficient in fiber,

antioxidants, vitamins, and minerals, and includes an imbalance in certain types of dietary fats. By 2025, Type II diabetes is projected to affect over 300 million people worldwide (Hays and others 2008). This chronic disease is the sixth leading cause of death in the United States (Pham and Kourlas, 2007). Decreasing the incidence of chronic disease caused by poor or deficient dietary practices, or maintaining optimal health for aiding in the prevention of developing such conditions, is a critical aim for research in functional foods.

A pro-inflammatory state developing within the body may contribute to development of chronic disease. Non-enzymatic oxidation of proteins, known as glycation of proteins, occurs within the body as a result of normal metabolism and homeostasis. The glycated proteins are known as advanced glycation endproducts (AGE) (Peyroux and Sternberg, 2006). This reaction occurs when a reducing sugar reacts with a free amino group on a protein molecule (McPherson and others 1988). In a pro-inflammatory state, the oxidation of proteins may occur at an accelerated rate. In diabetic patients, a clinical measure of blood glucose control over three months involves monitoring the oxidation of hemoglobin in the hemoglobin A1c test (HbA1c) (Boer and others 2006). Healthy individuals maintain levels of HbA1c, but diabetic patients may have higher than normal levels, which is an indication of accelerated oxidation of proteins in the body and sub-optimal blood glucose control (Pupillo and others 2008). Several chemical compounds including vitamins A and C, and flavonoids have demonstrated activity in inhibiting the glycation of proteins *in vitro* (Matsuda and others 2003; Peyroux and Sternberg, 2006; Dearlove and others 2008).

1.6 High-Procyanidin Sorghum Bran

Formulating functional food products such as beverages with polyphenolic compounds rich in antioxidants including flavonoids may contribute to chronic disease maintenance or maintaining optimal health, such as by reducing the effects from glycation on cellular proteins. High-procyanidin sorghum bran is high in condensed tannins, which are significant sources of monomeric and polymeric flavan-3-ol compounds (Awika and Rooney, 2004), which may inhibit the glycation of proteins. The condensed tannins are composed primarily of epicatechin monomeric units (Gu and others 2004). In the bran, they are composed predominantly of oligomeric procyanidins (OPC), but also contain significant contents of monomers, dimers, trimers, and OPC of 4-10 units (USDA, 2009).

In 2005, sorghum cultivation was 57 million tons worldwide (FAOSTAT, 2005; Dlamini and others 2007). Besides procyanidins, sorghum is also a significant source of flavonoids, anthocyanins, policosanols, and phytosterols (Awika and Rooney, 2004; Dlamini et al., 2007). Consumption of procyanidins is associated with health benefits that are chemopreventive (Nandakumar and others 2008) or may help prevent coronary heart disease resulting from inflammation (Cos and others 2004). High procyanidin sorghum bran extract has also shown anti-hyaluronidase activity, which has implications for those suffering from chronic inflammatory diseases such as osteoarthritis (Bralley and others 2008). It has also shown activity in inhibiting glycation of albumin protein (Farrar and others 2008). This has implications in aiding in the prevention of cellular protein damage associated with chronic inflammatory diseases such as Type II diabetes.

Similar health benefits are also conferred from consumption of other procyanidin-rich foods such as dark chocolate, grape seeds, or apples (Gu et al., 2004). Combining extracts from high-procyanidin sorghum bran that are high in flavonoids and phenolic acids (Awika and Rooney, 2004) with other polyphenolic-rich sources such as cinnamon, may provide complementary or enhanced antioxidant benefits to the consumer.

1.7 Cinnamon

Cinnamon is a common cooking spice derived from the bark of the tree C*innamomum cassia* or *C. zeylanicum*, which is indigenous to tropical regions of Asia (Marongiu and others 2007), and is available in many varieties such as Saigon, Korintje, or Ceylon. Recent evidence has shown the potential anti-diabetic effect that cinnamon may exert on humans. Type II diabetes mellitus, also known as adult-onset diabetes, is a condition caused by age, diet, excessive weight, metabolic syndrome, genetics, or a lack of exercise. In the early stages of Type II diabetes, the body becomes hyperinsulinemic and hyperglycemic as the beta islet cells of the pancreas must produce excessive amounts of insulin to counteract insulin insensitivity. Insulin insensitivity occurs as GLUT-4 receptor translocation to the cell membrane diminishes, and even after insulin binds to the cell, glucose uptake decreases (McGrane, 2006). Physicians recommend diabetic patients maintain levels of HbA1C at less than 7% and pre-prandial glucose levels at 70-110 mg/dL.

Methylhydroxychalcone polymer (MHCP) was originally considered to be the bioactive water-soluble compound in cinnamon, but is now thought to be Type-A procyanidin polyphenolics (Anderson and others 2004; Pham and Kourlas, 2007). These compounds are extractable in hot water and ethanol. A hot water extraction is relatively efficient at removing

the water-soluble compounds considered most desirable for blood glucose control, while excluding most lipid-soluble compounds.

Cinnamon contains both singly- and doubly-linked procyanidins (Lazarus and others 1999). Anderson et al. (2004) tested extracts of cinnamon, and they found insulin-like activity from aqueous extracts when evaluated using *in vitro* epididymal fat cells. In purified extracts, only doubly-linked procyanidin type-A polymers were found to exhibit insulin-like activity (Anderson et al., 2004). These compounds function by initiating phosphorylation of insulin receptor kinase, which causes increased glucose uptake by adipocytes. Glycogen synthesis may also increase with the water-soluble compounds. *In vitro* testing showed synergistic effects on glucose and glycogen production, which occurred when the purported MHCP and insulin were both present (Pham and Kourlas, 2007). Cinnamon also improves insulin receptor activity by activating insulin receptor PI 3-kinase, while inhibiting tyrosine phosphatases (Hlebowicz and others 2007). Cinnamon may also improve insulin receptor activity by increasing the concentration of phosphorylated protein IRS-1 and increasing the binding of PI 3-kinase (Hlebowicz et al., 2007).

1.8 Tea

Tea is a popular beverage consumed worldwide. It is derived from the dried leaves of the tea plant (*Camellia sinensis*). There are approximately 3 million metric tons of dried tea produced annually (Rio and others 2004), and green tea comprises about 20% of this amount (Rio et al., 2004; Lee and Ong, 2000). Green tea is widely consumed in China, Japan, Korea and Morocco, whereas black tea is consumed mostly in Europe, North America and North Africa. Oolong tea is considered a partially-fermented tea, and is popular in China and Taiwan

18

(Cabrera and others 2006). Tea leaves are grown predominantly in India, China, Kenya, and Sri Lanka (Committee, 1999; Rijken and others 2001).

Tea leaves typically contain: proteins (15-25%, dw), carbohydrates (45%, dw), linoleic and α-linolenic fatty acids, sterols and other lipids (5%, dw), vitamins B, C and E, xanthines such as caffeine (2%, dw) and theopylline, chlorophyll and carotenoids, volatiles such as aldehydes and lactones, minerals and trace elements (5%, dw), vitamins A and B (0.02%, dw), and vitamin C (0.25%, dw) (Cabrera et al., 2006; Rao and others 2003). In addition, tea also provides high concentrations of polyphenols (13%, dw) (Rao et al., 2003), which mainly consist of flavonoids that can comprise up to 30% (dw) of the water soluble material extracted from the tea leaf (Rijken et al., 2001; Lu and others 2003). The flavonoids contribute not only flavor, astringency (Thorngate and Noble, 1995), and bitterness (Cabrera et al., 2006), but are also associated with numerous health benefits stemming from their antioxidant capacity. Polyphenols also provide functional utility in food product formulations, such as how tea catechins can exhibit protective attributes for retaining color produced from β-carotene in beverages (Unten and others 1997).

Flavonoids are considered polyphenolics. There are different subclasses of flavonoids, which include flavanols (from tea, red grapes and red wine), flavanones (citrus), flavones (green leafy herbs), isoflavones (soybeans, legumes), flavonols (many foods), and anthocyanidins (blue, red, and purple berries) (Beecher, 2003). Flavan-3-ols are the predominant class of flavonoids in tea. Catechins as monomeric flavan-3-ols comprise 16-30% (dw) of non-fermented green tea, whereas fermented black tea contains only 3-10% catechins (dw) (Graham, 1992) and 4% theaflavins (Lee and others 2000). Oolong tea is considered an intermediate-fermented tea and thus contains 8-20% catechins (Graham, 1992).

Tea catechin concentrations vary extensively according to tea variety, age, leaf composition, cultivation practices, compound type, analysis method, storage conditions (Khokhar and Magnusdottir, 2002), and brewing (extraction) time. Green tea is composed predominantly of monomeric catechins (flavan-3-ols) such as (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), and (-)-epicatechin gallate (ECG) (Unno and others 2005). After thermal treatment, the catechins are converted by about one-half to their corresponding epimers of (-)-gallocatechin (GC), (-)-catechin (C), (-)-catechin gallate (CG), and (-)-gallocatechin gallate (GCG) (Wang and Helliwell, 2000; Ito and others 2003; Suzuki and others 2003), which comprise about 2-8% (dw) of green tea leaves (Friedman and others 2005; Neilson and others 2006). The compounds follow a diphenylpropane structure ($C_6C_3C_6$). The green tea polyphenols exhibit antibacterial, antiviral and antioxidant properties (Rao et al., 2003).

Depending on the extent of fermentation, the tea production process requires several processing stages including withering, rolling, and drying. Green tea is produced by rolling and steaming young tea leaves to minimize oxidation, while black tea leaves are rolled extensively to expose the polyphenols. The tea is then allowed to ferment by oxidizing the polyphenols for 90-120 min before being subsequently blanched and dried (Rio et al., 2004). During fermentation of black tea, the flavan-3-ols are oxidized to theaflavins and polymerization products such as thearubigins. Of the flavonoids, green tea is composed of about 77% flavan-3-ols, whereas black tea contains only about 3% of flavan-3-ols, but consists of mostly oxidation products (Rio et al., 2004). Other components in green tea include gallic acid, quinic esters of gallic, coumaric, and caffeic acids, and methyl xanthines which are purine alkaloids. In tea, these include theobromine, caffeine, and theophylline. In black tea, the flavonols that are oxidized or polymerized can form thearubigins, which have molecular weights between 1,000 and 40,000 Da

(Lee et al., 2000). Theaflavins also are found in tea and are orange-red in color, contain a benzotropolone group, and contribute to the brisk taste of tea (Wan and others 1997).

1.9 Nutritional Significance of Green Tea

Polyphenolic compounds are bioactive compounds that have antioxidant properties. Humans consume many polyphenols through ingestion of plant products, many of which are derived from fruits, vegetables, and grains. The polyphenols are produced as secondary stress metabolites from the plants and serve as colorants, insect attractants or repellants, or defense against herbivores (Nichenametla and others 2006). An antioxidant functions either by preventing reactive oxygen species (ROS) from forming or by quenching them before they oxidize other substances (Close and Hagerman, 2006), thus helping to prevent or minimize the accumulation of oxidative damage in the body. Prior et al. (2005) described how in humans, antioxidants are enzymes, macromolecular proteins, small molecules (glutathione or organic acids), or hormones such as phytoestrogens. These compounds work directly or indirectly in activating *in vivo* defense pathways in counteracting many different sources of free radicals or oxidants.

Various *in vitro* antioxidant assays are available that measure the ability of an antioxidant to quench oxidation induced by a free radical or oxidant. Multiple assays are often needed to analyze one type of antioxidant, since it may exert better action toward one type of free radical over another. When evaluating green tea catechins, researchers have used the Folin-Ciocalteu method for measuring total polyphenolic content (Aoshima and others 2007; Shishikura and Khokhar, 2005). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay measures the reducing power of catechins toward the organic DPPH nitrogen radical (Aoshima et al., 2007;

Gil and others 2000). The ABTS$^{\cdot+}$ test (TEAC) involves the oxidation of ABTS by peroxyl radicals or others to form ABTS$^{\cdot+}$ (Gil et al., 2000). The ferric reducing antioxidant power (FRAP) method measures the ability of antioxidants in a sample to reduce ferric to ferrous iron (Gil et al., 2000).

In the analysis of the total polyphenolic content of tea infusion determined by the Folin-Ciocalteu method (expressed as gallic acid equivalents – GAE), the total polyphenolic content of black and green teas ranged from 80-135 mg/g and 66-106 mg/g (dw), respectively, of which catechins contributed 50-98% in green tea (Khokhar and Magnusdottir, 2002). Claims should not be made specifying different tea varieties as being good or poor sources of polyphenols, because highly variable polyphenol contents may result from using different tea varieties, extraction techniques (Khokhar and Magnusdottir, 2002), or antioxidant capacity-determining assays.

Consumption of green tea is purported to have numerous positive health consequences. It is associated with a reduced risk of cardiovascular disease through the reduction of total plasma cholesterol levels (Bursill and others 2007). Other health-promoting properties include anti-hypertensive, anti-carcinogenic, anti-bacterial, and anti-fibrotic activities, while helping to maintain bone health and oral health, and exhibiting neuroprotective properties and anti-malarial properties (Sannella and others 2007; Wang and Helliwell, 2000; McKay and Blumberg, 2002; Cabrera et al., 2006). Green tea has also been shown to exhibit antiviral activity, such as in therapy for HIV treatment (Williamson and others 2006). Green tea extract is also useful in minimizing the occurrence of lipid oxidation and color degradation in foods (Tang and others 2006). On the other hand, catechins may also exhibit anti-nutritional factors by potentially

binding with digestive enzymes and some proteins (He et al 2006), and limiting absorption of iron in the gastrointestinal tract (GI) (Wang and Helliwell, 2000).

Green tea catechins are known to interact with some food components in the GI. Catechins can especially bind to divalent cations such as iron, and inhibit its absorption through the epithelium of the intestines (Zijp and others 2000). Catechins are also capable of binding to proteins such as in gelatin, and can inhibit digestive enzyme activity through similar non-covalent interactions (He and others 2006). Polyphenolic compounds are also known to interact with protein molecules, which are capable of forming haze in beverages (Siebert and others 1996).

The fate of catechins in the gut is also dependent on digestive conditions. This can include effects exerted by pH, digestive enzymes, metabolic rate, and fasting or non-fasting states. In the latter condition, other food components ingested with green tea catechins can affect the stability of the antioxidant compounds. In preliminary studies conducted by Green et al. (2006), beverage components such as bovine milk, ascorbic acid, and fruit juices were found to increase the digestive stability of catechins, whereas citric acid, metal chelators, and rice and soymilk were found to have negligible effects. Significant differences were even observed between catechin isomers, such as EGCG and ECG. This can have impact on the accuracy of health claims that appear on beverage product labels. As the market for functional beverages increases and as new beverage blends are created such as from fruit and vegetable juice sources, research is needed to elucidate how various beverage components will affect the digestion and absorption of green tea catechins.

Many American consumers consume a wide-array of beverages and foods over the course of a day, and are not likely to consume sufficient green tea products to obtain maximal health benefits. Consuming a large dose of green tea at one time may also not be very beneficial, since catechins are absorbed within 1-2 hours after consumption (Kotani and others 2003) and may have limited permeation through the intestinal mucosa. It would thus be beneficial to make available to consumers a wide variety of food products fortified with green tea catechins. Most importantly, the green tea catechins should be consumed as part of a diet full of antioxidant-rich foods.

1.10 Catechin Stability and Extraction

When extracting catechin flavonoids from tea leaves, an aqueous infusion is usually employed at elevated temperatures. In optimizing tea brewing conditions, extractions conducted at 85ºC and 100ºC for 4 min were found to produce the highest concentrations of catechins (Yang and others 2007). Khokar et al. (2002) found that extraction efficiency of individual catechins, total polyphenols, and caffeine increased with increasing extraction temperatures up to 100°C, and totals increased an additional 30-40% by increasing the extraction time from 5 to 10 minutes. The extraction of green tea at 60°C for 15 min, followed by additional extraction at 85-120°C for 4 minutes resulted in decreases in concentrations of EGCG, EGC, EC, and ECG. On the other hand, C and CG concentrations increased, while total catechin levels remained relatively constant (Kim and others 2007). This indicated that epimerization of catechin compounds may have occurred. Catechins may also have undergone oxidation to form larger molecular weight complexes. This was attributed to the green to yellow color change that occurred in tea during thermal processing (Kim et al., 2007). In a similar extraction optimization

experiment, heating at 80°C for 40 minutes was found to yield the highest levels of catechins while avoiding epimerization, which otherwise occurred at 100°C (Row and Jin, 2006).

In green tea, epimerization of monomeric flavan-3-ols may result in the decrease in contents of original catechin isomers. The process involves the isomerization of the catechin monomer to its non-epimeric form, such as EGCG to GCG. This process may occur if tap water is used instead of purified water, which is due to changes in pH and mineral content. It may also occur if tea extraction conditions are maintained at 40°C for a prolonged time (Wang and Helliwell, 2000). In another study, the catechin monomers EC and ECG epimerized to C and CG, having undergone extraction at 80°C and then storage. Hydrolysis of a gallate moiety may have also occurred, such as from CG to C (Ito et al., 2003).

Catechins show variable stability to beverage attributes and thermal processing conditions. They maintain relative stability, producing 80-90% recovery when stored in a high-acid environment (pH 3-4), whereas increasing pH rapidly can degrade catechin compounds. For example, raising the pH to 6 destroyed 80% of monomeric catechins (Chen and others 2001). Reduction in oxygen-scavenging ability of tea extracts can also occur from thermal treatment, while chain-breaking activity simultaneously increases. This may be indicative of polymerization reactions involving phenolic compounds where oxidation reactions promote the formation of macromolecular brown-colored molecules, which also exhibit significant antioxidant capacity (Manzocco and others 1998). Row et al. (2006) also described a solvent extraction system for developing a decaffeinated and concentrated extract of green tea.

Most conventional methods employed in separating and detecting catechins use high-performance liquid chromatography on a reversed-phase C_{18} column with photodiode array detection (Bronner and Beecher, 1998; Wang and others 2000a; Khokhar and Magnusdottir,

2002; Baranowski and others 2004; Rio et al., 2004; Sharma and others 2005; Benavides and others 2006; Garcia-Marino and others 2006; He et al., 2006; Heard and others 2006; Neilson et al., 2006; Row and Jin, 2006; Kim et al., 2007; Yang et al., 2007) or fluorescence detection (Gurbuz and others 2007; Lee and Ong, 2000). Similar methods were used for analyzing catechins in plasma (Chu and others 2004; Kotani et al., 2003; Masukawa and others 2006) by also using electrochemical detection (Kotani et al., 2003; Unno et al., 2005) or a combination of methods (Lee et al., 2000). Other analytical methods used for quantitating catechins include capillary electrophoresis (Dantuluri and others 2005), gas-chromatography mass-spectrometry (Soleas and others 2001), and micellular electrokinetic chromatography (Gotti and others 2006). Chromatographic methods varied in set wavelength for DAD detection, using 205 nm (Lee and Ong, 2000), 210-215 nm (Benavides et al., 2006; Wang and others 2000b; Bronner and Beecher, 1998; Wang et al., 2000a), 272 nm (Heard et al., 2006), or 278-280 nm for maximum absorbance (Wang et al., 2000b; Garcia-Marino et al., 2006; He et al., 2006; Khokhar and Magnusdottir, 2002; Kim et al., 2007; Neilson et al., 2006; Row and Jin, 2006; Yang et al., 2007). As for green tea flavan-3-ol compounds, apples are also composed of similar flavonoid compounds, and thus can utilize similar analytical separation and quantitation techniques (Boyer and Liu, 2004)**.**

1.11 Nutritional Properties of Apples

        Apples are a popular fruit consumed fresh, as juice, pureed, or as chips. Consumption of apples is associated with anti-carcinogenic and anti-oxidative properties in the body (Gerhauser, 2008), while it may reduce the risk of developing coronary heart disease, diabetes, asthma, and lowering total plasma cholesterol levels (Boyer and Liu, 2004). The phytochemicals present in apples include 70-90% as flavan-3-ols (C, EC, and procyanidins), 4-18% as hydroxycinnamic

acids (e.g. chlorogenic acid, *p*-coumaroylquinic acid), 1-10% as flavonols (e.g. quercetin 3-rhamnoside), 2-6% as dihydrochalcones (e.g. phloridzin) (Vrhovsek and others 2004; Hagen and others 2007; Khanizadeh and others 2008; Oszmianski and others 2008) among all apple varieties, and 1-3% as anthocyanins in the peel of red varieties (Awad and others 2000). Apples are also a significant source of ascorbic acid (Hagen et al., 2007). The ascorbic acid functions as an antioxidant in the xanthophyll cycle in apples, produced in response to exposure to light (Ma and Cheng, 2004). Ascorbic acid synthesis may occur from exposure to visible light, whereas flavonoid synthesis may occur from exposure to UV-B irradiation (Hagen et al., 2007). If an apple is peeled, then 30% of its antioxidant capacity, 100% of quercetin glycosides and anthocyanins, and 85% of ascorbic acid may be removed (Hagen et al., 2007).

The procyanidins in apples contribute color, bitterness, and astringency (Lea, 1990; Khanizadeh et al., 2008). Khanizadeh et al. (2008) analyzed the major phenolic compounds present in apples. Procyanidins comprised 40% of phenolic compounds in the peel and 53% in the flesh. EC and procyanidin B2 were the most abundant of the flavan-3-ol compounds, representing 24% and 19% in the peel, and 12% and 17% in the flesh, respectively. The concentration of hydroxycinnamic acids varied from 10% of total phenolic compounds in the peel to 39% of total phenolic compounds in the flesh. The flavonol contents ranged from 35% in the peel to 2% in the flesh. Dihydrochalcones comprised 7% in the peel and 6% in the flesh. During processing, flavan-3-ol compounds of catechins and procyanidins were affected by oxidation, while levels of caffeoylquinic acid were stable. Hamauzu et al. (2005) found that when comparing extracts of apple phenolics to other fruits, the DPPH radical scavenging activity was higher for fractions containing larger concentrations of oligomeric procyanidins (OPC) than

monomeric flavan-3-ols. This trend was also consistent with pears, where OPC fractions also

had higher DPPH radical scavenging activities than fractions richer in chlorogenic acid.

1.12 Moisture Mobility Properties

Foods such as apples are often multi-component systems that may behave in a manner

similar to polymers. Analysis of the glass transition temperature (T_g) by differential scanning

calorimetry (DSC) may help determine in which state an amorphous food may exist at a certain

temperature. It is often useful to evaluate T_g over a range of moisture contents at different

relative humidities. By determining the moisture content of each sample with respect to a_w, a

moisture sorption isotherm may be constructed, which may follow a mathematical relationship

such as the Guggeheim-Anderson-de Boer (GAB) model for apple slices (del Valle and others

1998; Sa and others 1999). As heating occurs, the T_g represents the temperature at which the

food or polymer transitions from a non-crystalline glass to a more liquid-like rubber. In multi-

component systems such as fruits, there exists two phases. The water-soluble components such

as sugars and pectins comprise one phase. Another portion consists of components that are

miscible with water but exist at different states of hydration. These two types of component

phases exist in a 12:1 ratio, respectively (Contreras and others 2005). The water-soluble

components determine the T_g (Contreras et al., 2005; Moraga and others 2004).

The T_g of a material is reached when its viscosity is 10^{13}-10^{14} Pa·s, where the rate of

molecular diffusion occurs over several years (Bai and others 2001). The T_g is also dependent

on time, temperature, and moisture (Bai et al., 2001). When a food is stored at a temperature

below its T_g, it is considered stable (Deng and Zhao, 2008), where mobility of constituents

including proteins and sugars is minimized (del Valle et al., 1998). Plasticizers such as water

can depress the T_g (Sa et al., 1999; Contreras et al., 2005). The amount of water present affects the extent of plasticization (Roos, 1995). When food products are lyophilized, the water is rapidly removed and affects the structure of the food, which may cause collapse or shrinkage of the product (del Valle et al., 1998; Cornillon, 2000; Deng and Zhao, 2008).

DSC is useful in investigating the effects of air drying or lyophilization and moisture content on T_g of apple products. In particular, increasing the moisture content significantly decreased the onset glass transition temperature (T_{go}) (Welti-Chanes and others 1999). In a dried apple product, only $a_w = 0$ produced a T_g greater than 25°C (Contreras et al., 2005). A linear relationship plotted between T_{go} and a_w of samples allowed for the correlation of slope and intercept constants with material properties. This can be applied to the Gordon-Taylor equation, with slope k correlating to the predominant sugar content or the water-soluble phase, and the intercept relating to the T_{go} of the material (Bai et al., 2001; Welti-Chanes et al., 1999). The Gordon-Taylor equation can show dependence of T_g on moisture content (Sa et al., 1999). Venir et al. (2007) found that the browning rate of freeze-dried apples was highest at a_w 0.50. At lower moisture contents, the higher viscosity may have limited the extent of diffusion of reactants through their immobilization (Bai et al., 2001). At higher a_w, reactants were more dilute (Loncin and others 1965; White and Bell, 1999). The browning rate can also be evaluated by measuring L*, a*, and b* color. A decrease in L* and an increase in a* provides an indication of brown color formation (Deng and Zhao, 2008).

Another tool useful for analysis of water mobility dynamics involves using low-field ^{1}H nuclear magnetic resonance (NMR) relaxation techniques. Low-field proton NMR is useful because it determines the mobility of water associated with solid phase or liquid phase protons (Ruan and Chen, 2001). NMR is useful for the evaluation of kinetic reactions to determine the

soluble solids or sugar content of a sample, and the state of water present in a sample such as apples (Cornillon, 2000). In food systems, it has been used for the evaluation of water mobility in mozzarella cheese (Kuo and others 2001), wheat starch (Choi and Kerr, 2003), fish (Aursand and others 2008), meat (Venturi and others 2007), and for bread staling (Ruan and Chen, 2001; Van Nieuwenhuijzen and others 2008).

Ruan and Chen (2001) described in detail nuclear magnetic resonance techniques applied to bread staling and moisture mobility. NMR employs a static magnetic field to induce polarized proton nuclei to orient in the same direction as the static field, which exists in a low energy state. Other nuclei will orient in a direction opposite to the static field, and this represents a higher energy state. The nuclei exist in an equilibrium state. When another magnetic field is imposed on the proton nuclei in the form of radiofreqency (RF) waves, this excites protons and increases their energy state. The protons will then return to a lower energy state through relaxation in equivalent proportion to the equilibrium state. As the protons decay, they release energy as RF waves. This relaxation process can be correlated to time constants, which follow an exponential relationship. Relaxation of protons occurs by longitudinal spin-lattice relaxation (T_1) or transverse spin-spin relaxation (T_2). The T_2 relaxation time can then be analyzed using the free induction decay (FID) pulse sequence or the Carr-Purcell-Meiboom-Gill (CPMG) sequence. The FID sequence emits pulses at 90°, and can detect decaying signals almost immediately. The CPMG sequence is useful for detecting proton nuclei that have a longer relaxation time. Multi-component systems such as dried apples will contain proton nuclei that will exist in a variety of environments within the sample; measurement of signal decay will thus represent a total function of decay from multiple environments. The type of signal decay that occurs can also be related to the type or state of polymer or water present in the sample. The FID sequence can provide a

measure of the difference between mobilized and immobile protons (Van Nieuwenhuijzen et al., 2008). Water content and type of free or immobile water may affect chemical reaction rates (Acevedo and others 2006). Water content and mobility thus play a crucial role in determining the physical state, chemical reactivity, and stability of a food product and its components.

REFERENCES

Acevedo N, Schebor C & Buera MP. 2006. Water-solids interactions, matrix structural properties and the rate of non-enzymatic browning. Journal of Food Engineering 77:1108-1115.

ADA. 1995. Position of the American Dietetic Association: phytochemicals and functional foods. Journal of the American Dietetic Association 95(4):493-496.

Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schoene NW & Graves DJ. 2004. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. Journal of Agricultural and Food Chemistry 52:65-70.

Aoshima H, Hirata S & Ayabe S. 2007. Antioxidative and anti-hydrogen peroxide activities of various herbal teas. Food Chemistry 103:617-622.

Aursand IG, Gallart-Jornet L, Erikson U, Axelson DE & Rustad T. 2008. Water distribution in brine salted cod (*Gadus morhua*) and salmon (*Salmo salar*): a low-field H NMR study. Journal of Agricultural and Food Chemistry 56(15):6252-6260.

Awad MA, Jager Ad & Westing LMv. 2000. Flavonoid and chlorogenic acid concentrations in apple fruit: characterisation of variation. Scientia Horticulturae. 83:249-263.

Awika JM & Rooney LW. 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65:1199-1221.

Bai Y, Rahman MS, Perera CO, Smith B & Melton LD. 2001. State diagram of apple slices: glass transition and freezing curves. Food Research International 34:89-95.

Baranowski R, Kabut J & Baranowska I. 2004. Analysis of mixture of catechins, flavones, flavanones, flavonols, and anthocyanidins by RP-HPLC. Analytical Letters 37(1):157-165.

Beecher GR. 2003. Proceedings of the Third International Scientific Symposium on Tea and Human Health: Role of Flavonoids in the Diet. The Journal of Nutrition:3248S-3254S.

Benavides A, Montoro P, Bassarello C, Piacente S & Pizza C. 2006. Catechin derivatives in *Jatropha macrantha* stems: Characterisation and LC/ESI/MS/MS quali-quantitative analysis. Journal of Pharmaceutical and Biomedical Analysis 40:639-647.

Beuchat LR & Cousin MA. 2001. Yeasts and Molds. In: Downes, F. P. & Ito, K., editors. Compendium of Methods for the Microbiological Examination of foods. Fourth ed. Washington, D.C.: American Public Health Association. p. 209-215.

Beuchat LR & Pitt JI. 2001. Detection and Enumeration of Heat-Resistant Molds. In: Downes, F. P. & Ito, K., editors. Compendium of Methods for the Microbiological Examination of Foods. Washington, D.C.: American Public Health Association. p. 217-222.

Boer HD, Keizers R, Jansen M, Verschoor L & Ruineman-Koerts J. 2006. Glycaemic control without weight gain in insulin requiring type 2 diabetes: 1-year results of the GAME regimen. Diabetes, Obesity and Metabolism 8:517-523.

Boyer J & Liu RH. 2004. Apple phytochemicals and their health benefits. Nutrition Journal 3.

Bralley E, Greenspan P, Hargrove JL & Hartle DK. 2008. Inhibition of hyaluronidase activity by select sorghum brans. Journal of Medicinal Food 11(2):307-312.

Bronner WE & Beecher GR. 1998. Method for determining the content of catechins in tea

infusions by high-performance liquid chromatography. Journal of Chromatography A

805:137-142.

Bull MK, Zerdin K, Howe E, Goicoechea D, Paramanandhan P, Stockman R, Sellahewa J, Szabo

EA, Johnson RL & Stewart CM. 2004. The effect of high pressure processing on the

microbial, physical and chemical properties of Valenical and Navel orange juice.

Innovative Food Science & Emerging Technologies 5:1335-1149.

Bursill CA, Abbey M & Roach PD. 2007. A green tea extract lowers plasma cholesterol by

inhibiting cholesterol synthesis and upregulating the LDL receptor in the cholesterol-fed

rabbit. Atherosclerosis 193(1):86-93.

Butz P, Edenharder R, Fister H & Tauscher B. 1997. The influence of high pressure processing

on antimutagenic activities of fruit and vegetable juices. Food Research International

30(3/4):287-291.

Cabrera C, Artacho R & Gimenez R. 2006. Beneficial effects of green tea--A review. Journal of

the American College of Nutrition 25(2):79-99.

Campos FP & Cristianini M. 2007. Inactivation of *Saccharomyces cerevisiae* and *Lactobacillus

plantarum* in orange juice using ultra high-pressure homogenisation. Innovative Food

Science & Emerging Technologies 8:226-229.

Castellari M, Arfelli G, Riponi C, Carpi G & Amati A. 2000. High hydrostatic pressure

treatments for beer stabilization. Journal of Food Science 65(6):974-977.

Charnock C, Finsrud T & Foss C. 2005. Controlling the proliferation of *Zygosaccharomyces

bailii* in ephedrine and anti-phlegm cough mixture. Journal of Clinical Pharmacy and

Therapeutics 30:329-335.

Chen S, Tang Q, Zhang X, Zhao G, Hu X, Liao X, Chen F, Wu J & Xiang H. 2006. Isolation and characterization of thermo-acidophilic endospore-forming bacteria from the concentrated apple juice-processing environment. Food Microbiology 23:439-445.

Chen Z-Y, Zhu QY, Tsang D & Huang Y. 2001. Degradation of green tea catechins in tea drinks. Journal of Agricultural and Food Chemistry 49:477-482.

Choi S-G & Kerr WL. 2003. 1H NMR studies of molecular mobility in wheat starch. Food Research International 36:341-348.

Chu KO, Wang CC, Chu CY, Rogers MS, Choy KW & Pang CP. 2004. Determination of catechins and catechin gallates in tissues by liquid chromatography with coulometric array detection and selective solid phase extraction. Journal of Chromatography B 810:187-195.

Clark JP. 2006. High-Pressure Processing Research Continues. Food Technology 2:63-65.

Close DC & Hagerman AE. 2006. Chemistry of reactive oxygen species and antioxidants. In: Alessio, H. M. & Hagerman, A. E., editors. Oxidative Stress, Exercising and Aging. London: Imperial College Press. p. 171.

Committee IT. 1999. Annual Bulletin of Statistics. Colombo, Sri Lanka: Aitken Spence Printing.

Contreras C, Martin ME, Martinez-Navarrete N & Chiralt A. 2005. Effect of vacuum impregnation and microwave application on structural changes which occurred during air-drying of apple. Lebensmittel-Wissenchaft und-Technologie 38:471-477.

Cornillon P. 2000. Characterization of osmotic dehydrated apple by NMR and DSC. Lebensmittel-Wissenchaft und-Technologie 33:261-267.

Cos P, Bruyne TD, Hermans N, Apers S, Berghe DV & Vlietinck AJ. 2004. Proanthocyanidins in health care: current and new trends. Current Medicinal Chemistry 11(10):1345-1359.

Dantuluri M, Gunnarsson GT, Riaz M, Nguyen H & Desai UR. 2005. Capillary electrophoresis of highly sulfated flavanoids and flavonoids. Analytical Biochemistry 336:316-322.

Dearlove RP, Greenspan P, Hartle DK, Swanson RB & Hargrove JL. 2008. Inhibition of protein glycation by extracts of culinary herbs and spices. Journal of Medicinal Food 11(2):275-281.

Dehnavi RA, Beishuizen ED, Reema VD, Cessie SL, Huisman MV, Kluft C, Princen HMG & Tamsma JT. 2008. The impact of metabolic syndrome and CRP on vascular phenotype in type 2 diabetes mellitus. European Journal of Internal Medicine 19:115-121.

del Valle JM, Cuadros TRM & Aguilera JM. 1998. Glass transitions and shrinkage during drying and storage of osmosed apple pieces. Food Research International 31(3):191-204.

Deng Y & Zhao Y. 2008. Effect of pulsed vacuum and ultrasound osmopretreatments on glass transition temperature, texture, microstructure and calcium penetration of dried apples (Fuji). LWT - Food Science and Technology 41:1575-1585.

Dickinson E & James JD. 1998. Rheology and flocculation of high-pressure-treated *B*-lactoglobulin-stabilized emulsions: comparison with thermal treatment. Journal of Agricultural and Food Chemistry 46:2565-2571.

DiGiacomo R & Gallagher P. 2001. Soft Drinks. In: Downes, F. P. & Ito, K., editors. Compendium of Methods for the Microbiological Examination of Foods. Fourth ed. Washington, D.C.: American Public Health Association. p. 569-571.

Dlamini NR, Taylor JRN & Rooney LW. 2007. The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. Food Chemistry 105:1412-1419.

Drake MA, Harrison SL, Asplund M, Barbosa-Canovas G & Swanson BG. 1997. High pressure treatment of milk and effects on microbiological and sensory quality of cheddar cheese. Journal of Food Science 62(4):843-845, 860.

Earnshaw R. 1996. HIgh pressure food processing. Nutrition & Food Science 2:8-11.

FAOSTAT. 2005. Food and Agricultural Organization of the United Nations.

Farr DR. 1997. Functional foods. Cancer Letters 114:59-63.

Farrar JL, Hartle DK, Hargrove JL & Greenspan P. 2008. A novel nutraceutical property of select sorghum (*Sorghum bicolor*) brans: inhibition of protein glycation. Phytotherapy Research 22:1052-1056.

FDA. 2004. Letter responding to health claim petition dated August 28, 2003: monounsaturated fatty acids from olive oil and coronary heart disease (Docket No 2003Q-0559). In: Nutrition, United States Food and Drug Administration: Center for Food Safety and Applied Nutrition.

Ford ES & Li C. 2008. Metabolic syndrome and health-related quality of life among U.S. adults. Annals of Epidemiology 18(3):165-171.

Friedman M, Kim SY, Lee SJ, Han GP, Han JS, Lee KR & Kozukue N. 2005. Journal of Food Science 70:C550.

Fujita K-I & Kubo I. 2005. Naturally occurring antifungal agents against *Zygosaccharomyces bailii* and their synergism. Journal of Agricultural and Food Chemistry 53:5187-5191.

Garcia-Marino M, Rivas-Gonzalo JC, Ibanez E & Garcia-Moreno C. 2006. Recovery of catechins and proanthocyanidins from winery by-products using subcritical water extraction. Analytica Chimica Acta 563:44-50.

Gerhauser C. 2008. Cancer chemopreventive potential of apples, apple juice, and apple components. Planta Medica 74(13):1608-1624.

Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM & Kader AA. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry 48(10):4581-4569.

Gliemmo MF, Campos CA & Gerschenson LN. 2006. Effect of several humectants and potassium sorbate on the growth of *Zygosaccharomyces bailii* in model aqueous systems resembling low sugar products. Journal of Food Engineering 77:761-770.

Gotti R, Furlanetto S, Pinzauti S & Cavrini V. 2006. Analysis of catechins in *Theobroma cacao* beans by cyclodextrin-modified micellar electrokinetic chromatography. Journal of Chromatography A 1112:345-352.

Graham HN. 1992. Green tea composition, consumption and polyphenol chemistry. Preventive Medicine 21:334-350.

Green RJ & Ferruzzi MG. 2006. Poster: Tea formulation modulates recovery of catechins following simulated digestion. Department of Food Science, Purdue University.

Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S & Prior RL. 2004. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. Journal of Nutrition 134(3):613-617.

Gurbuz O, Gocmen D, Dagdelen F, Gursoy M, Aydin S, Sahin I, Buyukuysal L & Usta M. 2007. Determination of flavan-3-ols and trans-resveratrol in grapes and wine using HPLC with fluorescence detection. Food Chemistry 100:518-525.

Hagen SF, Borge GIA, Bengtsson GB, Bilger W, Berge A, Haffner K & Solhaug KA. 2007. Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica*) Borkh., cv. Aroma. Postharvest Biology and Technology 45:1-10.

Hamauzu Y, Yasui H, Inno T, Kume C & Omanyuda M. 2005. Phenolic profile, antioxidant property, and anti-influenza viral activity of chinese quince (*Pseudocydonia sinensis* Schneid.), Quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. Journal of Agricultural and Food Chemistry 53(4):928-934.

Hatcher WS, Parish ME, Weihe JL, Splittstoesser DF & Woodward BB. 2001. Fruit Beverages. In: Downes, F. P. & Ito, K., editors. Compendium of Methods for the Microbiological Examination of Foods. Fourth ed. Washington, D.C.: American Public Health Association. p. 565-568.

Hays NP, Galassetti PR & Coker RH. 2008. Prevention and treatment of type 2 diabetes: Current role of lifestyle, natural product, and pharmacological interventions. Pharmacology & Therapeutics 118:181-191.

He Q, Lv Y & Yao K. 2006. Effects of tea polyphenols on the activities of alpha-amylase, pepsin, trypsin and lipase. Food Chemistry 101:1178-1182.

Heard CM, Johnson S, Moss G & Thomas CP. 2006. In vitro transdermal delivery of caffeine, theobromine, theophylline and catechin from extract of Guarana, *Paullinia Cupana*. International Journal of Pharmaceutics 317:26-31.

Henry CJK & Heppell N. 2002. Nutritional losses and gains during processing: future problems and issues. Proceedings of the Nutrition Society 61:145-148.

Hlebowicz J, Darwiche G, Bjorgell O & Almer L-O. 2007. Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects. American Journal of Clinical Nutrition 85:1552-1556.

Ito R, Yamamoto A, Kodama S, Kato K, Yoshimura Y, Matsunaga A & Nakazawa H. 2003. A study on the change of enantiomeric purity of catechins in green tea infusion. Food Chemistry 83:563-568.

Juan B, Trujillo AJ, Guamis V, Buffa M & Ferragut V. 2007. Rheological, textural and sensory characteristics of high-pressure treated semi-hard ewes' milk cheese. International Dairy Journal 17:248-254.

Khanizadeh S, Tsao R, Rekika D, Yang R, Charles MT & Rupasinghe HPV. 2008. Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. Journal of Food Composition and Analysis 21:396-401.

Khokhar S & Magnusdottir SGM. 2002. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. Journal of Agricultural and Food Chemistry 50:565-570.

Kim ES, Liang YR, Jin J, Sun QF, Lu JL, Du YY & Lin C. 2007. Impact of heating on chemical compositions of green tea liquor. Food Chemistry 103:1263-1267.

Kleef Ev, Trijp HCMv, Luning P & Jongen WMF. 2002. Consumer-oriented functional food development: how well do functional disciplines reflect the 'voice of the consumer'? Trends in Food Science & Technology 13:93-101.

Kotani A, Miyashita N & Kusu F. 2003. Determination of catechins in human plasma after commercial canned green tea ingestion by high-performance liquid chromatography with

electrochemical detection using a microbore column. Journal of Chromatography B 788:269-275.

Krebbers B, Matser AM, Hoogerwerf SW, Moezelaar R, Tomassen MMM & van den Berg RW. 2003. Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. Innovative Food Science and Emerging Technologies 4:377-385.

Kuo M-I, Gunasekaran S, Johnson M & Chen C. 2001. Nuclear magnetic resonance study of water mobility in pasta filata and non-pasta filata mozzarella. Journal of Dairy Science 84:1950-1958.

Lakshmanan R, Parkinson JA & Piggott JR. 2007. High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). Lebensmittel-Wissenchaft und-Technologie 40:544-551.

Lazarus SA, Adamson GE, Hammerstone JF & Schmitz HH. 1999. High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. Journal of Agricultural and Food Chemistry 47:3693-3701.

Lea AGH. 1990. Bitterness and astringency: the procyanidins of fermented apples cider. In: Roussef, R. L., editor). Bitterness in foods and beverages. Oxford, UK: Elsevier. p. 123-143.

Lee B-L & Ong C-N. 2000. Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis. Journal of Chromatography A 881:439-447.

Lee M-J, Prabhu S, Meng X, Li C & Yang CS. 2000. An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. Analytical Biochemistry 279:164-169.

Loncin M, Jackmain D, Provost AT, Lenges J & Bimbenet J. 1965. Influence de l'eau sur les reactions de Maillard. Critical Reviews of the Academy of Science 206:3208-3211.

Lopandic K, Zelger S, Banszky LK, Eliskases-Lechner F & Prillinger H. 2006. Identification of yeasts associated with milk products using traditional and molecular techniques. Food Microbiology 23:341-350.

Lu H, Meng X, Lee M-J, Li C, Maliakal P & Yang CS. 2003. Bioavailability and biological activity of tea polyphenols. In: Shahidi, F., Ho, C.-T., Watanabe, S. & Osawa, T., editors. Food Factors in Health Promotion and Disease Prevention. Washington, D.C.: American Chemical Society. p. 9-15.

Luca CD & Olefsky JM. 2008. Inflammation and insulin resistance. FEBS Letters 582:97-105.

Ma F & Cheng L. 2004. Exposure of the shaded side of apple fruit to full sun leads to up-regulation of both the xanthophyll cycle and the ascorbate-glutathione cycle. Plant Science 166:1479-1486.

Manzocco L, Anese M & Nicoli MC. 1998. Antioxidant properties of tea extracts as affected by processing. Lebensmittel-Wissenschaft und-Technologie 31(7-8):694-698.

Marongiu B, Piras A, Porcedda S, Tuveri E, Sanjust E, Meli M, Sollai F, Zucca P & Rescigno A. 2007. Supercritical CO_2 extract of *Cinnamomum zeylanicum*: chemical characterization and antityrosinase activity. Journal of Agricultural and Food Chemistry 55:10022-10027.

Masukawa Y, Matsui Y, Shimizu N, Kondou N, Endou H, Kuzukawa M & Hase T. 2006. Determination of green tea catechins in human plasma using liquid chromatography-electrospray ionization mass spectrometry. Journal of Chromatography B 834:26-34.

Matsuda H, Wang T, Managi H & Yoshikawa M. 2003. Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. Bioorganic & Medicinal Chemistry 11:5317-5323.

McGrane MM. 2006. Carbohydrate metabolism: synthesis and oxidation. In: Stipanuk, M. H., editor). Biochemical, physiological, and molecular aspects of human nutrition. United States: Saunders Elsevier. p. 257-318.

McKay DL & Blumberg JB. 2002. The role of tea in human health: an update. Journal of the American College of Nutrition 21(1):1-13.

McPherson JD, Shilton BH & Walton DJ. 1988. Role of fructose in glycation and cross-linking of proteins. Biochemistry 27:1901-1907.

Moraga G, Martinez-Navarrete N & Chiralt A. 2004. Water sorption isotherms and glass transition in strawberries: Influence of pretreatment. Journal of Food Engineering 62:315-321.

Mussa DM & Ramaswamy HS. 1997. Ultra high pressure pasteurization of milk: Kinetics of microbial destruction and changes in physico-chemical characteristics. Lebensmittel-Wissenchaft und-Technologie 30:551-557.

Nandakumar V, Singh T & Katiyar SK. 2008. Multi-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Letters 269:378-387.

Neilson AP, Green RJ, Wood KV & Ferruzzi MG. 2006. High-throughput analysis of catechins and theaflavins by high performance liquid chromatography with diode array detection. Journal of Chromatography A 1132:132-140.

Nichenametla SN, Taruscio TG, Barney DL & Exon JH. 2006. A review of the effects and mechanisms of polyphenolics in cancer. Critical Reviews in Food Science and Nutrition 46(2):161-183.

Nielsen MK & Arneborg N. 2007. The effect of citric acid and pH on growth and metabolism of anaerobic *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* cultures. Food Microbiology 24:101-105.

Nussinovitch A & Rosen B. 1989. Cloud destruction in aseptically filled citrus juice. Lebensmittel-Wissenschaft und-Technologie 22:60-64.

Oszmianski J, Wolniak M, Wojdylo A & Wawer I. 2008. Influence of apple puree preparation and storage on polyphenol contents and antioxidant activity. Food Chemistry 107:1473-1484.

Palou E, Lopez-Malo A, Barbosa-Canovas GV, Welti-Chanes J & Swanson BG. 1997. Kinetic analysis of *Zygosaccharomyces bailii* inactivation by high hydrostatic pressure. Lebensmittel-Wissenchaft und-Technologie 30:703-708.

Pascal G. 1996. Functional foods in the european union. Nutrition Reviews 54(suppl):S29-32.

Perrier-Cornet J-M, Tapin S, Gaeta S & Gervais P. 2005. High-pressure inactivation of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* at subzero temperatures. Journal of Biotechnology 115:405-412.

Peyroux J & Sternberg M. 2006. Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. Pathologie Biologie 54:405-419.

Pham AQ & Kourlas H. 2007. Cinnamon supplementation in patients with type 2 diabetes mellitus. Pharmacotherapy 27(4):595-599.

Piper P, Calderon CO, Hatzixanthis K & Mollapour M. 2001. Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. Microbiology 147:2635-2642.

Polydera AC, Stoforos NG & Taoukis PS. 2005. Quality degradation kinetics of pasteurised and high pressure processed fresh Navel orange juice: Nutritional parameters and shelf life. Innovative Food Science and Emerging Technologies 6:1-9.

Prior RL, Wu X & Schaich K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53:4290-4302.

Puel O, Tadrist S, Delaforge M, Oswald IP & Lebrihi A. 2007. The inability of *Byssochlamys fulva* to produce patulin is related to absence of 6-methylsalicylic acid synthase and isoepoxydon dehydrogenase genes. International Journal of Food Microbiology 115(2):131-139.

Pupillo M, Berardis GD, Antenucci D, Minnucci A & Nicolucci A. 2008. Glycated haemoglobin or mean blood glucose as indicators of metabolic control in Type 2 diabetes? Diabetes Research and Clinical Practice 80:e1-e3.

Rao TP, Okubo T, Chu D-C & Juneja LR. 2003. Pharmacological functions of green tea polyphenols. In: Watson, D. H., editor). Performance functional foods. Boca Raton: CRC Press. p. 200.

Raso J, Calderon ML, Gongora M, Barbosa-Canovas GV & Swanson BG. 1998. Inactivation of *Zygosaccharomyces bailii* in fruits juices by heat, high hydrostatic pressure and pulsed electric fields. Journal of Food Science 63(1):1042-1044.

Reyns KMFA, Soontjens CCF, Cornelis K, Weemaes CA, Hendricks ME & Michiels CW. 2000. Kinetic analysis and modeling of combined high-pressure-temperature inactivation of the yeast *Zygosaccharomyces bailii*. International Journal of Food Microbiology 56:199-210.

Rijken PJ, Wiseman SA, Weisgerber UM, Van Mierlo CAJ, Quinlan PT & Van de Put F. 2001. Ch. 19. Antioxidant and other properties of green and black tea. In: Wildman, E. C., editor). Handbook of nutraceuticals and functional foods. Boca Raton: CRC Press. p. 542.

Rio DD, Stewart AJ, Mullen W, Burns J, Lean MEJ, Brighenti F & Crozier A. 2004. HPLC-MS analysis of phenolic compounds and purine alkaloids in green and black tea. Journal of Agricultural and Food Chemistry 52:2807-2815.

Roberfroid MB. 2000. Concepts and strategy of functional food science: the European perspective. American Journal of Clinical Nutrition 71(suppl):1660S-1664S.

Roos YH. 1995. Phase transitions in foods. San Diego: Academic Press.

Ross S. 2000. Functional foods: the food and drug administration perspective. The American Journal of Clinical Nutrition 71(6):1735S-1738S.

Row KH & Jin Y. 2006. Recovery of catechin compounds from Korean tea by solvent extraction. Bioresource Technology 97:790-793.

Ruan RR & Chen PL. 2001. Nuclear magnetic resonance techniques. In: Chinachoti, P. & Vodovotz, Y., editors. Bread staling. Boca Raton: CRC Press. p. 113-128.

Ruiz-Capillas C, Carballo J & Jimènez-Colmenero F. 2007. Consequences of high-pressure processing of vacuum-packaged frankfurters on the formation of polyamines: effect of chilled storage. Food Chemistry 104:202-208.

Sa MM, Figueiredo AM & Sereno AM. 1999. Glass transitions and state diagrams for fresh and processed apple. Thermochimica Acta 329:31-38.

Sakanaka S, Juneja LR & Taniguchi M. 2000. Antimicrobial effects of green tea polyphenols on thermophilic spore-forming bacteria. Journal of Bioscience and Bioengineering 90(1):81-85.

Sanchez-Moreno C, Plaza L, de Ancos B & Cano MP. 2006. Impact of high-pressure and traditional thermal processing of tomato puree on carotenoids, vitamin C and antioxidant activity. Journal of the Science of Food and Agriculture 86(2):171-179.

Sanchez-Moreno C, Plaza L, Elez-Martinez P, Ancos Bd, Martin-Belloso O & Cano MP. 2005. Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. Journal of Agricultural and Food Chemistry 53:4403-4409.

Sannella AR, Messori L, Casini A, Vincieri FF, Bilia AR, Majori G & Severini C. 2007. Antimalarial properties of green tea. Biochemical and Biophysical Research Communications 353:177-181.

Schmidt BM, Erdman JW & Lila MA. 2005. Effects of food processing on blueberry antiproliferation and antioxidant activity. Journal of Food Science 70(6):S389-S394.

Sharma V, Gulati A, Ravindranath SD & Kumar V. 2005. A simple and convenient method for analysis of tea biochemicals by reverse phase HPLC. Journal of Food Composition and Analysis 18:583-594.

Shishikura Y & Khokhar S. 2005. Factors affecting the levels of catechins and caffeine in tea beverage: estimated daily intakes and antioxidant activity. Journal of the Science of Food and Agriculture 85:2125-2133.

Siebert KJ, Troukhanova NV & Lynn PY. 1996. Nature of polyphenol-protein interactions. Journal of Agricultural and Food Chemistry 44:80-85.

Smelt JPPM. 1998. Recent advances in the microbiology of high pressure processing. Trends in Food Science & Technology 9(4):152-158.

Soleas GJ, Yan J & Goldberg DM. 2001. Ultrasensitive assay for three polyphenols (catechin, quercetin and resveratrol) and their conjugates in biological fluids utilizing gas chromatography with mass selective detection. Journal of Chromatography B 757(1):161-172.

Spotts RA, Serdani M, Mielke EA, Bai J, Chen PM, Hansen JD, Neven LG & Sanderson PG. 2006. Effect of high-pressure hot water washing treatment on fruit quality, insects, and disease in apples and pears. Part II. Effect on postharvest decay of d'Anjou pear fruit. Postharvest Biology and Technology 40:216-220.

Suzuki M, Sano M, Yoshida R, Degawa M, Miyase T & Maeda-Yamamoto M. 2003. Epimerization of tea catechins and o-methylated derivatives of (-)-epigallocatechin-3-o-gallate: relationship between epimerization and chemical structure. Journal of Agricultural and Food Chemistry 51:510-514.

Tang SZ, Ou SY, Huang XS, Li W, Kerry JP & Buckley DJ. 2006. Effects of added tea catechins on colour stability and lipid oxidation in minced beef patties held under aerobic and modified atmospheric packaging conditions. Journal of Food Engineering 77:248-253.

Thorngate JH & Noble AC. 1995. Sensory evaluation of bitterness and astringency of 3*R*(-)-epicatechin and 3*S*(+)-catechin. Journal of the Science of Food and Agriculture 67:531-535.

Toledo RT & Moorman JE, inventors; Official Gazette of the United States Patent and Trademark Office Patents, assignee. 2000. Microbial inactivation by high-pressure throttling. United States patent US 6120732.

Unno T, Sagesaka YM & Kakuda T. 2005. Analysis of tea catechins in human plasma by high-performance liquid chromatography with solid-phase extraction. Journal of Agricultural and Food Chemistry 53:9885-9889.

Unten L, Koketsu M & Kim M. 1997. Antidiscoloring activity of green tea polyphenols on beta-carotene. Journal of Agricultural and Food Chemistry 45(6):2009-2012.

USDA. 2009. United States Department of Agriculture. USDA database for the proanthocyanidin content of selected foods.

Van Nieuwenhuijzen NH, Primo-Martin C, Meinders MBJ, Tromp RH, Hamer RJ & Vliet TV. 2008. Water content or water activity: What rules crispy behavior in bread crust? Journal of Agricultural and Food Chemistry 56(15):6432-6438.

Venir E, Munari M, Tonizzo A & Maltini E. 2007. Structure related changes during moistening of freeze dried apple tissue. Journal of Food Engineering 81:27-32.

Venturi L, Rocculi P, Cavani C, Placucci G, Rosa MD & Cremonini MA. 2007. Water absorption of freeze-dried meat at different water activities: a mutlianalytical approach using sorption isotherm, differential scanning calorimetry, and nuclear magnetic resonance. Journal of Agricultural and Food Chemistry 55(26):10572-10578.

Verhagen H, Coolen S, Duchateau G, Hamer M, Kyle J & Rechner A. 2004. Assessment of the efficacy of functional food ingredients -- introducing the concept "kinetics of biomarkers". Mutation Research 551:65-78.

Vrhovsek U, Rigo A, Tonon D & Mattivi F. 2004. Quantitation of polyphenols in different apple varieties. Journal of Agricultural and Food Chemistry 52:6532-6538.

Wan X, Nursten HE, Cai Y, Davies AL, Wilkins JPG & Davies AP. 1997. Journal of the Science of Food and Agriculture 74:401-408.

Wang H & Helliwell K. 2000. Epimerisation of catechins in green tea infusions. Food Chemistry 70:337-344.

Wang H, Helliwell K & You X. 2000a. Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. Food Chemistry 68:115-121.

Wang L-F, Kim D-M & Lee CY. 2000b. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. Journal of Agricultural and Food Chemistry 48(9):4227-4232.

Welti-Chanes J, Guerrero JA, Barcenas ME, Aguilera JM, Vergara F & Barbosa-Canovas GV. 1999. Glass transition temperature (T_g) and water activity (a_w) of dehydrated apple products. Journal of Food Process Engineering 22:91-101.

White K & Bell L. 1999. Glucose loss and Maillard browning in solids as affected by porosity and collapse. Journal of Food Science 64:1010-1014.

Wildman REC. 2001. Nutraceuticals: A brief review of historical and teleological aspects. In: Wildman, R. E. C., editor). Handbook of nutraceuticals and functional foods. Boca Raton: CRC Press. p. 1-12.

Williamson MP, McCormick TG, Nance CL & Shearer WT. 2006. Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: Potential for HIV-1 therapy. Journal of Allergy and Clinical Immunology 118(6):1369-1374.

Yang D-J, Hwang LS & Lin J-T. 2007. Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. Journal of Chromatography A 1156:312-320.

Yen G-C & Lin H-T. 1999. Changes in volatile flavor components of guava juice with high-pressure treatment and heat processing and during storage. Journal of Agricultural and Food Chemistry 47:2082-2087.

Zijp IM, Korver O & Tijburg LBM. 2000. Effect of tea and other dietary factors on iron absorption. Critical Reviews in Food Science and Nutrition 40(5):371-398.

Zook CD, Parish ME, Braddock RJ & Balaban MO. 1999. High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juices. Journal of Food Science 64(3):533-535.

CHAPTER 2. INACTIVATION OF *ZYGOSACCHAROMYCES BAILII* IN APPLE JUICE BY CONTINUOUS-FLOW HIGH-PRESSURE THROTTLING

ABSTRACT

The purpose of this study was to evaluate continuous-flow high-pressure throttling (CFHPT) as an alternative processing technique to conventional hot-filling in reducing populations of *Zygosaccharomyces bailii* in apple juice. In experimentation, log-7 concentrations of washed, pelletized vegetative cells of *Z. bailii* were inoculated into calcium-fortified apple juice and processed by a conventional hot-fill method (90°C for 2 min holding time), or by CFHPT operated at 140 MPa (CFHPT:140) or 280 MPa (CFHPT:280). Samples were stored at 25°C for up to 14 d. Samples were serially diluted and spread plated on acidified YM agar (pH 4.0). The CFHPT process produced a 2-log reduction for CFHPT:140 and a 7-log reduction for CFHPT:280. After 3 d of non-refrigerated storage, populations increased to log-7 concentrations for all pressure-treated samples. This experiment demonstrated the CFHPT process in reducing populations of vegetative cells of *Z. bailii* in fruit juice processing.

Keywords: high pressure processing; *Zygosaccharomyces bailii*; apple juice; yeast

Industrial relevance: Continuous-flow high-pressure throttling, a non-thermal processing technique, is important to industry as a means to reduce populations of spoilage microorganisms, impart homogenization to liquids, and operate in a continuous processing stream.

INTRODUCTION

High-pressure processing (HPP) serves as an alternative processing-method to traditional hot-fill methods in beverage manufacture. High hydrostatic pressure methods have been used to process beer (Castellari, Arfelli, Riponi, Carpi & Amati, 2000), milk (Drake, Harrison, Asplund, Barbosa-Canovas & Swanson, 1997; Mussa & Ramaswamy, 1997), and emulsions (Dickinson & James, 1998). HPP helps prevent the occurrence of adverse compositional product attributes often associated with thermal processing. This occurs through either preserving the fresh character of raw food product by preventing formation of heat-induced colors or flavors, or by protecting thermally-labile compounds including volatile flavors, vitamins, and antioxidants (Schmidt, Erdman & Lila, 2005).

In HPP, pressures of 100-1,000 MPa (Clark, 2006) are typically created and maintained, typically for less than 40 min. This may be combined with moderate heating to temperatures of 20-95ºC. HPP systems operate using hydrostatic or throttling pressure. Most scientific literature has reported on high pressure treatment of food products using hydrostatic pressure. This is a batch process, and the food is normally pre-packaged in flexible or semi-rigid bags or plastic containers and placed into a sealed chamber that is then pressurized (Clark, 2006). In a continuous-flow high-pressure throttling system (CFHPT), a continuous stream of liquid is compressed through a throttling valve, similar to a homogenizer (Figure 2.1). High-pressure throttling has been used to process a blueberry-whey beverage (Peck, 2004), milk (Adapa, Schmidt & Toledo, 1997; Sivanandan, Toledo & Singh, 2008), or citrus juice (Amornsin, 1999). Some heat is generated in liquids among both types of HPP as a result of adiabatic heating (on the order of 3ºC for every 100 MPa) (Butz, Edenharder, Fister & Tauscher, 1997). This occurs as a result of the compression of the food and is a function of its thermal and physical properties.

For example, air found in a food matrix can compress significantly and contribute to temperature rise (Earnshaw, 1996). Some high-pressure units can also regulate temperature, and thus can combine pressure, temperature, and holding time to exert processing effects. The CFHPT system is especially suited for beverage production. It allows for easier conversion to a continuous process as pre-packaging of materials is not necessary and the unit can be connected with fittings for pumping product. Having the ease of pumping liquid product in between the processing unit and holding tanks is especially desirable for industrial processors.

Ready-to-drink (RTD) beverages composed of fruit juices typically range in pH from 2.4-4.2 (Hatcher, Parish, Weihe, Splittstoesser & Woodward, 2001). Spoilage in RTD beverages may occur from aciduric bacteria, yeasts, or molds that are often ubiquitous to the surface skin or peel of the fruit (Spotts et al, 2006). *Zygosaccharomyces bailii* is an osmotolerant, spoilage yeast that is able to grow in acidic conditions pH 2.5-6.0 and environments high in sugars (Fujita & Kubo, 2005), such as cough syrup (Charnock, Finsrud & Foss, 2005) or fruit and vegetable juices (Raso, Calderon, Gongora, Barbosa-Canovas & Swanson, 1998). *Z. bailii* spoilage may produce off-odors, off-tastes, sedimentation and increased pressure on packaging (DiGiacomo & Gallagher, 2001). It is also capable of forming haploid ascospores that are generally more resistant to processing variables of pressure, heat, and holding time than vegetative cells (Raso et al, 1998). High-pressure processing has been used to evaluate inactivation kinetics of *Z. bailii* using a hydrostatic system (Raso et al, 1998; Reyns, Soontjens, Cornelis, Weemaes, Hendrickx & Michiels, 2000). During high pressure processing, dissolved solutes may exert baroprotective properties on spoilage yeasts (Goh, Hocking, Stewart, Buckle & Fleet, 2007).

The purpose of this study was to evaluate the effectiveness of continuous-flow high-pressure throttling compared to conventional hot-fill processing in reducing populations of vegetative cells of *Zygosaccharomyces bailii* in apple juice, which was evaluated over abusive shelf-life conditions. As industrial convention is to process fresh-pressed juice immediately, this study focused primarily on demonstration of processing efficacy of CFHPT on populations of *Z. bailii*.

MATERIALS AND METHODS

2.1 Materials

Frozen 100% apple juice concentrate fortified with calcium (Kroger Co., Cincinnati, OH) was purchased from a local supermarket and diluted to single strength volume with deionized water according to package instructions. The yeast used was *Zygosaccharomyces bailii* FR-1299 donated by Dr. Larry Beuchat (Department of Food Science & Technology, University of Georgia, Griffin, Georgia, United States). Stock cultures were maintained at 4°C on acidified YM agar (41 g powder·L^{-1}: 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g dextrose, 20.0 g agar; acidified to pH 4.0±0.1 with 10% filter sterilized tartaric acid) (Teknova, Hollister, CA, United States) or at -80°C as a frozen suspension in Microbank® cryopreservative capsules (Pro-Bank Diagnostics, Richmond Hill, Ontario, Canada).

2.2 Preparation of inoculum

For inoculation cultures, YM broth (21 g powder·L^{-1}: 3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g dextrose) (Difco Laboratories, Detroit, MI, United States) was used. A 9 mL tube of YM broth was inoculated with cells isolated from YM agar streak plates and incubated at 25°C for 24 h. A 0.1 mL aliquot was then transferred to inoculate another YM

broth tube that was also incubated at 25°C for 24 h. The procedure was repeated three times

prior to the inoculation experiments.

For growth of cells, a 0.1 mL aliquot of inoculated YM broth was added to 500-mL flasks

of sterile apple juice. An equal volume of apple juice was inoculated for pelletization as was

subsequently used for processing by CFHPT. The inoculated apple juice was then incubated for

24 h at 25°C with agitation (120 rpm). Juice was then centrifuged at 3,500 rpm for 20 min

(Forma Scientific, Inc.,Waltham, MA, United States). Cells were washed three times with 250

mL aliquots of 0.1% peptone broth (BBL/Difco, Spark, MD, United States) and pelletized. Cells

were then re-suspended in 50 mL of 0.1% peptone broth and maintained at 4°C overnight until

processed.

2.3 Continuous-Flow High-Pressure Throttling

Apple juice (4°C) was used for the processing medium. Just prior to processing, the

cultures stored overnight were added to apple juice and agitated to suspend the cultures.

Inoculated juice was then immediately processed by continuous-flow high-pressure throttling in

a stainless steel Microfluidizer Processor M-140K (Microfluidics Corp., Newton, MA, United

States) that was fitted with a 7.5 horsepower electric-hydraulic module, double-acting intensifier

pump, product flow interaction chamber for development of shear forces, cold-water jacketed

heat exchanger for processed product, 2 L pressurized feed reservoir, and retrofitted with a

proprietary external split-flow throttling valve that induced homogenization and instantaneous

pressure drop (Figure 2.1). Inoculated juice was processed at pressures of 280-310 MPa and

140-170 MPa (at a flow rate of 100 mL/min). Product was filled into pre-sterilized screw-capped

50-mL glass test tubes, leaving a headspace of about 3 mL in each tube.

Hot-fill samples were processed by passing inoculated apple juice heated to 90°C with a flow rate of 250 mL·min⁻¹ through a stainless steel heat exchanger immersed in boiling water, which was contained within a steam-jacketed kettle. Samples were filled into tubes as for CFHPT, held for 5 minutes, and then immediately cooled in an ice slurry until reaching 4°C. These samples served as a negative control. Inoculated but unprocessed juice was also filled into tubes and sealed and stored with other samples. These served as a positive control.

All tubes were then stored in duplicate in racks at $25° \pm 0.5°C$ in incubators (Model HEC10R, HotPack, Warmisnster, PA, United States) and held for 0, 3, 7, and 14 d of storage. The entire processing procedure was replicated on three separate days.

The pH of reconstituted apple juice was measured using pH meter model AR15 (Accumet, Fisher Scientific, Pittsburg, PA, United States).

2.4 Analysis of Growth

At either 0, 3, 7, or 14 d of storage, populations of *Z. bailii* were enumerated. Tubes were vortexed to suspend any pelletized material, and then 1 mL samples were pipetted and serially diluted in test tubes containing 9 mL of 0.1% peptone broth. Dilutions were plated using an Autoplate 4000 spiral plating system (Spiral Biotech, Inc., Norwood, MA, United States) on acidified YM agar plates in duplicate and incubated at 25°C for 3 days. Colonies were counted on a 100 mm counting grid, which was correlated with a population concentration. Populations were reported as CFU·mL⁻¹.

An additional enrichment step was also performed to detect for presence of injured cells, surviving vegetative cells, or ascospores of *Z. bailii* that existed at concentrations below the limit of detection. At the time of sampling, three 0.1 mL aliquots of sample were transferred to sterile YM broth tubes. Tubes were incubated for 24-72 h, and a turbid, cloudy appearance was a

presumptive indication of *Z. bailii* growth from injured cells, surviving vegetative cells, or ascospores. Turbid tubes were serially diluted with 0.1% peptone broth and spread plated on acidified YM agar to confirm presence of acid-tolerant mold.

2.5 Statistical Analyses

Statistical analysis to compare factors (process method, duration of sample storage, and interaction of process × storage) was conducted using a stripped-split plot design on SAS (SAS Institute, Inc., Cary, NC). To compare treatment means within level of processing method (i.e., unprocessed sample, hot-fill, CFHPT:140, and CFHPT:280) over storage time (in days), a One-Way ANOVA was conducted followed by the Tukey's Honestly Significant Difference Test (α = 0.05) on Minitab software (Minitab, Inc., State College, PA).

RESULTS

This study compared factors of processing, storage time, and experimental trials using a stripped-split plot design. This statistical design allowed for analysis of significance by ANOVA for processing, storage time, and interaction of processing × storage time. Analysis showed that processing, storage time, and interaction of these two were significant (α = 0.01).

Initial inoculation loads of pelletized, washed cells were at least log 7 concentration when analyzed over three separate trials (Table 2.1). In preliminary research, several commercial apple juices were inoculated and consistently supported growth of *Z. bailii* to log 7 levels. Growth curves were also initially established to determine log phase of growth of vegetative cells, which was used for inoculation experiments (data not shown).

The initial inoculation loads of *Z. bailii* increased only modestly over storage in the unprocessed sample (log 7.21 to log 7.56), although means were significantly different ($\alpha = 0.05$) (Table 2.1). The hot-fill technique produced concentrations that were not detectable (ND) by the sampling and enumeration technique employed. CFHPT processing at 140 MPa produced about a 2-log reduction. However, concentrations of *Z. bailii* reached log-7 growth after three days of storage. CFHPT processing at 280 MPa produced a 7-log reduction at processing, but was also followed by growth to log 7 by 3 d of storage. Initial reductions in populations of *Z. bailii* processed by CFHPT at the lower (*Z. bailii* reduced to log 5.7 CFU·mL^{-1}) and higher pressure (populations were not detectable) conditions were significantly different compared to growth observed after 3 d of storage. Unprocessed and CFHPT samples processed at 140 MPa and 280 MPa maintained log 7 concentrations from 3 through 14 d of storage, therefore only data through 7 d is shown. Refrigerated storage at 4°C was sufficient to inhibit its growth (data not shown).

After initial reductions in *Z. bailii* by two processing pressures operated by the CFHPT, growth occurred very rapidly within 3 d of product storage when stored at 25°C. As a qualitative enrichment step for evaluating for the survival of injured cells, surviving vegetative cells, or ascospores of *Z. bailii* in apple juice before and after processing, aliquots of samples were also enriched in YM broth. As expected, unprocessed samples produced positive growth (+) through indication of turbidity in the broth. On the other hand, the hot-fill process showed effectiveness at preventing growth of either injured cells or ascospores initially and throughout storage (Table 2.2), as indicated by the absence of turbidity. Whereas the CFHPT:140 treatment produced growth that corresponded with results observed with the enumeration plating technique in time zero samples, the CFHPT:280 treatment did not produce growth on the plating enumeration procedure but produced growth in YM broth tubes. This is an indication that either *Z. bailii*

concentrations existed below the limit of detection, or the plating enumeration technique was not able to detect the presence of injured cells or ascospores. Growth occurred when *Z. bailli* was provided with a more optimal environment in the YM broth. The limit of detection was 20 CFU·mL^{-1} of sample. The turbid YM broth tubes were also serially diluted in 0.1% peptone broth and plated on acidified YM agar. These colonies appeared with similar color and morphology as other colonies in the experiment. The pH of the apple juice was 3.91.

DISCUSSION

Continuous-flow high-pressure throttling demonstrated applicability for processing of batch or continuous liquid processing streams, such as in reducing populations of the spoilage organism *Z. bailii* by 7-logs in apple juice at an operating pressure of 280 MPa. In a typical batch hydrostatic high-pressure processing system, the pressure exerted on microbial cells damages cell membranes, and disrupts enzyme, protein and ribosomal subunit conformations, adversely affecting cell nutrient uptake and waste removal (Kalchayanand et al, 2002; Torres & Velazquez, 2005). Morphological changes in the cell membrane and cell wall may induce instability, producing changes in pH and membrane potential gradient (Kalchayanand, Frethem, Dunne, Sikes & Ray, 2002). Water and acid molecules can become ionized, which may become lethal to microbial cells (Earnshaw, 1996). The minimal rise in temperature due to adiabatic heating is usually not sufficient to induce negative loss of flavor, or nutritional or quality attributes of the product (Moorman, 1997). The limitation of hydrostatic high-pressure processing involves dependence on batch-scale production. This requires that foods are either processed in thick-walled, high-pressure vessels, or pre-packaged into pouches prior to processing.

60

The use of CFHPT is a novel extension of high-pressure processing. It creates moderate pressures (up to 300 MPa), while also inducing a homogenization effect. A typical hydrostatic process uses a high-pressure homogenization valve configured in a split-flow arrangement, which produces back pressure. This CFHPT instrument uses a novel high-pressure needle throttling valve that releases pressure instantly. This creates an additional shear stress that is unique to CFHPT, and which is lacking in HHP (Peck, 2004). In CFHPT, a liquid medium is drawn through pressure inducers and pulled into a dual-action pressure intensifier. Product then passes through the needle valve before entering a cold-water jacketed heat exchanger. This CFHPT instrument is unique from a conventional microfluidics homogenizer in that the interaction chamber was removed and replaced with a throttling valve. As the pressure intensifier pressurizes and expels product from opposing sides of the chamber in an alternating fashion, air ball valves control the pressure differential. At the time between product exit and entry, there is a momentary drop in operating pressure of up to 35 MPa. During processing of CFHPT, the operating pressure was actually 35 MPa higher than target. This additional pressure was used to ensure all product was exposed to target operating pressure during momentary pressure drop. In addition, using a throttling valve in place of an interaction chamber downstream minimized the pressure difference encountered by the product.

In high-pressure processing, operating pressures above 100 MPa are generally detrimental to vegetative cells, whereas spores are resistant to pressures even above 1000 MPa. Some spores will germinate in response to pressure treatment, especially for pressures between 50 and 300 MPa (Earnshaw, 1996). The spores are most resistant to pressure treatment at neutral pH. They can then be destroyed by another subsequent thermal or pressure treatment, or controlled through use of preservatives, controlled-temperature storage, or high-acid pH;

sterilization of product is not possible (Smelt, 1998). This research showed that while there was over a 5-log reduction in the population of *Z. bailii* in samples processed by CFHPT at 280 MPa, growth of surviving vegetative cells, injured cells or ascospores of *Z. bailii* after enrichment in YM broth occurred during storage at 25°C. At this operating pressure, it is conceivable that ascospores from such an aciduric strain of yeast were induced to germinate due to the pressure and shear forces exerted on the liquid media. It is also possible that injured cells were present after processing, but were incapable of growth on the acidified YM agar. Temperature-stressed cells from *Z. bailii* show varying levels of ability to recover on acidified media (Makdesi & Beuchat, 1996). High hydrostatic pressure processing at 276 MPa of *Z. bailii* in Sabourad Dextrose broth prepared with sucrose for adjustment of media to a_w 0.98 had a D-value of 4.82 min (Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes & Swanson, 1997).

 Z. bailii cultures grow well at 25-27°C (Palou et al, 1997; Raso et al, 1998, Reyns et al, 2000), and a few strains can grow at 37°C (Martorell, Stratford, Steels, Fernandez-Espinar & Querol, 2007). On the other hand, *Z. bailii* can show resistance to acidic pH 2.2 (Martorell et al, 2007). Preliminary research showed optimal growth conditions for this strain of *Z. bailii* occurred at 25-27°C. Growth did not occur at 4°C storage (Martorell et al, 2007). Little growth occurred in YM broth, apple juice, or acidified YM agar at temperatures below 22°C or above 30°C. The storage temperature of 25°C was selected for storage of samples since it was considered optimal for growth of surviving vegetative cells, injured cells, or ascospores. More research is needed to show if a hurdle technology approach such as refrigeration storage temperatures and use of chemical preservatives would be sufficient to prevent growth of *Z. bailii* after processing by CFHPT in apple juice.

REFERENCES

Adapa, S., Schmidt, K.A., & Toledo, R.T. (1997). Functional properties of skim milk processed with continuous high pressure throttling. *Journal of Dairy Science, 80*(9), 1941-1948.

Amornsin, A. (1999). Effect of high pressure throttling on ascorbic acid, pectin esterase activity and limonin content in citrus juice. University of Georgia. Athens, Georgia. Master of Science, 92 pgs.

Butz, P., Edenharder, R., Fister, H., & Tauscher, B. (1997). The influence of high pressure processing on antimutagenic activities of fruit and vegetable juices. *Food Research International*, *30*(3/4), 287-291.

Castellari, M., Arfelli, G., Riponi, C., Carpi, G., & Amati, A. (2000). High hydrostatic pressure treatments for beer stabilization. *Journal of Food Science, 65*(6), 974-977.

Charnock, C., Finsrud, T., & Foss, C. (2005). Controlling the proliferation of *Zygosaccharomyces bailii* in ephedrine anti-phlegm cough mixture. *Journal of Clinical Pharmacy and Therapeutics, 30*, 329-335.

Clark, J.P. (2006). High-pressure processing research continues. *Food Technology*, *2,* 63-65.

Dickinson, E., & James, J.D. (1998). Rheology and flocculation of high-pressure-treated *β*-lactoglobulin-stabilized emulsions: Comparison with thermal treatment. *Journal of Agricultural and Food Chemistry*, *46,* 2565-2571.

DiGiacomo, R., & Gallagher, P. (2001). Soft Drinks. Compendium of Methods for the Microbiological Examination of Foods. F.P. Downes and K. Ito. Washington, D.C., American Public Health Association: 569-571.

Drake, M.A., Harrison, S.L., Asplund, M., Barbosa-Canovas, G. & Swanson, B.G. (1997). High pressure treatment of milk and effects on microbiological and sensory quality of cheddar

cheese. *Journal of Food Science, 62*(4), 843-845, 860.

Earnshaw, R. (1996). High pressure food processing. *Nutrition & Food Science, 2,* 8-11

Fujita, K.-I., & Kubo, I. (2005). Naturally occurring antifungal agents against
Zygosaccharomyces bailii and their synergism. *Journal of Agricultural and Food
Chemistry, 53,* 5187-5191.

Goh, E.L.C., Hocking, A.D., Stewart, C.M., Buckle, K.A., & Fleet, G.H. (2007). Baroprotective
effect of increased solute concentrations on yeast and moulds during high pressure
processing. *Innovative Food Science and Emerging Technologies, 8,* 535-542.

Hatcher, W.S., Parish, M.E., Weihe, J.L., Splittstoesser, D.F., & Woodward, B.B. (2001). Fruit
Beverages. Compendium of Methods for the Microbiological Examination of Foods.
F.P. Downes and K. Ito. Washington, D.C., American Public Health Association, 565-
568.

Kalchayanand, N., Frethem, C., Dunne, P., Sikes, A., & Ray, B. (2002). Hydrostatic pressure
and bacteriocin-triggered cell wall lysis of *Leuconostoc mesenteroides*. *Innovative Food
Science and Emerging Technologies, 3,* 33-40.

Makdesi, A.K., & Beuchat, L.R. (1996). Improved selective medium for enumeration of
benzoate-resistant, heat-stressed *Zygosaccharomyces bailii*. *Food Microbiology, 13,* 281-
290.

Martorell, P., Stratford, M., Steels, H., Fernandez-Espinar, M.T., & Querol, A. (2007).
Physiological characterization of spoilage strains of *Zygosaccharomyces bailii* and
Zygosaccharomyces rouxii isolated from high sugar environments. *International Journal
of Food Microbiology*, *114,* 234-242.

Moorman, J.E. (1997). Microbicidal and rheological effects of high-pressure throttling (HPT).

University of Georgia. Athens, Georgia. Master of Science, 74 pgs.

Mussa, D.M., & Ramaswamy, H.S. (1997). Ultra high pressure pasteurization of milk: Kinetics

of microbial destruction and changes in physico-chemical characteristics. *Lebensmittel-*

Wissenchaft und-Technologie, 30, 551-557.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J., & Swanson, B.G.   (1997).

Kinetic analysis of *Zygosaccharomyces bailii* inactivation by high hydrostatic pressure.

Lebensmittel-Wissenchaft und-Technologie, 30, 703-708.

Peck, D.C. (2004). The effects of high-pressure throttling versus thermal pasteurization on a

blueberry-whey beverage. University of Georgia. Athens, Georgia. Master of Science, 67

pgs.

Raso, J., Calderon, M.L., Gongora, M., Barbosa-Canovas, G.V., & Swanson, B.G. (1998).

Inactivation of *Zygosaccharomyces bailii* in fruit juices by heat, high hydrostatic pressure

and pulsed electric fields. *Journal of Food Science*, *63*(1), 1042-1044.

Reyns, K.M.F.A., Soontjens, C.C.F., Cornelis, K., Weemaes, C.A., Hendrickx, M.E., &

Michiels, C.W. (2000). Kinetic analysis and modeling of combined high-pressure-

temperature inactivation of the yeast *Zygosaccharomyces bailii*. *International Journal of*

Food Microbiology, 56, 199-210.

Schmidt, B.M., Erdman, J.W., & Lila, M.A. (2005). Effects of food processing on blueberry

antiproliferation and antioxidant activity. *Journal of Food Science, 70*(6), S389-S394.

Sivanandan, L., Toledo, R.T., & Singh, R.K. (2008). Effect of continuous flow high-pressure

throttling on rheological and ultrastructural properties of soymilk. *Journal of Food*

Science, 73(6), E288-E296

Smelt, J.P.P.M. (1998). Recent advances in the microbiology of high pressure processing.

Trends in Food Science & Technology, 9(4), 152-158.

Spotts, R.A., Serdani, M., Mielke, E.A., Bai. J., Chen, P.M., Hansen, J.D., Neven, L.G., & Sanderson, P.G. (2006). Effect of high-pressure hot water washing treatment on fruit quality, insects, and disease in apples and pears. Part II. Effect on postharvest decay of d'Anjou pear fruit. *Postharvest Biology and Technology, 40,* 216-220.

Torres, J.A., & Velazquez, G. (2005). Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering*, *67,* 95-112.

Table 2.1. Concentration (mean log CFU·mL^{-1}) of *Z. bailii* recovered from samples stored at 25°C (n = 3)

Process[1]	Storage Time (d)		
	0	3	7
Unprocessed	7.21 a	7.56 b	7.51 b
Hot fill	<1.30[2] a	<1.30 a	<1.30 a
CFHPT 140 MPa[2]	5.64 a	7.60 b	7.50 b
CFHPT 280 MPa[2]	<1.30 a	7.31 b	7.47 b

[1]Process: Tukey's Honestly Significant Difference Test performed within levels of process, where different letters following concentrations of *Z. bailii* indicate a significant difference among means within row (α = 0.05)

[2]CFHPT = Continuous-Flow High-Pressure Throttling operated at 140 or 280 MPa

Table 2.2. Growth of injured cells or ascospores of *Z. bailii* after enrichment of samples immediately after processing or after storage at 25°C

Process	Storage (days)		
	0	3	7
Unprocessed	+[1]	+	+
Hot fill	–	–	–
CFHPT 140 MPa[2]	+	+	+
CFHPT 280 MPa[2]	+	+	+

[1](+) = Turbid, cloudy appearance of YM broth culture tubes after inoculation with 0.1 mL of sample; (-) = No visible growth
[2]CFHPT = Continuous-Flow High-Pressure Throttling operated at 140 or 280 MPa

Figure 2.1. Schematic of a continuous-flow high-pressure throttling system
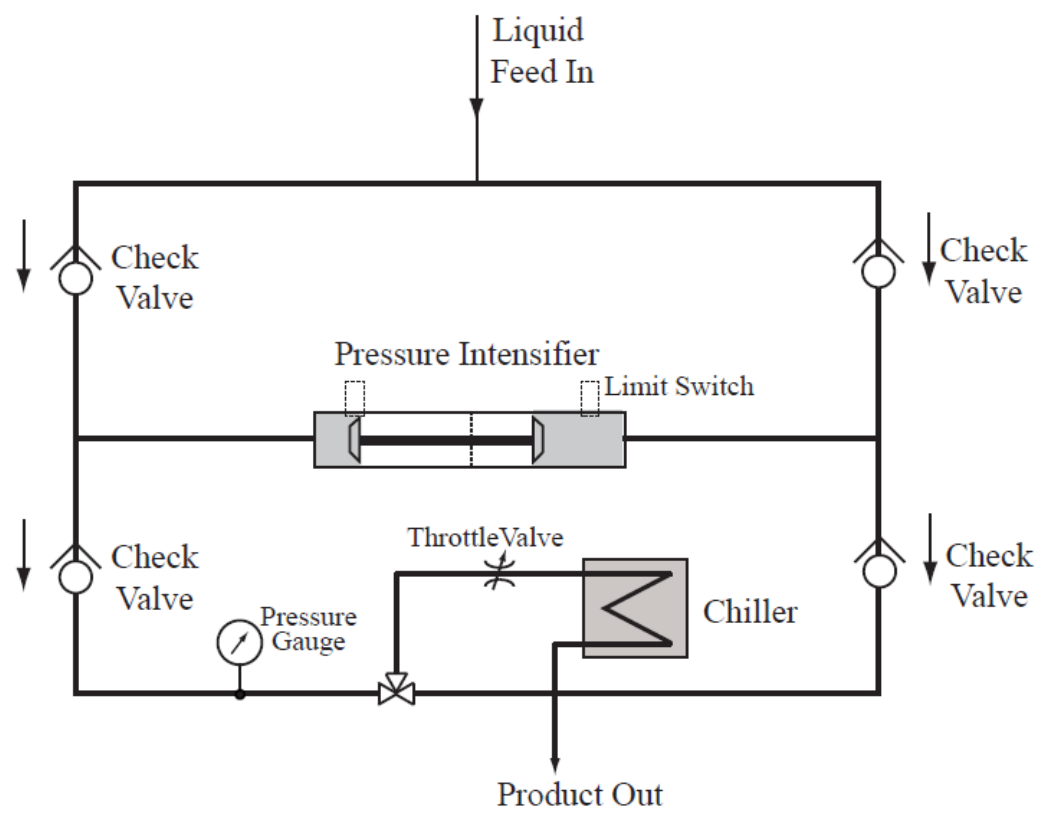
Figure 2.1

CHAPTER 3. NOVEL FUNCTIONAL BEVERAGES WITH ANTI-GLYCATION

PROPERTIES FROM HIGH-PROCYANIDIN SORGHUM BRAN

ABSTRACT

The purpose of this research was to evaluate the acceptability and stability of a functional beverage containing white grape juice, 50% ethanolic or hot-water extract of high-proanthocyanidin sorghum bran, and hot water extracts of cinnamon and allspice. The formulation was designed for consumers concerned with metabolic syndrome and diabetes, or those desiring a high antioxidant, high-proanthocyanidin dietary supplement. Antioxidant analyses including Folin-Ciocalteu and FRAP (ferric reducing/antioxidant power) showed beverage formulations had 1.82 or 3.35 mg of gallic acid eq per mL and FRAP values of about 9.73 or 10.53 mmol of ferrous sulfate eq per mL. The beverages showed inhibition of fructose-induced glycation of bovine serum albumin by 50 % (IC_{50}) at dilutions of 217 to 489 times original strength. Sensory attributes were evaluated for overall acceptability, flavor, color, aroma, mouthfeel, and likelihood of purchase using 5- or 9-point structured hedonic scales. Sensory results demonstrated overall acceptability of the formulation containing hot-water sorghum extract and that consumers "probably would buy" the product.

Keywords: Glycation, Metabolic Syndrome, Type II diabetes, high-procyanidin sorghum bran, procyanidin, functional beverage

INTRODUCTION

The Western diet is characterized by relatively high intake of red meat, sugary foods, high fat, and refined grains. Such diets may encourage the development of chronic health conditions characterized by a pro-inflammatory state, which may include metabolic syndrome, Type II diabetes mellitus, insulin resistance, or atherosclerosis (Dehnavi and others 2008; Ford and Li 2008; Luca and Olefsky 2008). The incidence of Type II diabetes is expected to affect over 300 million worldwide by 2025 (Hays and others 2008) and is the sixth leading cause of death in the United States (Pham and Kourlas, 2007). Incorporation of a diet rich in fruits, vegetables and whole grains, which provide a significant source of antioxidants, is thought to help prevent such conditions from developing.

In pro-inflammatory stress states such as Type II diabetes mellitus, cellular protein aging occurs at an accelerated rate due primarily to glycation of proteins by higher sugar concentrations (McPherson and others 1988). These modified proteins are termed advanced glycation endproducts or AGE proteins. Hemoglobin A1c (HbA1c) provides a clinical measure of non-enzymatic oxidation of hemoglobin protein in blood, which serves as a longer-term indicator (that is 3-4 months) of blood glucose control in diabetic patients (Pupillo and others 2008). Elevated blood glucose levels may account for only 30% of the incidence of glycated hemoglobin in HbA1c (Yudkin and others 1990). Antioxidants including vitamins A and C, and particularly flavonoids inhibit formation of AGE proteins (Matsuda and others 2003; Peyroux and Sternberg 2006). Proanthocyanidins are associated with chemopreventive (Nandakumar and other 2008) and anti-inflammatory properties, and with reduced risk of coronary heart disease (Cos et al, 2004). The major sources of proanthocyanidins in the U.S. diet include apples, chocolate, and grapes (Gu and others 2004).

Sorghum is a cereal-grain grown in arid regions of the world. Its global production was 58 million tons in 1990 (FAOSTAT; Dlamini and others 2007). Antioxidant compounds present in sorghum include proanthocyanidins, flavonoids, anthocyanins, phytosterols, and policosanols (Awika and Rooney 2004; Dlamini and others 2007). The proanthocyanidin content of conventional sorghum bran varieties is estimated at 4.6 mg/g (d.w.) (USDA-ARS) but may range upward from 10.0 to 68.0 mg/g (d.w.) (Jambubathan and Mertz, 1973; Hahn and Rooney, 1986; Agullo and Rodriguez, 1995; Awika, 2000; Awika and Rooney, 2004). The polyphenolic fractions extracted from high-proanthocyanidin (50–70 mg phenolics/g) sorghum cultivars possess significantly higher antioxidant capacity than conventional varieties (Dykes and Rooney 2006). Polyphenolic compounds of sorghum are readily extractable in various solvent systems (Kamath and others 2004). Hot water or aqueous ethanolic solutions would be most practical for commercial-scale extraction.

Functional and nutraceutical beverages formulated to combat metabolic syndrome and diabetes or to improve overall health are becoming attractive to health-conscious consumers (Verhagen and others 2004). In recent diabetes research, water-soluble proanthocyanidins extracted from cinnamon have shown effectiveness in decreasing insulin resistance and increasing glucose utilization by cells (Anderson and others 2004), while aiding in blood glucose control and slowing rates of gastric emptying (Hlebowicz and others 2007). Extracts from cinnamon and allspice have shown activity in inhibition of AGE proteins (Dearlove and others, 2008). Combining multiple bioactive sources in a single food source may enhance complementary health benefits. Combining flavonoid polyphenolic compounds for reducing AGE formation, and cinnamon for increasing insulin sensitivity and affecting glucose uptake and

74

utilization by cells, may address health maintenance needs for sufferers of Type II diabetes or metabolic syndrome.

This study focused on developing a nutraceutical beverage formulated from white grape juice, high-proanthocyanidin sorghum bran, and complementary ingredients such as cinnamon and allspice that may benefit Type II diabetic, pre-diabetic, or normal consumers. This study involved characterizing beverage ingredient extracts and formulations for antioxidant activity, evaluating the ability of the beverage and its components to inhibit formation of fructose-induced AGE proteins, and assessing consumer acceptability of a beverage formulation made from a hot water extract of high-proanthocyanidin sorghum bran.

MATERIALS AND METHODS

Materials

High-proanthocyanidin sorghum bran (v. Early Sumac) was donated by Dr. Lloyd L. Rooney of Texas A&M University. Gallic acid, Folin-Ciocalteu reagent, sodium acetate, iron (II) sulfate heptahydrate, 2,4,6-tris(2-pyridyl)-*s*-triazine, bovine serum albumin (97% essentially fatty acid free), quercetin, and *D*-(−)-fructose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Anhydrous sodium carbonate and anhydrous ferric chloride were from Fisher Scientific (Pittsburgh, PA). Anhydrous iron (III) chloride was from Riedel-deHaën® (Germany). Hydrochloric acid, dibasic potassium phosphate, and monobasic potassium phosphate were purchased from J.T. Baker, Inc. (Phillipsburg, NJ). Potassium chloride was from EM Science (Gibbstown, NJ). Chelex® 100 Resin was from Bio Rad Laboratories (Hercules, CA).

Ground cinnamon from Great Value brand (Bentonville, AR), ground allspice from McCormick and Co, Inc. (Hunt Valley, MD), white grape juice from Welch's® (Concord, MA), and unsalted Kroger brand Saltines (Cincinnati, OH) were purchased at local supermarkets.

Extract and beverage preparation

Following good manufacturing practices, hot water extracts of high-proanthocyanidin sorghum bran (1:10, w/v), cinnamon (1:100, w/v), and allspice (1:100, w/v) were prepared by adding ground material to previously boiled deionized water. The extracts were heated to 80ºC for 1 h and continuously mixed. The ethanolic extracts of sorghum bran (1:10, w/v) were prepared using 50% aqueous ethanolic solutions of vodka (Frïs®, Denmark). The suspensions were stirred continuously for 1 h at room temperature (22°C). Extracts were then centrifuged at $1,000 \times g$ at 4°C for 10 min. Supernatants were collected and used for experimental or sensory evaluation.

For sensory evaluation, beverages were prepared by adding white grape juice to either aqueous or ethanolic sorghum bran extracts (60:40, v/v). Cinnamon and allspice (1.468% and 0.49% w/w, based on total volume of beverage preparation) were then pre-hydrated with additional white grape juice and added to the grape juice/sorghum mixture. Mixtures were heated to 75°C with constant stirring, and then cooled to 22°C. The beverages were then centrifuged at $1,000 \times g$ at 4°C for 10 min to remove any suspended solids. The supernatant was used for sensory evaluation. For antioxidant analysis, samples were serially diluted with deionized water to the appropriate concentration for the FRAP evaluation. Samples were stored before use at 4°C for less than one day or frozen at -20°C for up to 9 months in airtight high-density polyethylene plastic gallon jugs in the dark.

Physicochemical characterization

Beverage formulated with hot water sorghum extract was evaluated at 22°C for pH using an AR15 pH meter (Accumet, Fisher Scientific, Pittsburgh, PA), titratable acidity as tartaric acid (AOAC 942.15), and total soluble solids by refractometry (PR-201 Palette, Atago, Japan). Color was evaluated using a handheld Chroma Meter CR-410 (Minolta, Co., Osaka, Japan). In a dark room, 50 mL of juice was poured into a Petri plate (87 mm internal diameter) and placed onto a black background. The sample was rotated 90° between color measurements. Three measurements were taken per sample, and L*, a*, and b* values were recorded. Particle size analysis was conducted using a Malvern Laser Particle Size Analyzer (Mastersizer S with 300 mm lens and dispersion unit controller and Mastersizer-S v. 2.18 software, Malvern Instruments, Southborough, MA). For each physicochemical characterization, three samples were analyzed with mean values reported.

Folin-Ciocalteu analysis

Folin-Ciocalteu analysis was conducted following the method of Singleton and Rossi (1965) with modifications. One mL of deionized water was pipetted into a 1.5 mL plastic microcentrifuge tube (VWR, West Chester, PA). Next, 12.7 µL of standard or sample was pipetted to each tube, followed by 63.3 µL of Folin-Ciocalteu reagent. Tubes were then vortexed. After one min, 189.9 µL of 20% sodium carbonate solution (w/v) was added to each tube. The microcentrifuge tubes were then vortexed and incubated at 22°C for 45 min. Samples were transferred to plastic cuvettes and the absorbance at 765 nm was measured on a Beckman DU Series 600 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA) against a deionized water blank. A standard curve was created using gallic acid at concentrations of 0.05, 0.10, 0.15,

0.25, and 0.50 mg/mL. All samples and standards were analyzed in triplicate. Results were reported as mg of gallic acid equivalents per mL of sample.

FRAP analysis

The ferric reducing/antioxidant power (FRAP) analysis followed methods of Benzie and Strain (1996) with modification. To 1.5 mL microcentrifuge tubes, 30 µL of standard or sample was added. Iron (II) sulfate heptahydrate solutions were used as standard solutions (0.1, 0.2, 0.4, 0.6, and 1.0 mM). To each tube, 1 mL FRAP reagent (300 mM sodium acetate buffer, pH 3.6; 10 mM TPTZ [2,4,6-tris(2-pyridyl)-*s*-triazine]; 20 mM ferric chloride; deionized water; 758:76:76:91, v/v/v/v). Tubes were vortexed, and then incubated in a 37°C water bath for 4 min. Samples were then transferred to plastic cuvettes, and the absorbance at 593 nm was measured against a deionized water blank. All samples and standards were measured in triplicate. FRAP results were reported as mmol of ferrous sulfate eq per mL of sample.

Fructose-induced glycation of bovine serum albumin

The glycation of bovine serum albumin was conducted based on the methods of McPherson and others (1988) with modification. To 1.5 mL microcentrifuge tubes, 0-50 µL of sample or standard was added to each tube, followed by the addition of 900 µL of phosphate buffer (200 mM potassium phosphate buffer, pH 7.4 with 0.02% sodium azide, treated with Chelex resin), 300 µL of albumin solution (50 mg/mL bovine serum albumin in chelex-treated phosphate buffer) (BSA), and 300 µL of fructose solution (1.25 M fructose in chelex-treated phosphate buffer). Tubes were capped and incubated in a 38°C incubator for 72 h. Following incubation, each sample was analyzed for fluorescence intensity on an LS 55 Luminescence

Spectrometer (Perkin-Elmer, Waltham, MA) with an excitation/emission wavelength pair $\lambda =$ 370/440 nm with 3 nm slit width, read against a phosphate buffer blank. Each standard or sample was evaluated at five dilutions in triplicate over two separate days. Extract without added fructose or BSA was evaluated for fluorescence and used as a correction factor. Quercetin was evaluated as an external standard. Data points of concentration versus fluorescence were plotted and fitted by linear regression using Excel® 2003 software (Microsoft Corporation, Redmond, WA). Results were reported as 50% inhibition of fructose-induced advanced glycation protein endproduct formation (IC_{50}), based on the estimate from the regression curve specific to each sample. Internal controls (fructose only, BSA only, fructose + BSA) were used to determine endpoints from which to calculate % inhibition of glycation.

Sensory evaluation

For the survey of consumer acceptability, the sorghum beverage was evaluated using hedonic testing based on the methods of Meilgaard and others (1999). All sensory evaluation protocol, advertisements, and handling of research data were approved by the University of Georgia's Institutional Review Board. Panelists were excluded from participation if they were pregnant or breast-feeding, under the age of 18, a prisoner, over the age of 64, a non-English speaker, physically or mentally incapacitated, or had allergies or sensitivities to sorghum, gluten, apples, grapes, cinnamon, allspice, or exposure to peanut processing equipment. The evaluation was conducted in a sensory laboratory equipped with individual testing booths having white fluorescent lighting. Untrained subjects (n = 54 panelists) that consisted of university faculty, staff, students, and the public participated in the test after completion of informed consent forms.

In the session, subjects were presented with a 4-oz beverage sample at 4°C contained in a white plastic cup, crackers, and a cup of water. No additional cup was provided for expectoration.

Using paper ballots containing three pages, panelists were first instructed to complete the sensory test in chronological page order. On the first page, they were instructed to chew some crackers and rinse their mouth with filtered tap water, and then indicate gender, age, and frequency of bottled juice consumption using a 5-point purchase intent scale (Figure 3.1). On page two, panelists then evaluated the beverage sample for sensory attributes of overall acceptability, flavor, color, and aroma/smell using a 9-point structured hedonic scale marked with word descriptors (Figure 3.1). On the third page, panelists were presented with a succinct description of the potential health benefits derived from consuming the product. The statement declared,

> "If the sample was sold in a small, 4 or 6-ounce single serving bottle, and its packaging
> label was to state the following: This product provides a rich source of antioxidants that
> are known to fight free radicals. Its unique formulation may help in the maintenance of
> blood sugar levels and may reduce oxidative damage associated with Type II diabetes.
> …how likely would you be to purchase this product (for yourself or for someone in your
> family) assuming it was priced competitively to similar products?"

Likelihood of product purchase was then indicated using a 5-point structured purchase intent scale (Figure 3.1). Upon completion of the test, subjects were offered a piece of candy for their participation. The sensory evaluation was conducted over two replicate sessions timed nine months apart.

Statistical analysis

Statistical analysis of data was performed using ANOVA with Tukey's Honestly

Significant Difference Test (α = 0.05) on Minitab software (Minitab, Inc., State College, PA).

RESULTS AND DISCUSSION

Physicochemical characterization of hot water sorghum beverage

The sorghum beverage formulated with hot water sorghum bran extract had a relatively

low pH of 3.70 (Table 3.1). The titratable acidity was 4.01 ± 0.03 g tartaric acid per liter. This

is based on tartaric acid representing the predominant acid in the beverage, as it is the

predominant acid in grape juice (Haard and Chism, 1996). Ascorbic acid present in the white

grape juice is also thought to have contributed to the total titratable acidity. The color evaluation

showed moderate lightness (L* = 47.3). There was also a slight tendency towards the red (a* =

2.79) and yellow (b*= 2.71) axes. The soluble solids content of the beverage was 13.6°Brix. The

sugar content in the grape juice likely contributed to most of the total soluble solids content.

 The hot water sorghum beverage had suspended particles originating from sorghum bran,

cinnamon, and allspice. The particle size distribution (volume-length diameter) had a Gaussian

shape with center around 46 μm.

Folin-Ciocalteu analysis

The total polyphenolic content and FRAP values are shown in Table 3.2. Values are

listed for component extracts and beverage formulations. For comparison, values are also shown

for a commercial Concord (red) grape juice and white tea, beverages known to contain

81

significant proanthocyanidins. The 50% EtOH sorghum extract contained 5.78 mg/ml total phenolics (expressed in terms of gallic acid) as compared to 2.13 mg/ml for the hot-water extract, indicating that the 50% ethanol was more effective at recovering polyphenolic compounds. The total polyphenolics content for the white grape juice was 0.97 mg/ml, while that for the cinnamon and allspice extracts were 1.12 and 0.80 mg/ml. Values for the beverage based on hot-water extract and that based on 50% EtOH extract were 1.82 and 3.35 mg/ml, respectively. It should be noted that the beverage is a blend of 60% by volume of white grape juice, and 40% by volume of sorghum extract, plus 1.47% and 0.49% by weight of cinnamon and allspice. Thus, the juice contributed approximately 40.1% to the total phenolics, while the hot-water sorghum extract contributed 58.7% and the cinnamon 1.1%.

The total phenolics assay does not distinguish between particular antioxidants, but it is known that sorghum extracts contain proanthocyanidins, anthocyanins, and flavonoids (Awika and Rooney 2004). In the beverage formulations, there were also proanthocyanidins from cinnamon (Anderson and others 2004), and polyphenolic compounds from allspice (Broadhurst and others 2000) and white grape juice (Frankel and others 1998). In comparison, the beverage made from hot-water sorghum extract had greater total phenolics than white tea, while that made from the 50% EtOH extract had greater total phenolics than both white tea and Concord grape juice.

FRAP analysis

Results from the FRAP assay are shown in Table 3.2. The 50% EtOH and hot-water sorghum extracts had FRAP values of 17.29 and 11.82 mmol $FeSO_4$ equivalents per ml, respectively. Values for the white grape juice, cinnamon and allspice were 3.54, 20.78 and 3.05

mmol $FeSO_4$ equivalents per ml, respectively. Accounting for the beverage formulation, the juice contributed approximately 29.7% to the FRAP values, while the hot-water sorghum extract contributed 66.1% and the cinnamon 4.3%, respectively. In general, FRAP values were linearly correlated with the total phenolics, except for the cinnamon extract, which had comparatively higher FRAP values. The FRAP assay measures the ability of phenolic compounds to quench free radicals by delaying the oxidation of ferric to ferrous iron complexed to tripyridyltriazine (Benzie and Strain 1996), providing a measure of their antioxidant potential. Cinnamon thus contains polyphenolic compounds that have potent ability in exerting antioxidant capacity, when compared to contents for total polyphenolic compounds.

FRAP values for beverages made from hot water (9.73 mmol $FeSO_4$/ml) or 50% aqueous ethanolic (10.53 mmol $FeSO_4$/ml) sorghum extracts were not significantly different from those for Concord grape juice (8.78 mmol $FeSO_4$/ml) or white tea (10.11 mmol $FeSO_4$/ml).
It has been estimated that a Western-style diet provides about 150-200 mg of flavonoids per day (Manach et al, 2004; Gu et al, 2004). An 8 fluid ounce serving of a beverage formulated with hot water or 50% aqueous ethanolic extract from high-proanthocyanidin sorghum bran may provide up to 400-800 mg of flavonoids. As large quantities of bran are available as a by-product from cereal grain processing, sorghum bran can serve as an inexpensive raw ingredient, which may provide consumers with a significant source of antioxidants.

High-proanthocyanidin sorghum bran is high in polyphenolic compounds including proanthocyanidins, anthocyanins, phenolic acids, phytosterols, and policosanols.   The proanthocyanidins are predominantly oligomeric or polymeric condensed tannins, composed of linked flavan-3-ols or flavan-3,4-diols (Awika and Rooney 2004). The proanthocyanidins of sorghum are composed almost entirely of epicatechin units (Gu and others 2004).   High-

proanthocyanidin sorghum bran is highest in larger oligomeric proanthocyanidins (OPC), but also contains lesser amounts of monomers, dimers, trimers, and OPC chain lengths of 4-10 units (USDA Database 2004).

Inhibition of fructose-induced advanced glycation endproduct (AGE) formation

The inhibition of formation of fructose-induced advanced glycation endproducts (AGE) was measured in the presence of extracts of beverage components and beverage formulations (Table 3.3). In the glycation experiment, standards (BSA only, BSA + fructose) were used as endpoints from which to estimate the ability of beverage formulation component extracts to inhibit glycation of BSA. To compare the effectiveness of different samples in inhibiting the reaction, sample dilutions were determined that inhibited glycation of protein by 50% (IC_{50}), based upon creation of dose-response curves for each sample tested. The correlation coefficient (R^2) is shown for each sample from which the estimate was calculated using a linear fitted mathematical relationship (Table 3.3).

The results showed that a 210:1 dilution of hot-water sorghum extract and a 781:1 dilution of the ethanolic extract would inhibit 50% of fructose-induced AGEs. That is, less of the ethanolic extract was needed to attain the same anti-glycation effect. For the white grape juice, cinnamon extract, and allspice extract, those values were 180, 100 and 63 to 1, respectively. Interestingly, the IC_{50} values were linearly related to the total phenolic values ($R^2=0.97$) but not to the FRAP values ($R^2=0.20$). When the hot water cinnamon extract was omitted, the relationship increased ($R^2=0.72$).

The beverage made from 50% EtOH sorghum extract had the highest IC_{50} (489), and was about twice as effective at inhibiting formation of AGE as beverage formulated with hot water extract (IC_{50}=217). The beverage made from 50% EtOH sorghum extract also showed greater inhibitory activity than Concord grape juice (IC_{50}=319) and white tea (IC_{50}=223). The beverage made from hot-water extract had similar effectiveness to that of white tea.

Glycation of protein occurs when sugar carbonyl groups react with amine groups of amino acids, creating a Schiff base. When in the presence of free metal ions such as iron, the Schiff base can readily convert to an Amadori product, following a pathway similar to that in the Maillard reaction (McPherson and others 1988). Certain compounds are capable of chelating free metal ions, such as the chemically synthesized ethylenediamine-tetraacetic acid (EDTA) commonly used in manufactured food products, or flavonoids such as quercetin, green tea catechins (Nest and others 2004), or proanthocyanidins (Zijp and others 2000).

The glycation of protein may proceed more rapidly in the presence of excess fructose or glucose (McPherson and others 1988). In persons suffering from Type II diabetes mellitus, or those consuming an excessive amount of fructose, such as through products containing high-fructose corn syrup (HFCS), similar oxidative damage may occur to cellular proteins through formation of advanced-glycation end products (Peyroux and Sternberg 2006). This is often measured using a hemoglobin A1c test (Boer and others 2006). Many polyphenolic compounds such as extracts rich in flavonoids and other phenolic compounds have demonstrated effectiveness in inhibiting the glycation process (Dearlove and others 2008).

Glycation of protein occurs even in healthy individuals. Consuming a product rich in phytochemicals capable of inhibiting oxidative damage to protein is of great interest. As the incidence of Type II diabetes mellitus is expected to increase, there will be an increased demand

for nutraceutical products that can help prevent or alleviate the symptoms of the disease. An added benefit of the beverage formulation studied is that it includes cinnamon extract. Cinnamon supplementation has been shown to help control blood glucose levels in adult Type II diabetic patients (Mang and others 2006; Hlebowicz and others 2007; Pham and others 2007). This occurs because cinnamon increases glucose utilization by cells, increasing translocation of the GLUT-4 transporter to the cell membrane. This is especially beneficial in reducing the effects of postprandial glucose rise following food consumption.

Cinnamon contains both singly- and doubly-linked proanthocyanidins (Lazarus and others 1999). Anderson and others (2004) tested extracts of cinnamon and found insulin-like activity from aqueous extracts when evaluated using *in vitro* epididymal fat cells. In purified extracts, only doubly-linked proanthocyanidin type-A polymers were found to exhibit insulin-like activity. These compounds function by initiating phosphorylation of insulin receptor kinase, leading to increased glucose uptake by adipocytes. Glycogen synthesis may also increase with the water-soluble compounds. *In vitro* testing showed synergistic effects on glucose and glycogen production, which occurred when the purported methylhydroxy-chalcone polymer (MHCP) and insulin were both present (Pham and Kourlas 2007).   Cinnamon also improves insulin receptor activity by activating insulin receptor PI 3-kinase, while inhibiting tyrosine phosphatases (Hlebowicz and others 2007). Cinnamon may also improve insulin receptor activity, since it was shown to increase concentrations of phosphorylated protein IRS-1 and increased binding of PI 3-kinase, which helps increase cellular uptake of glucose (Hlebowicz et al, 2007).

Sensory analysis

Sensory results for the beverage formulated with hot water extracts of high-proanthocyanidin sorghum bran, cinnamon, allspice, and white grape juice are shown in Table 3.4. On average, panelists consumed similar single-serve bottled teas or juices a few times per month. The mean overall acceptability (6.8) and flavor (6.8) were rated as "Like Moderately". The mean scores for color (6.2) were "Like Slightly". Some comments indicated that the brown color was not appealing, and therefore a commercialized product would most likely need added colorants. The mean aroma/smell (7.0) of the beverage was rated as "Like Moderately". When asked about intent to purchase, panelists indicated they Probably Would Buy the product for themselves or for someone in their family. This was based on having read the health benefits statement, having already evaluated product for sensory attributes, and assuming the product was priced competitively to similar products.

CONCLUSION

As the incidences of obesity, metabolic syndrome, and Type II diabetes continue to increase, the market continues to grow for novel functional foods and beverages that may help reverse these chronic disease states. A high-proanthocyanidin sorghum-based functional beverage containing complementary ingredients formulated especially for health maintenance shows considerable promise for mainstream market appeal. The phenolic contribution from high-proanthocyanidin sorghum bran combined with cinnamon, allspice, and white grape juice may provide complementary health benefits for reduced protein damage from glycation and greater utilization and uptake of glucose by cells. High-proanthocyanidin sorghum bran is high

in polyphenolic compounds and is significantly cheaper than comparable fruit or vegetable sources when considering proanthocyanidin content.

REFERENCES

Agullo E, Rodriguez MS. 1995. Evaluation of 3 rapid methods to determine tannins in sorghum grains. Anales de la Asociacion Quimica Argentina 83: 83-87.

Anderson RA, Broadhurst, CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schoene NW, Graves DJ. 2004. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem 52: 65-70.

AOAC International. 2000. Official Methods of Analysis (Method 942.15). Association of Official Analytical Chemists: Maryland.

Awika JM. 2000. Sorghum phenols as antioxidants. M.S. Thesis, Texas A&M University, College Station, TX.

Awika JM, Rooney LW. 2004. Sorghum phytochemicals and their potential impact on human health. Phytochem 65: 1199-1221.

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. Anal Biochem 239: 70-76.

Boer HD, Keizers R, Jansen M, Verschoor L, Ruineman-Koerts J. 2006. Glycaemic control without weight gain in insulin requiring type 2 diabetes: 1-year results of the GAME regimen. Diabetes, Obesity and Metabolism 8: 517-523.

Broadhurst CL, Polansky MM, Anderson RA. 2000. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J Agric Food Chem 48: 849-852.

Cos P, De Bruyne T, Hermans N, Apers S, Berghe DV, Vlietinck AJ. 2004. Proanthocyanidins in

health care: current and new trends. Curr Med Chem 11(10): 1345-59.

Dearlove RP, Greenspan P, Hartle DK, Swanson RB, Hargrove JL. 2008. Inhibition of protein
glycation by extracts of culinary herbs and spices. J Med Food 11(2): 275-281.

Dehnavi RA, Beishuizen ED, Reema VD, Cessie SL, Huisman MV, Kluft C, Princen HMG,
Tamsma JT. 2008. The impact of metabolic syndrome and CRP on vascular phenotype in
type 2 diabetes mellitus. Eur J Internal Medicine 19: 115-121.

Dlamini NR, Taylor JRN, Rooney LW. 2007. The effect of sorghum type and processing on the
antioxidant properties of African sorghum-based foods. Food Chem 105: 1412-1419.

Dykes L, Rooney LW. 2006. Sorghum and millet phenols and antioxidants. J Cereal Sci 44: 236-
251.

FAOSTAT: Food and Agricultural Organization of the United Nations. Sorghum and millets in
human nutrition [Internet]. Rome, Italy: FAO Food and Nutrition Series, No. 27; c 1995
[Accessed 2009 Jun 18]. Available from:
http://www.fao.org/docrep/T0818E/T0818E00.HTM

Ford ES, Li C. 2008. Metabolic syndrome and health-related quality of life among U.S. adults.
Annals of Epidemiology 18(3): 165-171.

Frankel EN, Bosanek CA, Meyer AS, Silliman K, Kirk LL. 1998. Commercial grape juices
inhibit the in vitro oxidation of human low-density lipoproteins. J Agric Food Chem 46:
834-838.

Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL.
2004. Concentrations of proanthocyanidins in common foods and estimations of normal
consumption. J Nutr 134(3): 613-617.

Haard NF, Chism TP. 1996. Characteristics of edible plant tissues. In: *Food Chemistry*, 3rd ed,

Ed: Fennema OR., Marcel Dekker, Inc., New York.

Hahn DH, Rooney LW. 1986. Effects of genotype on tannins and phenols of sorghum. Cereal Chem 63: 4-8.

Hays NP, Galassetti PR, Coker RH. 2008. Prevention and treatment of Type 2 diabetes: Current role of lifestyle, natural product, and pharmacological interventions. Pharmacology & Therapeutics 118: 181-191.

Hlebowicz J, Darwiche G, Bjorgell O, Almer L-O. 2007. Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in health subjects. The American Journal of Clinical Nutrition 85: 1552-1556.

Jambubathan R, Mertz ET. 1973. Relationship between tannin levels, rat growth, and distribution of proteins in sorghum. J Agric Food Chem 21: 692-696.

Kamath VG, Chandrashekar A, Rajini PS. 2004. Antiradical properties of sorghum (*Sorghum bicolor* L. Moench) flour extracts. J Cereal Sci 40: 283-288.

Lazarus SA, Adamson GE, Hammerstone JF, Schmitz HH. 1999. High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. J Agric Food Chem 47: 3693-3701.

Luca CD, Olefsky JM. 2008. Inflammation and insulin resistance. FEBS Letters 582: 97-105.

Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. 2004. Polyphenols: food sources and bioavailability. Am J Clin Nutr 79(5): 727-747.

Mang B, Wolters M, Schmitt B, Kelb K, Lichtinghagen R, Stichtenoth DO, Hahn A. 2006. Effects of a cinnamon extract on plasma glucose, HbA1c, and serum lipids in diabetes mellitus type 2. Eur J Clinical Investigation 36: 340-344.

Matsuda H, Wang T, Managi H, Yoshikawa M. 2003. Structural requirements of flavonoids for

inhibition of protein glycation and radical scavenging activities. Bioorganic & Medicinal Chemistry 11: 5317-5323.

McPherson JD, Shilton BH, Walton DJ. 1988. Role of fructose in glycation and cross-linking of proteins. Biochem 27: 1901-1907.

Meilgaard M, Civille GV, Carr BT. 1999. Ch. 12 Affective Tests: Consumer tests and in-house panel acceptance tests. Sensory Evaluation Techniques. 3rd ed. Boca Raton: CRC Press. p. 231-264.

Miles MV, Morrison JA, Horn PS, Tang PH, Pesce AJ. 2004. Coenzyme Q10 changes are associated with metabolic syndrome. Clinica Chimica Acta 344: 173-179.

Nandakumar V, Singh T, Katiyar SK. 2008. Multi-targeted prevention and therapy of cancer by proanthocyanidins. Cancer Letters 269: 378-387.

Nest GL, Caille O, Woudstra M, Roche S, Burlat B, Belle V, Guigliarelli B, Lexa D. 2004. Zn-polyphenol chelation: complexes with quercetin, (+)-catechin, and derivates: II Electrochemical and EPR studies. Inorganica Chimica Acta 357: 2027-2037.

Peyroux J, Sternberg M. 2006. Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. Pathologie Biologie 54: 405-419.

Pham AQ, Kourlas H. 2007. Cinnamon supplementation in patients with type 2 diabetes mellitus. Pharmacotherapy 27(4): 595-599.

Pupillo M, Berardis GD, Antenucci D, Minnucci A, Nicolucci A. 2008. Glycated haemoglobin or mean blood glucose as indicators of metabolic control in Type 2 diabetes? Diabetes Research and Clinical Practice 80: e1-e3.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16(3): 144-

158.

USDA-ARS. United States Department of Agriculture-Agricultural Research Service. USDA
database for the proanthocyanidin content of selected foods [Internet]. Washington, D.C.:
United States Department of Agriculture-Agricultural Research Service; c 2005
[Accessed 2009 Feb 9]. Available from: http://www.nal.usda.gov/fnic/foodcomp

Verhagen H, Coolen S, Duchateau G, Hamer M, Kyle J, Rechner A. 2004. Assessment of the
efficacy of functional food ingredients—introducing the concept "kinetics of
biomarkers". Mutation Research 551: 65-78.

Yudkin, J.S., Forrest, R.D., Jackson, C.A., Ryle, A.J., Davie, S., Gould, B.J. 1990. Unexplained
variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia.
Diabetologia 33: 208-215.

Zijp IM, Korver O, Tijburg LBM. 2000. Effect of tea and other dietary factors on iron
absorption. Critical Reviews in Food Science and Nutrition 40(5): 371-398.

ACKNOWLEDGEMENTS

Table 3.1. Physicochemical properties of a white grape juice beverage formulated with hot water sorghum bran extract

pH	3.70
Titratable acidity (g tartaric acid·l^{-1})	4.01 ± 0.03
Color	
L*	47.3 ± 0.022
a*	2.79 ± 0.011
b*	2.71 ± 0.006
Soluble solids (°Brix)	13.6
Particle size (μm):	
D$_{(50)}$: diameter of particle below which 50% of volume of particles are found	42.5
D$_{(3,2)}$: mean diameter (area/volume)	35.3
D$_{(4,3)}$: mean diameter (volume/length)	46.3

Table 3.2. Total phenolic and FRAP values of beverages from Early Sumac sorghum bran (ES), their component extracts, and references

Sample	Total Phenolics (mg gallic acid per·ml)	FRAP (mmol $FeSO_4$ per ml)
Beverage components		
Early Sumac sorghum, hot water (1:10, w/v)	$2.13^a \pm 0.10$	$11.82^i \pm 0.73$
Early Sumac sorghum, 50% aq. EtOH (1:10, w/v)	$5.78^b \pm 0.14$	$17.29^j \pm 0.12$
White grape juice	$0.97^c \pm 0.04$	$3.54^k \pm 0.10$
Cinnamon, hot water (1:100, w/v)	$1.12^d \pm 0.04$	$20.78^l \pm 0.38$
Allspice, hot water (1:100, w/v)	$0.80^e \pm 0.03$	$3.05^k \pm 0.08$
Beverage formulations		
Beverage, hot water	$1.82^f \pm 0.04$	$9.73^m \pm 0.34$
Beverage, 50% aq. ethanol	$3.35^g \pm 0.05$	$10.53^{imn} \pm 0.94$
Commercial references		
Concord grape juice	$2.57^h \pm 0.08$	$8.78^{mo} \pm 0.49$
White tea, hot water (1 bag/8 fl. oz)	$1.20^d \pm 0.03$	$10.11^{mno} \pm 0.90$

Values in the same column followed by different letters are significantly different at $p < 0.05$

Table 3.3. Extract dilution required to achieve 50% inhibition (IC_{50}) of formation of fructose-induced advanced glycation endproducts from bovine serum albumin

Sample	IC_{50}[a]	Regression Equation[b]	Correlation Coefficient (R^2)
Beverage components			
Early Sumac sorghum, hot water (1:10, w/v)	210	y = 0.0441x - 0.1298	0.99
Early Sumac sorghum, 50% aq. ethanol (1:10, w/v)	781	y = 0.2042x - 0.2847	1.00
White grape juice	180	y = 0.0078x + 0.4349	0.93
Cinnamon, hot water (1:100, w/v)	100	y = 0.0165x + 0.0065	0.90
Allspice, hot water (1:100, w/v)	63	y = 0.019x + 0.0486	0.88
Beverage formulations			
Beverage, hot water	217	y = 0.0639x - 0.3844	0.78
Beverage, 50% aq. ethanol	489	y = 0.0775x + 0.0241	0.80
Commercial references			
Concord grape juice	319	y = 0.0618x - 0.0809	0.99
White tea, hot water (1 bag/8 fl. oz.)	223	y = 0.0156x + 0.395	0.89

[a]Extract dilution that inhibits glycation by 50% was determined by estimating 50% inhibition based on dose response curve.

[b]Regression equation fit to datapoints derived from analysis of samples at different dilutions.

Table 3.4. Consumer sensory results for a white grape juice/sorghum based beverage (n = 54 panelists; Male = 21, Female = 33)

	Mean ± SE	(Range)
Age (years)	33 ± 2	(20-62)
Frequency of Consumption[a] (5-point scale)	2.1 ± 0.2	(1-5)
Overall Acceptability[b] (9-point scale)	6.8 ± 0.2	(2-8)
Flavor (9-point scale)	6.8 ± 0.2	(2-9)
Color (9-point scale)	6.2 ± 0.2	(3-8)
Aroma/Smell (9-point scale)	7.0 ± 0.2	(4-9)
Purchase Intent[c] (5-point scale)	3.9 ± 0.1	(2-5)

[a]Freqency of consuming single-serve teas or juices
  1 = less than once per month, 2 = few times per month, 3 = one or two times per week,
  4 = three to five times per week, and 5 = daily
[b]Beverage attributes were evaluated using a 9-point hedonic scale from 1 = dislike
  extremely to 9 = like extremely.
[c]Purchase intent evaluated on a 5-point scale from 1 = definitely would not buy to 5 =
  definitely would buy.

Figure Captions

Figure 3.1. Frequency of consumption 5-point scale (a), 9-point structured hedonic scale for

acceptability (b), and 5-point purchase-intent scale (c)

(a)

| | < Once/ Month | Few Times/ Month | 1-2 Times/ Week | 3-5 Times/ Week | Daily |

(b)

Dislike Extremely — Dislike Very Much — Dislike Moderately — Dislike Slightly — Neither Like nor Dislike — Like Slightly — Like Moderately — Like Very Much — Like Extremely

(c)

Definitely Would Not Buy — Probably Would Not Buy — Maybe/ Maybe Not — Probably Would Buy — Definitely Would Buy
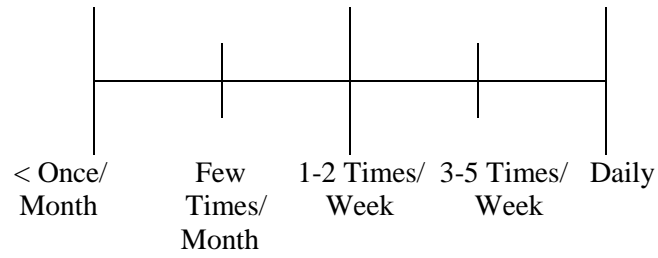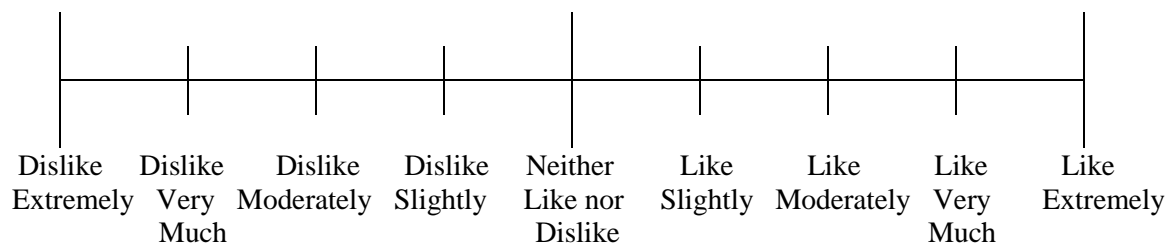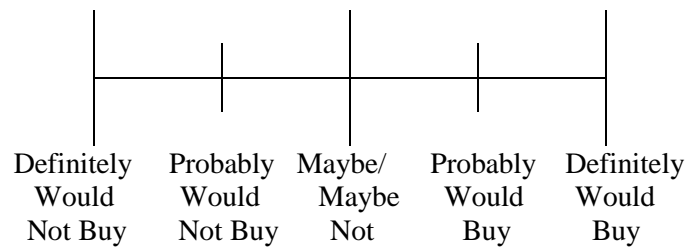
Figure 3.1

CHAPTER 4. ANTIOXIDANT PROPERTIES OF LOW-MOISTURE APPLE AND GREEN

TEA PRODUCTS

ABSTRACT

The purpose of this study was to evaluate the stability of phytochemicals and antioxidant activity in dried green tea (GT)-fortified apple products at low water activity. Slurry from blanched apple flesh was supplemented with a hot water green tea-extract and freeze-dried. Freeze-dried samples were stored at $a_w = 0.113$, 0.216, and 0.324 and 30°C for up to 52 days. The corresponding equilibrium moisture contents were 2.81, 4.50 and 6.87 g water/100 g for apples and 2.63, 4.76 and 6.95 g water/100 g for the GT-apple samples. The pH of apple and GT-apple products were 3.58 and 3.72, respectively. Color measurements showed that L* decreased and a* increased for all samples. Degradation of flavan-3-ol compounds and caffeine, ascorbic acid, chlorogenic acid, and total dihydrochalcones followed a pseudo-first-order kinetic model. There were differing rates of degradation amongst analytes, although there was a general trend of increasing magnitude of k with increasing moisture content. Antioxidant activity measured by the 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH) scavenging activity showed initial activity values of 15.5 and 72.1 mmol Trolox eq/kg of product for apple and GT-apple products, respectively, with $k = 0.008$ to 0.010 and 0.003 to 0.004, respectively.

Keywords: Low moisture foods, Green tea, Apple, Polyphenolic compounds, Pseudo-first-order rate kinetic

INTRODUCTION

Green tea is harvested from the leaves and buds of the plant *Camellia sinensis.* Its popularity as a beverage originated in China over 2000 years ago. Green tea has many health benefits, and its consumption may provide protection against cancer (Tang, Yuemin, Zhou, Wang & Rongbin, 2008), atherosclerosis (Zaveri, 2006), metabolic syndrome (Thielecke & Boschmann, 2009), coronary heart disease, damage to skin from UV radiation (Nagle, Ferreira & Zhou, 2006), increasing abdominal and body fat (Maki et al., 2009; Wolfram, Wang & Thielecke, 2006), high cholesterol, Parkinsons' disease, Alzheimer's disease, and some neurological disorders (Zaveri, 2006). The leaves of green tea are composed of 15-20% protein; 5-7% carbohydrates including cellulose and simple sugars; lipids including linoleic and α-linolenic acids; xanthines such as caffeine and theophylline; chlorophyll and carotenoids; several volatile compounds; and 5% trace minerals (Cabrera, Artacho & Gimenez, 2006). The polyphenolic fraction of green tea is composed of monomeric flavan-3-ols including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), and (-)-epicatechin gallate (ECG), which typically comprise about 50-80% of the catechin content (Chen, Zhu, Tsang & Huang, 2001; Green, Murphy, Shulz, Watkins & Ferruzzi, 2007). The monomers may also epimerize when exposed to heat or high pH (≥ 5), forming (-)-catechin (C), (-)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), and (-)-catechin gallate (CG) (Wang, Zhou & Jiang, 2008). In response to storage, hydrolysis of the gallate moiety may also occur (Ito et al., 2003). Green tea also contains procyanidins, which have implications in inhibiting glycation of protein (Nakagawa, Yokozawa, Terasawa, Shu & Juneja, 2002). EGCG is the predominant monomeric flavonoid, comprising 60% of the total catechins (Cabrera et al., 2006).

EGCG may also increase thermogenesis and fat oxidation (Boschmann & Thielecke, 2007) and has anti-obesity and anti-diabetic properties (Kao, Chang, Lee & Chen, 2006).

The stability of green tea catechins is dependent on factors of pH, temperature, time, moisture, and presence of other chemical compounds (Chen, Zhu, Wong, Zhang & Chung, 1998; Wang et al., 2008). Ascorbic acid has shown protective effects for catechin stability, whereas citric acid has not (Chen et al., 1998; Zhu, Hammerstone, Lazarus, Schmitz & Keen, 2003). pH stability also affects recovery of catechins through digestion (Green et al., 2007). Degradation of green tea catechins in an aqueous system followed pseudo-first-order rate kinetics (Wang et al., 2008). When studied in dry powder beverage mixes, minimal degradation occurred at relative humidity < 40%. Rates of degradation then increased with increasing relative humidity of the storage environment (Ortiz, Ferruzzi, Taylor & Mauer, 2008).

Apples are popular fruits that are often consumed fresh, in juice or sauce, and are available in the marketplace as chips or dried slices. Consumption of apples may decrease chronic disease risk associated with coronary heart disease, asthma, diabetes, and cancer (Boyer & Liu, 2004). Apples are high in phenolic content, such as chlorogenic acid, hydroxycinnamic acids, catechin, epicatechin, procyanidins, quercetin, and dihydrochalcones (Guyot, Marnet, Sanoner & Drilleau, 2003; Khanizadeh, Tsao, Rekika, Yang, Charles & Rupasinghe, 2008). Polyphenolic compounds such as in apples provide color, bitterness, and astringency (Khanizadeh et al., 2008; Lea, 1990). During processing, the total phenolic content of apple pomace was the same when either air-dried or lyophilized (Schieber, Keller & Carle, 2001).

There is increased interest in formulating functional foods that provide the benefits of bioactive compounds from more than one source. In this study, we investigated combinations of an apple and green tea product processed to low moisture content. The water activity levels were

chosen to simulate a product that would be used as a dry ingredient or part of a crisp, dry snack. The stability of the overall antioxidant potential, and that of key bioactive compounds, was studied over a 52 day period.

MATERIALS & METHODS

Samples of apple and green tea supplemented apple (GT-apple) were prepared and stored at low water activity (0.113, 0.216 and 0.324) at 30°C for up to 52 days. The pH, moisture content, titratable acidity and soluble solids were determined. At regular intervals, samples were analyzed for the content of select bioactive compounds, as well as total phenolic and FRAP antioxidant potential.

Preparation of Green Tea Extract

A hot water green tea extract was prepared using Java Green Tea (Twinings, London, UK). Extract was prepared by steeping 25 g of dried tea leaves in 500 mL of pre-heated 85°C deionized water for 5 min. The extract was then immediately chilled in ice slurry and filtered through Whatmann no. 4 filter paper.

Preparation of Apples and Green Tea-Supplemented Apples

Fresh apples (var. *Golden Delicious*) (Melinda, Revo, Italy) were peeled, cored, and quartered. Two homogeneous batches of apple pieces were created by manually cutting fruit into two portions and randomly allocating quartered portions to each batch. After each apple was peeled and cut, portions were immediately immersed in deionized water. Each batch (2 kg) of apples was suspended in a wire basket and then blanched in boiling deionized water for 4 min.

Apple batches were then rapidly cooled in new containers of fresh, 4°C deionized water and transferred onto paper towels to drain. Apple slices were then pureed for 3 min in a K 3000 Braun Multisystem blender (Braun, Kronberg, Germany). To batches of apples designated for supplementation, 384 mL of green tea extract was added. The control batch had 384 mL of water added. Control and green tea-supplemented (GT-apple) batches were then lyophilized in a Lyoflex Edwards freeze-drier (Crawley, UK). Freeze-dried material was then ground into powder and sieved manually (800 μm mesh size).

Storage Study

Approximately 0.141 g apple or GT-apple powders were weighed into Petri dishes. Duplicate samples were placed into airtight plastic boxes onto platforms suspended above a saturated salt solution (Sigma-Aldrich, Milan) to maintain a specific relative humidity. The saturated salt solutions and target water activities were: lithium chloride, $a_w = 0.113$; potassium acetate, $a_w = 0.216$; and magnesium chloride, $a_w = 0.324$. Samples were stored at 30°C in an environmental chamber. The a_w of salt solutions was checked using an Aqualab water activity meter (Decagon Devices, WA, USA) and values were consistent with those published by Greenspan (Greenspan, 1977).

Physicochemical Properties

The moisture content of apple and GT-apple samples was determined by drying samples in a vacuum oven at 70°C and 6.7 kPa for 18 h (AOAC, 1998). For soluble solids determination, samples were diluted with deionized water (0.5 g of sample in 20 mL of water) and allowed to equilibrate to 20°C. The total soluble solids was determined at 20°C using an

RFM 340 refractometer (Bellingham & Stanley, Ltd., Tunbridge Wells, UK). Results were expressed as °Brix (g of sucrose·100 g^{-1}, dry weight). The pH of solutions was determined using a model 62 pH meter (Radiometer, Copenhagen, Denmark). Titratable acidity of products was determined by performing a titration with 0.1 N sodium hydroxide solution that was standardized to pH 8.1. Results were reported as g of malic acid·100g^{-1}, dry weight. L*, a*, and b* color of samples was determined using a model SL-2000 Chromameter (Labo scientifica, Parma, Italy), which was standardized against a white standard tile.

Analysis of Bioactive Compounds

Prior to analysis on each sampling day, three separate extractions were performed on samples: 0.5 g of sample was extracted with either 10 mL methanol (Van der Sluis, Dekker, Skrede & Jongen, 2002), 10 mL of aqueous acetone (70:30, acetone:water, v/v) (Vanzani et al., 2005), or 5 mL of 6% metaphosphoric acid (containing 1 g·L^{-1} of potassium metabisulphite) (Vrhovsek, Rigo, Tonon & Mattivi, 2004). The extracts were vortexed for 2 min and centrifuged (10,000 × g for 10 min at 15°C). Supernatants were then transferred and filtered through Whatmann no. 4 filter paper.

Phenolic Compounds. Phenolic compounds were determined using the methods of Tomas-Barberan et al (Tomas-Barberan, Gil, Cremin, Waterhouse, Hess-Pierce & Kader, 2001). For HPLC analysis, an aliquot of extract was diluted in water:methanol (95:5, v/v) containing 5% formic acid. A Waters HPLC was used with a 250 × 4.6 mm i.d., 5 µm, Symmetry C18 reverse-phase column (Waters, Vimodrone, Italy). The HPLC included a model 600 HPLC pump, a model 2996 photodiode array detector and Empower software for data collection and analysis. Stock solutions of methanol and water with 5% formic acid were made before

preparation of quaternary mobile phases. These included (A) water/methanol (95:5, v/v), (B) water/methanol (88:12, v/v), (C) water/methanol (20:80, v/v), and (D) methanol. The gradient elution profile consisted of: 0-5 min with 100% A; 5.1-10 min following a linear gradient to reach 100% B; 10.1-13 min at 100% B; 13.1-35 min following a linear gradient to reach 75% B and 25% C; 35.1-50 min following a linear gradient to reach 50% B and 50% C; 50.1-52 min following a linear gradient to reach 100% C; 52.1-57 min at 100% C; 57.1-60 min at 100% D. The flow rate was 1 mL·min^{-1}, and the injection volume was 10 µL.

Standards of caffeine, chlorogenic acid, and phloridzin were from Sigma-Aldrich (Milan, Italy); standards of C, EC, ECG, EGC, EGCG, GCG, and procyanidin B2 were from Extrasynthese (Lyon, France). Standards for caffeine, chlorogenic acid, EC, and phloridzin (phloretin 2'O-glucoside) were used for peak identification and for quantification using calibration curves. Standards for C, ECG, EGC, EGCG, and GCG were used for peak identification. The catechins were quantified based on a calibration curve of EC, and then by using relative response factors (Wang, Provan & Helliwell, 2003). Chlorogenic acid was measured at $\lambda = 330$ nm, whereas other standards were measured at $\lambda = 280$ nm.

Ascorbic Acid. Standards for ascorbic acid were purchased from Sigma-Aldrich (Milan, Italy). The ascorbic acid content of extracts containing 6% metaphosphoric acid was determined following the methods of Mannino and Pagliarini (Mannino & Pagliarini, 1988). The Waters model HPLC had a Bio-Rad Fruit Quality Analysis column (300×7.8 mm, i.d.). An isocratic mobile phase consisted of 1 mM sulfuric acid operated at 22°C with a flow rate of 1 mL·min^{-1}. Ascorbic acid was detected at $\lambda = 245$ nm and quantified based on a calibration curve. The concentration of ascorbic acid was expressed as mg·kg^{-1}, dry weight of product.

Procyanidins. The estimation of total procyanidin content was performed using the vanillin assay (Sun, Ricardo-da-Silva & Spranger, 1998). To prepare sample extracts for analysis, the extracts of 70% aqueous acetone were dried under nitrogen gas. Samples were then reconstituted in 1 mL of 0.1 M phosphate buffer (pH 7.0) and filtered through 500 mg Sep-pak C18 cartridges (Waters, Vimodrone, Italy). Analytes were eluted with 1 mL methanol (Vrhovsek et al., 2004). For extracts containing green tea, the chlorophyll interfered with the assay. It was removed by mixing 1 mL of sample extract with 1 mL of hexane, and then discarding the top layer. In the vanillin assay, 0.5 mL of filtered extract or standard (dissolved in methanol) was combined with 1.25 mL of 1% vanillin in methanol and 1.25 mL of 9N sulfuric acid in methanol (Sun et al., 1998). The reaction proceeded at 25°C and was monitored at 500 nm in a Jasco UVDEC-610 spectrophotometer (Jasco Europe, Cremelia, LC, Italy) until maximum absorbance was achieved. Estimation of procyanidin content was performed based on a calibration curve using catechin. The procyanidin contents were reported as mg of catechin equivalents·kg^{-1}, dry weight.

Folin-Ciocalteu Analysis

The total polyphenolic content of methanol and 70% acetone extracts were estimated using the Folin-Ciocalteu method following the methods of Singleton et al. (1999). The reaction mixture consisted of 76 µL of either standard or apple extract, or 38 µL of GT-apple extract with 38 µL of solvent, which was added to 6 mL of deionized water. Next, 380 µL of Folin Ciocalteu reagent was added along with 1,140 µL of 20% sodium carbonate. Samples were then incubated at room temperature for 45 min before evaluation of absorbance at $\lambda = 760$ nm on the spectrophotometer against a deionized water blank. All samples were evaluated in three

replicates. Results were estimated based on a calibration curve of gallic acid. The total polyphenolic content was reported as mg of catechin equivalents·kg^{-1}, dry weight of sample.

Ferric Reducing/Antioxidant Power (FRAP)

The ferric reducing/antioxidant power method (FRAP) was performed on the 70% acetone extracts following the methods described by Benzie and Strain (Benzie & Strain, 1996). In brief, the FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 20 mM ferric chloride, and 2.5 mL of 10 mM 2,4,6-tripyridyl-*s*-triazine in 40 mM hydrochloric acid (Sigma-Aldrich, Milan). The reaction mixture consisted of 3 mL of FRAP reagent and 1 mL of sample extract evaluated in different dilutions. The reaction mixtures were then incubated at 37°C for 4 min before measuring absorbance at λ = 593 nm against a deionized water blank. Iron (II) sulfate heptahydrate was used as a standard. Results were reported as mmol of ferrous sulfate equivalents·kg^{-1}, dry weight of sample.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was performed on the 70% acetone extracts based on the methods of Lavelli (Lavelli, 2002). The reaction mixture consisted of 2.5 mL of 6.35 × 10^{-5} M DPPH (Sigm-Aldrich, Milan) solution (DPPH dissolved in methanol) and 1 mL of 70% aqueous acetone extract from samples that were analyzed at several dilutions. The initial absorbance of the reaction mixtures was evaluated at λ = 515 nm and then after 30 min of incubation at room temperature. The 30 min incubation yielded constant absorbance values. Four dilutions of each sample were analyzed. Trolox (Sigm Aldrich, Milan) was used as a standard. Results were calculated as % decrease in DPPH concentration, which corresponded to

initial and final absorbance. Results were reported as Trolox equivalents, which represents the

ratio of I_{50} of Trox (nmol) per I_{50} of sample (mg, dry weight of sample).

Activity of Polyphenoloxidase and Peroxidase Enzymes

Determination of polyphenoloxidase activity (PPO) was conducted following the

methods of Alvarez-Parilla et al (Alvarez-Parrilla et al., 2007). For preparation of extracts, 0.10

g of apple powder was added to 1 mL of buffer (0.03 M acetic acid, 0.14 M dipotassium

phosphate, 1 M sodium chloride, and 5% (w/w) polyvinylpolypyrrolidone, pH 6.5). After

centrifugation (10,000 × g for 10 min at 20°C), the supernatant was then filtered through

Whatman no. 1 filter paper. For the evaluation of PPO activity, 0.1 mL of filtered supernatant

was added to 0.9 mL of 10 mM chlorogenic acid in filtered buffer. The reaction mixture was

incubated at 25°C, and the absorbance was measured at $\lambda = 400$ nm.

The activity of peroxidase enzymes was determined by adding a 200 µL of filtered

supernatant extract to 100 µL of 640 mM guaiacol, 100 µL of 400 mM hydrogen peroxide, and

3.6 mL of 100 mM phosphate buffer (pH 6.5) (Ahn, Paliyath & Murr, 2007). The mixture was

then incubated at 25°C and monitored for absorbance at 470 nm. The linear increase in

absorbance was then calculated to estimate the reaction rate.

Statistical Analysis

One-way Analysis of Variance was conducted using Tukey's Honestly Significant Difference

(HSD) test ($p < 0.05$) with Minitab 15 software (State College, PA).

RESULTS

Physicochemical Properties

The pH of apple and GT-apple samples was 3.58 and 3.72, respectively (Table 4.1). The pH of green tea is typically 4-6.5, so the slightly higher pH of GT-apple samples likely reflects the contribution of tea to overall pH. After lyophilization, the moisture contents of the products were significantly different ($p < 0.05$) (Table 4.1). After samples were stored in different relative humidity environments of a_w 0.113, 0.216 and 0.324, the equilibrium moisture contents were 2.81, 4.50 and 6.87 g H_2O/100 g for apple, and 2.63, 4.76 and 6.95 g H_2O/100 g for GT-apple, respectively. The titratable acidity was 2.52 and 2.76 g of malic acid/100 g for the apple and GT-apple products. Results were reported based on malic acid, the predominant organic acid in apples (Haard & Chism, 1996). The apple product had 74.1 g sucrose/100 g while the GT-apple had 76.1 g sucrose/100 g.

The L*, a*, and b* color values are shown for apple and GT-apple samples stored at a_w 0.33 in Figure 4.1. Initial L* values were 74.9 for apple and 79.8 for GT-apple. The L* values decreased for both apple and GT-apple during storage, reaching steady values after 15-20 days, indicating that samples became darker during storage. For both apple and GT-apple, the a* values increased from negative to positive values, indicating that samples lost green attributes and became redder. The b* values decreased over storage, while retaining positive values, indicating that samples lost yellow color. These color values show that samples became darker over storage, which may indicate formation of Maillard reaction products. No polyphenol oxidase activity was measured in the samples after blanching or storage.

Bioactive Compounds

To evaluate the storage stability of bioactive compounds, methanol extracts were evaluated by HPLC from 0 d (C_0) through 45 d (C_{45}) of storage. A pseudo-first-order kinetic equation was used to model degradation rates:

$$\ln C = Ae^{-kt} \tag{1}$$

where C is the concentration of bioactive compound, k is the rate constant, and A is a pre-exponential constant. In general, analytes showed either no change in concentration or decreased during storage (Table 4.2).

Initial concentrations of EC were higher for GT-apple (860 mg/kg) than for apple (277 mg/kg). EC decreased during storage in both apple and GT-apple samples, and the reaction rate constant k increased slightly with a_w. For example, at $a_w = 0.113$ EC in apple had decreased from 277 to 194 mg/kg after 45 days, while at $a_w = 0.324$ it had decreased to 160 mg/kg.

For apple, initial catechin levels were 56 mg/kg while for GT-apple they were 301 mg/kg. In apple, catechin content had decreased to 38 and 43 mg/kg (at $a_w = 0.113$ and 0.216), and the reaction rate constant increased with a_w. For GT-apple, initial catechin levels were 301 mg/kg, but significant changes were not consistently measured through storage.

Only green tea-supplemented samples contained EGC, EGCG, ECG and GCG. Initial levels were 2066, 2317, 548 and 542 mg/kg, respectively. Levels decreased somewhat during storage. For example, at $a_w = 0.216$ EGC, EGCG, ECG and GCG decreased to 1618, 1569, 295 and 455 mg/kg after 45 days. The highest rate constants were observed for ECG (0.011-0.016 d^{-1}). In general, rate constants increased with a_w, except for GCG in which they decreased slightly with a_w.

For procyanidin B2, similar levels were found initially in apple (498 mg/kg) and GT-apple (507 mg/kg) products. After 45 days, levels in apple had decreased to 464, 391 and 245 mg/kg (at a_w 0.113, 0.216 and 0.324). For GT-apple, only measurable changes could be found for the 0.324 a_w sample after 45 days (that is 310 mg/kg). More rapid degradation occurred in the apple product with increasing a_w than in GT-fortified product. It should be noted that for the HPLC analysis of procyanadin B2, samples were extracted in methanol. In subsequent analysis of total procyanidins extracted in 70% acetone and measured by the vanillin assay, there was greater degradation in the GT-apple product than in the apple. As shown in Table 4.2, initial levels of total procyanidins were substantially lower in the apple (1505 mg/kg) than in GT-apple (2438 mg/kg). At a_w = 0.324, total procyanidins in apple had decreased to 1064 mg/kg after 45 days and in GT-apple to 1562 mg/kg.

The contents of other analytes including caffeine, ascorbic acid, chlorogenic acid, and total dihydrochalcones are shown in Table 4.3. Caffeine is not found in apples, whereas GT provides a considerable source. Initial levels in GT-apple were 1429 mg/kg and had decreased to 1048, 1229 and 1211 mg/kg after 45 days (at a_w = 0.113, 0.216 and 0.324). Caffeine showed greater stability than other flavan-3-ol compounds, although decreases still occurred with storage.   Interestingly, the highest reaction rate occurred at the lowest a_w.

Contents of ascorbic acid were similar between apple (118 mg/kg) and GT-apple (120 mg/kg) products initially, and the rates of degradation were only slightly higher for apple during storage. The rates of degradation also increased with increasing a_w. After 45 days, ascorbic acid had decreased to 85, 82 and 76 mg/kg (at 0.113, 0.216 and 0.324 a_w) in the apple, and to 92, 91 and 80 mg/kg for the GT-apple.

Chlorogenic acid was very stable throughout storage. A slight decrease in contents occurred, but decreases were not sufficient to fit a first-order kinetic model to the data. Initial levels were 1061 and 1110 mg/kg for the apple and GT-apple. After 45 days, these had decreased to 969-1026 mg/kg for apple and 949-983 for GT-apple.

Initial levels of total dihydrochalcones, which includes phloridzin and phloretin 2'-O-xyloglucoside, were 135 mg/kg for apple and 142 mg/kg for GT-apple. Total dihydrochalcone degradation was similar for the apple treatments among all a_w, with levels of 101-112 mg/kg after 45 days. Greater decreases in total dihydrochalcones were seen in GT-apple treatments. After 45 days, levels were 75 and 98 mg/kg at 0.113 and 0.216 a_w, and were undetectable at 0.324 a_w.

In Vitro Antioxidant Activity

The fortification of apple products with GT-extract significantly increased all measured antioxidant activity levels (Table 4.4). The total phenolic content of lyophilized samples, as measured by the Folin-reducing capacity, was 8082 mg catechin eq/kg for apple and 17,476 mg catechin eq/kg for GT-apple. During storage, the levels of Folin-reducing capacity did not change for either apple or GT-apple samples.

The ferric reducing/antioxidant power (FRAP) assay showed that lyophilized GT-fortified apple samples had FRAP values that were 3-4 fold greater than that of the apple control (Table 4.4). Initial values for apple were 79 mmol $FeSO_4$/kg for apple and 272 mmol $FeSO_4$/kg for GT-apple. FRAP values also did not change during storage for either apple or GT-apple treatments.

113

Based on DPPH radical scavenging activity, the GT-apple sample had 4-5 fold greater radical scavenging activity than the apple control (Table 4.4). Initial values for apple were 15.5 mmol TE/kg, and for GT-apple 72.1 mg TE/kg. Degradation was also modeled using a pseudo-first-order kinetic model. During storage, decreases in radical scavenging activity occurred (Table 4.5), though a_w was not a determining factor. In the estimation of Trolox equivalents, the GT-apple samples had activity that was about six-fold greater than the apple control. During storage, degradation for apple treatments occurred at a more rapid rate than in GT-apple samples. Rates of degradation among a_w were consistent within treatments.

DISCUSSION

Low moisture foods that are dry, nonpliable, crisp and uncaked are usually at a_w levels of 0-0.45. Typical foods include dried ingredients, crackers, bread crust, milk powder, breakfast cereal, dried pasta, powdered cheese, or potato chips (Fennema, 1996). The apple and GT-apple products studied here fall into that range. The evaluation of apple and GT-apple products stored in low moisture environments provided insight into effects of storage on product attribute stability. This included physicochemical properties, phytochemical stability, and antioxidant activity. The total content of green tea catechins (GTC) retained after lyophilization was used to estimate desirable levels of GT needed for fortification purposes. A single serving of apple products (50g), which was comparable in size to similar commercially available products, was fortified with GT in order to provide the equivalent GTC content as that found in a standard cup of green tea. A single GT-fortified apple serving contained 330 mg of monomeric flavonoids and 120 mg of total procyanidins. This content was also estimated based on 120 mg of EGCG. This content is similar to a conventional cup of GT (Chen et al., 2001). EGCG was selected as a

114

marker for GTC estimation purposes, since it has significant antioxidative properties (Nagle et al., 2006; Wang et al., 2008).

There was slight darkening of both the dried apple and GT-apple during storage. As initial raw apples were blanched, any degradative enzymes should have been denatured. In fact, there was no measurable activity of polyphenol oxidase or peroxidase enzymes. Thus, the reactions that contributed to degradation in stored materials were attributed to non-enzymatic mechanisms. These reactions may include caramelization, ascorbic acid degradation, and the Maillard reaction (Burdurlu & Karadeniz, 2003).

One major series of pathways is the Maillard reaction. When the reaction proceeds at pH < 5, a reducing sugar reacts with an amine to form a glycosylamine, which can then undergo several reactions to produce products such as furans, pyrroles, or thiophenes (BeMiller & Whistler, 1996). At these acidic conditions, 5-Hydroxymethylfurfural is a common product and is an indication of quality deterioration in apple juice concentrate (Burdurlu et al., 2003). Further polymerization may induce formation of higher molecular weight substances known as melanoidins, which may possess a brown color. EC may inhibit the Maillard reaction by binding to sugar fragments (Totlani & Peterson, 2005). At low moisture contents (or a_w 0.1-0.4), the Maillard reaction proceeds at a slower rate compared to intermediate-moisture foods of a_w 0.6-0.8 (Fennema, 1996).

The low moisture conditions had variable effects on antioxidant stability. Similar rates of degradation occurred for EC in both apple and GT-apple. The catechin (C) content degraded significantly faster in apple products with increasing a_w, whereas no change occurred in GT-fortified products. EC and C are found naturally in apples and green tea (Hagen et al., 2007; Wang et al., 2003). During thermal processing or storage, flavan-3-ol compounds may isomerize

into a non-epimeric form (for example, epicatechin epimerizes to catechin) (Wang et al., 2008). The fortification of apple products with GT extract significantly increased concentrations of C and EC. This could explain why concentrations of C in GT-fortified product remained constant during storage. Matrix interaction effects, through the presence of multiple compounds, may have produced differences observed in stability. At 45 d, the C content in the apple control was below the limit of detection.

The flavan-3-ols of EGC, EGCG, ECG, and GCG are found only in GT (Boschmann et al., 2007), and thus were quantified in GT-fortified apple products. At lower reaction rates k, the correlation coefficient (R^2) did not always show a strong relationship when fit to a pseudo-first-order model; at higher degradation rates, the fit was generally better. Degradation rates of EGC were similar at all a_w. The rate constant k for EGCG increased with increasing a_w. This also occurred for ECG. The concentrations of GCG changed very little even with increasing a_w. As concentrations of EGCG decrease over storage, as with what occurred with increasing a_w, then the content of GCG could increase, since EGCG may isomerize into its non-epimeric form. In another mechanism, GCG may also epimerize to EGCG. Wang et al (2008) found that these pathways are temperature dependent. When held at 25°C for 16 d, EGCG contents decreased from 100 to 15%, and GCG contents increased to a maximum of 0.5% at 8 d before falling thereafter.

In a complex food matrix such as the GT-apple product, the presence of different food components such as ascorbic acid may affect the stability of GTC and other polyphenolic compounds. In this study, ascorbic acid levels decreased 20-40% over three months of storage. Rates of degradation increased with a_w for both GT-fortified and unfortified apple products. Ortiz et al (2008) evaluated the stability of GTC in dry beverage powders stored at relative

humidity from near 0 to 85%. At relative humidity < 43%, minimal degradation occurred when stored at 22°C for 3 months. Contents of EGC, EC, EGCG, ECG, and total catechins remained stable, although stability varied with the presence of sugar, ascorbic or citric acids. Over relatively short periods, the incorporation of ascorbic acid contributed to the stability of green tea flavan-3-ols or dimers in aqueous solutions (Chen et al., 1998; Zhu et al., 2003). In one study, ascorbic acid acted as an antioxidant for GTC stability during the first month of storage in aqueous conditions, but then theoretically reverted to a pro-oxidant status during subsequent months of product storage (Zhu et al., 2003).

Procyanidin B2 is also a flavan-3-ol compound that exists as a dimer. In apples, procyanidins comprise 40-90% of the total phenolic content (Khanizadeh et al., 2008). Procyanidin B2 was more stable than most other flavan-3-ol compounds, although degradation was greatest at a_w 0.33. A similar pattern was observed for the total procyanidin content. In our study, the greatest stability of procyanidins after 45 days was observed at a_w 0.11.

Adding green tea to apples also provided a source of caffeine. Caffeine contents degraded only modestly through storage. Ortiz et al (2008) found that caffeine was stable in all GT dry beverage powders stored at 0-85% relative humidity through three months of storage at room temperature.

In Vitro Antioxidant Activity

The fortificiation of apple products with green tea extract significantly increased *in vitro* antioxidant activity. This was analyzed as total polyphenolic compounds by the Folin-Ciocalteu method, by the FRAP assay for reducing potential of the antioxidants present, and by the DPPH radical scavenging activity.

In initial freeze-dried products, addition of green tea doubled the Folin reducing capacity over the control apple products (Table 4.4). These levels did not change significantly over storage. The levels measured in this study were consistent with that found in apple pulp (Hagen et al., 2007; Hamauzu, Yasui, Inno, Kume & Omanyuda, 2005). The Folin reducing capacity is a measure of total polyphenolic compounds present in the sample. However, the levels measured in this study were lower than the total individual polyphenolic contents measured by HPLC. There may also be interactions with other compounds such as Maillard reaction products, which may interfere with Folin-reducing capacity by contributing to the total content of oxidizable substances in the samples.

The FRAP assay measures the reducing potential of the antioxidants present in the sample to reduce ferric to ferrous iron. This assay is unique among antioxidant activity procedures in how it measures the reducing capacity of the antioxidants present in the system, rather than measuring the ability of the antioxidants in the system to survive an influx of reactive oxygen species (Benzie and Strain, 1996). In this study, GT addition increased FRAP values by about three-fold over the apple control (Table 4.4). These levels did not significantly change during storage. The FRAP values measured in this study were similar to those found for apple pulp (Khanizadeh et al., 2008).

In the DPPH radical scavenging activity evaluation, GT-fortification increased activity about 4-5 fold over the apple control (Table 4.4). Unlike the other *in vitro* analyses, the DPPH radical scavenging activities for apple and GT-apple decreased during storage. Degradation occurred about twice as rapidly for apple than for GT-apple products. Rates of degradation, as measured by *k,* did not show any trends among different storage humidities.

CONCLUSIONS

This research demonstrated the applicability of fortifying apple products with GT extract. Producing dried apples requires minimal thermal processing, and produces products that are microbiologically stable, while preserving the nutritional and original characteristics of the raw product. It would be beneficial to increase apple consumption among consumers, and increase the availability of GT polyphenols into the diet. Both apples and GT are antioxidant-rich food sources. When creating novel products, it is important to evaluate the antioxidant content and antioxidant activity in order to ensure stability and estimate shelf-life. A low-moisture product has limited free water available. It also provides a novel environment in which to study GT stability, which has mostly been studied in beverage or aqueous model systems.

TABLES

Table 4.1. pH, moisture content, titratable acidity, and soluble solids of apple and GT-apple products

Evaluation	Sample	
	Apple	GT-Apple
pH	$3.58^a \pm 0.01$	$3.72^a \pm 0.06$
Moisture Content		
after lyophilization	$3.71^b \pm 0.06$	$3.17^c \pm 0.17$
equilibrium a_w 0.113	$2.81^d \pm 0.03$	$2.63^d \pm 0.26$
equilibrium a_w 0.216	$4.50^e \pm 0.07$	$4.76^e \pm 0.12$
equilibrium a_w 0.324	$6.87^f \pm 0.25$	$6.95^f \pm 0.23$
Titratable Acidity (g malic acid·100g^{-1} dry product)	$2.52^g \pm 0.03$	$2.76^h \pm 0.01$
Soluble Solids (g sucrose·100g^{-1} dry product)	$74.08^i \pm 1.68$	$76.06^i \pm 1.21$

Values in the same row followed by different letters are significantly different at $p < 0.05$

Table 4.2. Initial (C_0) and 45 d (C_{45}) concentrations of flavan-3-ols in apple and GT-apple products at different a_w, along with rate constant (k). n.d.- below limit of detection; n.c.- k could not be calculated due to limited concentration change in analyte

Flavan-3-ol	a_w	C_0 (mg·kg^{-1})	(mg·kg^{-1})	C_{45} (mg·kg^{-1})	(mg·kg^{-1})	k (d^{-1})	R^2	k (d^{-1})	R^2
		Apple	GT-Apple	Apple	GT-Apple	Apple		GT-Apple	
EC	0.113	277[a] ± 18	860[b] ± 42	194[c] ± 7	585[e] ± 28	0.008	0.94	0.009	0.88
	0.216	277[a] ± 18	860[b] ± 42	187[c] ± 8	570[e] ± 20	0.009	0.91	0.010	0.96
	0.324	277[a] ± 18	860[b] ± 42	160[d] ± 6	555[e] ± 32	0.013	0.98	0.010	0.84
C	0.113	56[f] ± 4	301[g] ± 17	43[h] ± 2	278[i] ± 14	0.006	0.83	n.c.	
	0.216	56[f] ± 4	301[g] ± 17	38[h] ± 2	308[i] ± 13	0.008	0.87	n.c.	
	0.324	56[f] ± 4	301[g] ± 17	n.d.	249[i] ± 29	0.022	0.87	n.c.	
EGC	0.113	n.d.	2066[j] ± 157	n.d.	1513[k] ± 58	n.d.		0.006	0.55
	0.216	n.d.	2066[j] ± 157	n.d.	1556[k] ± 58	n.d.		0.007	0.67
	0.324	n.d.	2066[j] ± 157	n.d.	1514[k] ± 50	n.d.		0.007	0.74
EGCG	0.113	n.d.	2317[l] ± 63	n.d.	1779[m] ± 113	n.d.		0.006	0.47
	0.216	n.d.	2317[l] ± 63	n.d.	1569[m] ± 100	n.d.		0.009	0.83
	0.324	n.d.	2317[l] ± 63	n.d.	1478[m] ± 118	n.d.		0.012	0.89
ECG	0.113	n.d.	548[n] ± 28	n.d.	339[o] ± 31	n.d.		0.011	0.79
	0.216	n.d.	548[n] ± 28	n.d.	295[o] ± 25	n.d.		0.012	0.81
	0.324	n.d.	548[n] ± 28	n.d.	291[o] ± 22	n.d.		0.016	0.95
GCG	0.113	n.d.	542[p] ± 32	n.d.	426[q] ± 12	n.d.		0.005	0.54
	0.216	n.d.	542[p] ± 32	n.d.	455[q] ± 15	n.d.		0.004	0.33
	0.324	n.d.	542[p] ± 32	n.d.	473[q] ± 12	n.d.		0.003	0.41
Procyanidin B$_2$	0.113	498[r] ± 5	507[s] ± 6	464[t] ± 127	524[w] ± 26	n.c.		n.c.	
	0.216	498[r] ± 5	507[s] ± 6	391[u] ± 131	485[w] ± 28	0.007	1.00	n.c.	
	0.324	498[r] ± 5	507[s] ± 6	245[v] ± 145	310[x] ± 22	0.016	0.92	0.011	0.85
Total Procyanidins	0.113	1505[y] ± 257	2438[z] ± 89	1278[αγ] ± 127	2161[δ] ± 58	n.c.		n.c.	
	0.216	1505[y] ± 257	2438[z] ± 89	1551[α] ± 131	1797[δζ] ± 48	n.c.		0.007	0.93
	0.324	1505[y] ± 257	2438[z] ± 89	1064[βγ] ± 145	1562[εζ] ± 46	0.007	0.38	0.010	0.97

Values followed by different lowercase letters within same flavan-3-ol compound, day, and apple or apple-GT treatment by a_w are significantly different (p < 0.05). Flavan-3-ol monomers include epicatechin (EC), catechin (C), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and gallocatechin gallate (GCG).

Table 4.3. Initial (C_0) and 45 d (C_{45}) concentrations of caffeine, ascorbic acid, chlorogenic acid, and total dihydrochalcones in apple and GT-apple products at different a_w, along with rate constant (k). n.d.- below limit of detection; n.c.- k could not be calculated due to limited change in analyte.

Analyte	a_w	C_0 (mg·kg^{-1})	(mg·kg^{-1})	C_{45} (mg·kg^{-1})	(mg·kg^{-1})	k (d^{-1})	R^2	k (d^{-1})	R^2
		Apple	GT-Apple	Apple	GT-Apple	Apple		GT-Apple	
Caffeine	0.113	n.d.	1429[a] ± 61	n.d.	1093[b] ± 54	n.d.		0.006	0.45
	0.216	n.d.	1429[a] ± 61	n.d.	1262[b] ± 26	n.d.		0.003	0.71
	0.324	n.d.	1429[a] ± 61	n.d.	1163[b] ± 30	n.d.		0.004	0.68
Ascorbic acid	0.113	118[c] ± 4	120[d] ± 3	86[e] ± 1	95[f] ± 2	0.007	0.94	0.005	0.85
	0.216	118[c] ± 4	120[d] ± 3	80[e] ± 2	93[f] ± 1	0.008	0.84	0.006	0.97
	0.324	118[c] ± 4	120[d] ± 3	76[e] ± 2	82[g] ± 1	0.009	0.96	0.009	0.99
Chlorogenic acid	0.113	1061[h] ±18	1110[i] ± 41	984[j] ± 32	965[l] ± 21	n.c.		n.c.	
	0.216	1061[h] ±18	1110[i] ± 41	1026[k] ± 38	983[l] ± 24	n.c.		n.c.	
	0.324	1061[h] ±18	1110[i] ± 41	968[j] ± 7	949[l] ± 18	n.c.		n.c.	
Total dihydrochal-cones	0.113	135[m] ± 7	142[n] ± 7	101[o] ± 0	75[p] ± 4	0.007	0.87	0.012	0.83
	0.216	135[m] ± 7	142[n] ± 7	112[o] ± 7	98[q] ± 0	0.006	0.49	0.007	0.62
	0.324	135[m] ± 7	142[n] ± 7	109[o] ± 4	n.d.	0.005	0.90	n.d.	

Values followed by different lowercase letters within same analyte, day (C_0 or C_{45}), and apple or GT-apple treatment by a_w are significantly different ($p < 0.05$).

Table 4.4. Antioxidant activity of freeze-dried apple and GT-apple products

Evaluation	Apple	GT-Apple
Folin-reducing capacity (mg catechin eq·kg^{-1}, d.w.)	8082[a] ± 277	17476[b] ± 421
FRAP value (mmol ferrous sulfate eq·kg^{-1}, d.w.)	79[c] ± 7	272[d] ± 9
DPPH radical scavenging activity (mmol TE·kg^{-1}, d.w.)	15.5[e] ± 0.6	72.1[f] ± 1

Values followed by different letters within the same row are significantly different ($p < 0.05$).

Table 4.5. First-order rate constants for DPPH radical scavenging activity of apple and GT-apple products during storage, and Trolox equiv after 45 d of storage

| a_w | Apple | | | GT-Apple | | |
	k (d^{-1})	R^2	TE (mmol Trolox·kg^{-1},d.w.)	k (d^{-1})	R^2	TE (mmol Trolox·kg^{-1}, d.w.)
0.113	0.010	0.94	$10.0^a \pm 0.1$	0.004	0.88	$58.8^b \pm 1.0$
0.216	0.008	0.81	$10.4^a \pm 1.0$	0.003	0.98	$62.0^b \pm 0.6$
0.324	0.008	0.92	$10.8^a \pm 0.0$	0.004	0.80	$61.2^b \pm 1.3$

Values in columns followed by different letters are significantly different ($p < 0.05$).

Figure Captions

Figure 4.1. L* (a), a* (b), and b* (c) color values for GT-fortified (♦) and unfortified (◊) apple

products stored at a_w 0.33.
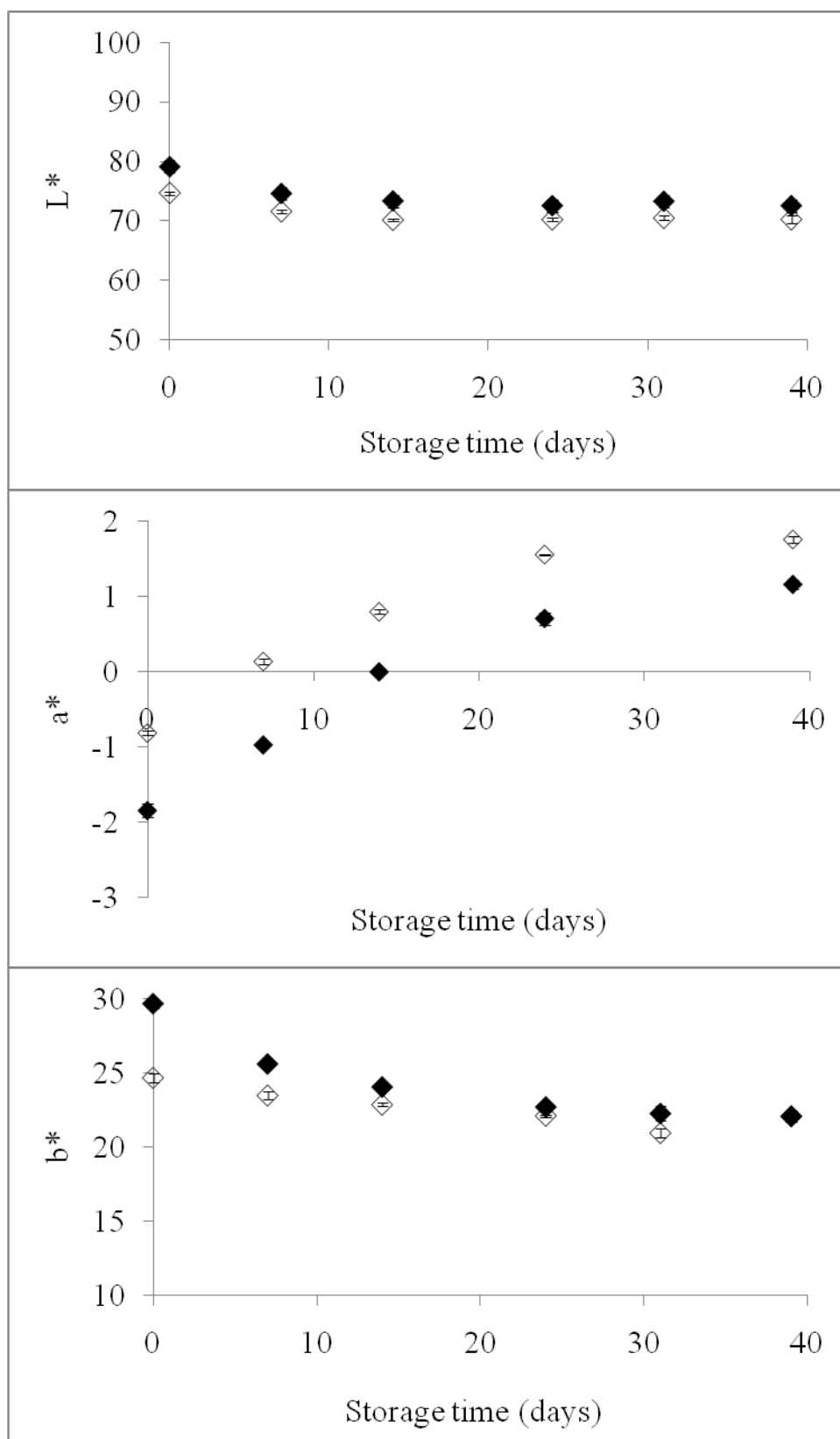
Figure 4.1

126

REFERENCES

AOAC 1998 International. *Official Methods of Analysis (Method 934.06).* Maryland, United States.

AOAC 2000 International. *Official Methods of Analysis (Method 942.15).* Maryland, United States.

Ahn, T., Paliyath, G., & Murr, P. D. (2007). Antioxidant enzyme activities in apple varieties and resistance to superficial scald development. *Food Research International*, *40*, 1012-1019.

Alvarez-Parrilla, E., de la Rosa, L. A., Rodrigo-Garcia, J., Escobedo-Gonzalez, R., Mercado-Mercado, G., Moyers-Montoya, E., Vazquez-Flores, A., & Gonzalez-Aguilar, G. A. (2007). Dual effect of β-cyclodextrin (β-CD) on the inhibition of apple polyphenol oxidase by 4-hexylresorcinol (HR) and methyl jasmonate (MJ). *Food Chemistry*, *101*, 1346-1356.

BeMiller, J. N., & Whistler, R. L. (1996). Carbohydrates. In: O. R. Fennema, *Food Chemistry* (pp. 157-224). New York: Marcel Dekker, Inc.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. *Analytical Biochemistry*, *239*, 70-76.

Boschmann, M., & Thielecke, F. (2007). The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: a pilot study. *Journal of the American College of Nutrition*, *26*(4), 389S-395S.

Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal*, *3*.

Burdurlu, H. S., & Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, *80*, 91-97.

Cabrera, C., Artacho, R., & Gimenez, R. (2006). Beneficial effects of green tea--A review. *Journal of the American College of Nutrition*, *25*(2), 79-99.

Chen, Z.-Y., Zhu, Q. Y., Tsang, D., & Huang, Y. (2001). Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry*, *49*(1), 477-482.

Chen, Z.-Y., Zhu, Q. Y., Wong, Y. F., Zhang, Z., & Chung, H. Y. (1998). Stabilizing effect of ascorbic acid on green tea catechins. *Journal of Agricultural and Food Chemistry*, *46*(7), 2512-2516.

Fennema, O. R. (1996). *Food Chemistry* (p. 1067). New York: Marcel Dekker, Inc.

Green, R. J., Murphy, A. S., Shulz, B., Watkins, B. A., & Ferruzzi, M. G. (2007). Common tea formulations modulate *in vitro* digestive recovery of green tea catechins. *Molecular Nutrition in Food Research*, *51*, 1152-1162.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards -- A. Physics and Chemistry*, *81A*(1), 89-96.

Guyot, S., Marnet, N., Sanoner, P., & Drilleau, J.-F. (2003). Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. *Journal of Agricultural and Food Chemistry*, *51*(21), 6240-6247.

Haard, N. F., & Chism, G. W. (1996). Ch. 16. Characteristics of edible plant tissues. In: O. R. Fennema, *Food Chemistry* (pp. 943-1012). New York: Marcel Dekker, Inc.

Hagen, S. F., Borge, G. I. A., Bengtsson, G. B., Bilger, W., Berge, A., Haffner, K., & Solhaug, K. A. (2007). Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica*) Borkh., cv. Aroma. *Postharvest Biology and Technology*, *45*, 1-10.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C., & Omanyuda, M. (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of chinese quince (*Pseudocydonia sinensis*

Schneid.), Quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *Journal of Agricultural and Food Chemistry*, *53*(4), 928-934.

Ito, R., Yamamoto, A., Kodama, S., Kato, K., Yoshimura, Y., Matsunaga, A., & Nakazawa, H. (2003). A study on the change of enantiomeric purity of catechins in green tea infusion. *Food Chemistry*, *83*, 563-568.

Kao, Y.-H., Chang, H.-H., Lee, M.-J., & Chen, C.-L. (2006). Tea, obesity, and diabetes. *Molecular Nutrition in Food Research*, *50*, 188-210.

Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M. T., & Rupasinghe, H. P. V. (2008). Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *Journal of Food Composition and Analysis*, *21*, 396-401.

Lavelli, V. (2002). Comparison of the antioxidant activities of extra virgin olive oils. *Journal of Agricultural and Food Chemistry*, *50*(26), 7704-7708.

Lea, A. G. H. (1990). Bitterness and astringency: the procyanidins of fermented apples cider. In: R. L. Roussef, *Bitterness in foods and beverages* (pp. 123-143). Oxford, UK: Elsevier.

Maki, K. C., Reeves, M. S., Farmer, M., Yasunaga, K., Matsuo, N., Katsuragi, Y., Komikado, M., Tokimitsu, I., Wilder, D., Jones, F., Blumberg, J. B., & Cartwright, Y. (2009). Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. *The Journal of Nutrition*, *139*, 264-270.

Mannino, S., & Pagliarini, E. (1988). A rapid HPLC method for the determination of vitamin C in milk. *Lebensmittel-Wissenchaft und-Technologie*, *21*, 313-314.

Nagle, D. G., Ferreira, D., & Zhou, Y.-D. (2006). Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. *Phytochemistry*, *67*, 1849-1855.

Nakagawa, T., Yokozawa, T., Terasawa, K., Shu, S., & Juneja, L. R. (2002). Protective activity of green tea against free radical- and glucose-mediated protein damage. *Journal of Agricultural and Food Chemistry*, *50*, 2418-2422.

Ortiz, J., Ferruzzi, M. G., Taylor, L. S., & Mauer, L. J. (2008). Interaction of environmental moisture with powdered green tea formulations: effect on catechin chemical stability. *Journal of Agricultural and Food Chemistry*, *56*(11), 4068-4077.

Schieber, A., Keller, P., & Carle, R. (2001). Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *Journal of Chromatography A*, *910*, 265-273.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152-178.

Sun, B., Ricardo-da-Silva, J. M., & Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry*, *46*, 4267-4274.

Tang, N., Yuemin, W., Zhou, B., Wang, B., & Rongbin, Y. (2008). Green tea, black tea consumption and risk of lung cancer: A meta-analysis. *Lung Cancer*.

Thielecke, F., & Boschmann, M. (2009). The potential role of green tea catechins in the prevention of the metabolic syndrome - A review. *Phytochemistry*, *70*(1), 11-24.

Tomas-Barberan, F. A., Gil, M. I., Cremin, P., Waterhouse, A. L., Hess-Pierce, B., & Kader, A. A. (2001). HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *Journal of Agricultural and Food Chemistry*, *49*, 4748-4760.

Totlani, V. M., & Peterson, D. G. (2005). Reactivity of epicatechin in aqueous glycine and glucose Maillard reaction models: Quenching of C2, C3, and C4 sugar fragments. *Journal of Agricultural and Food Chemistry*, *53*(10), 4130-4135.

Van der Sluis, A. A., Dekker, M., Skrede, G., & Jongen, W. M. F. (2002). Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *Journal of Agricultural and Food Chemistry*, *50*, 7211-7219.

Vanzani, P., Rossetto, M., Rigo, A., Vrhovsek, U., Mattivi, F., D'Amato, E., & Scarpa, M. (2005). Major phytochemicals in apple cultivars: Contribution to peroxyl radical trapping efficiency. *Journal of Agricultural and Food Chemistry*, *53*, 3377-3382.

Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry*, *52*, 6532-6538.

Wang, H., Provan, G. J., & Helliwell, K. (2003). HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry*, *81*, 307-312.

Wang, R., Zhou, W., & Jiang, X. (2008). Reaction kinetics of degradation and epimerization of epigallcatechin gallate (EGCG) in aqueous system over a wide temperature range. *Journal of Agricultural and Food Chemistry*, *56*(8), 2694-2701.

Wolfram, S., Wang, Y., & Thielecke, F. (2006). Anti-obesity effects of green tea: from bedside to bench. *Molecular Nutrition in Food Research*, *50*, 176-187.

Zaveri, N. T. (2006). Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sciences*, *78*, 2073-2080.

Zhu, Q. Y., Hammerstone, J. F., Lazarus, S. A., Schmitz, H. H., & Keen, C. L. (2003). Stabilizing effect of ascorbic acid on flavan-3-ols and dimeric procyanidins from cocoa. *Journal of Agricultural and Food Chemistry*, *51*(3), 828-833.

CHAPTER 5. KINETIC MODELING OF ANTIOXIDANT AND ANTI-GLYCATION PROPERTIES OF INTERMEDIATE-MOISTURE APPLE AND GREEN TEA PRODUCTS

ABSTRACT

This study evaluated the effects of storage (a_w 0.736, 30°C) on attributes of intermediate moisture products made with apple (control) or apple and green tea extract. Products were evaluated for soluble solids, pH, titratable acidity, color (L*, a*, b*), flavanol and ascorbic acid contents, total polyphenols, ferric reducing antioxidant potential (FRAP), 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH) scavenging activity, and ability to inhibit formation of fructose-induced advanced glycation endproducts (AGE). A single serving of the fortified product (50 g) provided equivalent catechin content to one cup of green tea. Total phenolics, FRAP values, DPPH radical scavenging activity and inhibition efficiency of fructose-induced AGE formation were about 3-5 times greater for the fortified product than control. Over storage for 45 days, antioxidant contents generally decreased. However, the fortified product maintained much higher antioxidant and anti-AGE formation activities than the control, suggesting that it could have potential benefits for metabolic syndrome or diabetic consumers.

Keywords: Intermediate moisture foods, Green tea, Apple, Glycation, Metabolic syndrome

133

INTRODUCTION

A diet rich in fruits and vegetables that is high in phytochemicals may contribute to health maintenance by reducing risk of chronic disease (*1*). Apples are rich in phytochemicals such as flavan 3-ols (oligomeric procyanidins, epicatechin and catechin), hydroxycinnamic acids (chlorogenic acid and a *p*-coumaric acid derivative), dihydrochalcones (phloridzin and phloretin 2'*O*-xyloglucosidee), flavonols (quercetin glycosides) and anthocyanins (cyanidin glycosides) (*2-4*).   Their polyphenolic content is comprised by 70-90% of flavan-3-ols, 4-18% as hydroxycinnamates, 1-10% as flavonols, 2-6% as dihydrochalcones, and 1-3% as anthocyanins in some apple varieties (*5*). Apples have anti-carcinogenic properties (*6*) and may help prevent development of chronic diseases such as cardiovascular disease, diabetes, asthma, pulmonary dysfunction, while aiding in weight management (*2*).

Another food that is high in dietary sources of phytochemicals and surpasses apples in antioxidant content and potential health related properties is green tea. The high polyphenolic content of GT contributes health-promoting effects. GT leaves are composed of about 10% (w/w) flavan 3-ols, which are referred to as catechins (*7*).   The most prevalent compounds include (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), and (-)-epicatechin gallate (ECG) (*8*).   When exposed to sub-optimal conditions of increasing processing temperature or pH > 5 epimerized catechins may degrade to become undetectable, or to a corresponding isomer that is present in a non-epicatechin form, such as (-)-gallocatechin gallate (GCG), (-)-gallocatechin (GC), (-)-catechin gallate (CG), or (-)-catechin (C) (*8-10*).

Health benefits associated with GT consumption include anti-diabetic, anti-obesity (*11*), anti-oxidative (*12*), anti-inflammatory, anti-mutagenic, anti-carcinogenic properties (*13, 14*), and prevention of metabolic syndrome (12, 13). Additional GT properties may include prevention of

dental caries (*15*) and protection of skin from UV exposure (*12*). EGCG, which is often the most predominant flavan-3-ol monomer in GT extract, may possess thermogenic properties (*16*). Metabolic syndrome and obesity are becoming increasingly prevalent in Western societies.

Metabolic syndrome is associated with multiple risk factors including age, gender, hypertension, hypercholesterolemia, dyslipidemia, hyperglycemia, abdominal obesity, and insulin resistance (*17, 18*). When these risk factors are combined, there is a synergistic risk association toward increased cardiovascular morbidity. This may also increase disposition of risk for Type II diabetes. GT extract has anti-diabetic properties, which may result from modulation of energy balance, metabolism, and endocrine factors (*11*), or insulin-potentiating activity (*19*).

In humans, the cells of the body undergo a glycation process in response to oxidative damage. This is part of normal physiology, but in diabetic patients or those suffering from metabolic syndrome, the process may occur at an accelerated rate. It can be evaluated using the hemoglobin A1c test as a measure of 90-day blood glucose control (*2*). The glycation process occurs when a reducing sugar reacts with an amino group to form AGE and protein cross-linking (*20*), which are thought to contribute to development of chronic disease. Polyphenols found in GT may inhibit this reaction. In fact, extracts from polyphenol-rich spices and fruits (among which GT was not comprised) and purified phenolic compounds, including EGCG, were demonstrated to inhibit protein glycation (*21-23*).

The food industry has recently regarded GT as a promising source for food fortification, having the intention of improving the health-promoting properties of food products. Depending on the stability of GT flavonoids during storage in a fortified food, GT fortification could potentially become a dietary aid for diabetic patients, or those having metabolic syndrome.

The purposes of this study were: a) to produce a novel intermediate moisture (IM) apple product fortified with GT, and b) to determine over storage the stability of phenolic compounds, *in vitro* antioxidant activity, and anti-glycation properties.

MATERIALS AND METHODS

Materials

The following reagents sodium chloride, 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-*s*-triazine, $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, Folin-Ciocalteu phenol reagent, bovine serum albumin (97% fatty acid free), Chelex resin, fructose, sodium azide, sodium phosphate monobasic, sodium phosphate dibasic, and standards of ascorbic acid, caffeine, chlorogenic acid, and phloridzin were purchased from Sigma Aldrich (Milan, Italy). Standards of C, EC, EGC, EGCG, ECG, GCG, and procyanidin B2 were purchased from Extrasynthese (Lyon, France).

Sample Preparation

Java Green Tea (Twinings, London, UK) was purchased at a local supermarket. In a one-liter beaker, green tea GT extract was prepared by extracting 25 g of dried leaves in 500 mL of pre-heated 85°C deionized water. The extraction proceeded for 5 min before immersion of the beaker in ice/water slurry. The extract was then filtered through Whatmann no. 4 filter paper. A sample of extract was removed and diluted 1:20 (v/v) in water:methanol (95:5, v/v) containing 5% formic acid, which was analyzed by HPLC for phenolic content.

Fresh apples of the variety Golden Delicious (Melinda, Revo, Italy) were purchased from a local supermarket. Each apple was peeled, cored with seeds removed, and quartered. One half portion was designated for GT addition, and the other for control. Two-kg batches of apple

portions were placed into a wire mesh basket and immediately boiled in a deionized water pot at 100°C for 4 minutes. After cooking, the basket was rapidly removed from boiling water and immersed in a 4°C deionized water bath. Once cooled, the apple pieces were transferred onto paper towels to allow for drainage of excess water. After 5 min, apple slices were blended to a puree consistency in a K 3000 Braun Multisystem blender (Braun, Kronberg, Germany) for three min. To a 2-kg batch of apple slurry was added 384 mL of GT extract, which was manually blended with a spoon. Control and GT fortified apple slurries were then spread over stainless steel trays and freeze-dried in a Lyoflex Edwards freeze drier (Crawley, UK). The freeze-dried powders were ground in the food processor and sieved (800 μm pan sieve mesh size).

Storage Study

For the storage study, powders of apple and apple fortified with GT were weighed into Petri dishes (0.141 g of powder·cm^{-1}). Following the methods of Greenspan (*24*), the dishes were then placed onto wire mesh racks that were suspended within airtight plastic boxes above a sodium chloride saturated salt solution (a_w of 0.7509 ± 0.0011). The boxes were prepared in duplicate for each experimental treatment and stored at 30°C in a thermostated heating cabinet.   Water activity of the samples and of the saturated salt solutions was measured on an Aqualab water activity meter (Decagon Devices, WA, USA). Samples were periodically evaluated for color, antioxidant content and related properties.

Physicochemical Characterization

The moisture content of samples at equilibrium with the environmental chambers was determined by drying samples in a vacuum oven (*25*). Results were reported as g of water·100 g^{-1} sample, dry weight. Color evaluation of samples was performed using a model SL-2000 Chromameter (Labo scientifica, Parma, Italy) with calibration using a white standard   The

Hunter L*, a*, and b* coordinates were recorded, which indicated lightness and darkness (L*), redness (+a*), greenness (-a*), yellowness (+b*), and blueness (-b*). Mean values were reported.

Freeze-dried samples were diluted 1:20 (w/v) with deionized water held at 20°C. pH was determined using a model 62 pH meter (Radiometer, Copenhagen, Denmark). Soluble solids was determined using a model RFM 340 refractometer (Bellingham & Stanley, Ltd, Tunbridge Wells, UK). Results were reported as °Brix (g sucrose·100g^{-1} dry product). Titratable acidity was determined by performing a titration with 0.1 N sodium hydroxide solution to reach pH 8.1 (*26*). Results were reported as g of malic acid·100 g^{-1} of dry product.

Sample Extraction

Extractions of samples were performed with 0.5 g of sample in either 10 mL of methanol (*4*), 10 mL of acetone: water (70:30, v/v) (*27*), or 5 mL of 6% metaphosphoric acid (containing 1 g·L^{-1} of sodium metabisulphite) (*5*). The extracts were then vortexed for 2 min and centrifuged (10,000 × *g* for 10 min at 15°C). The supernatant was then removed and filtered through Whatman no. 4 filter paper. Methanolic extracts were evaluated for individual phenolic content and total phenolics, antioxidant activity, and anti-glycation activity. Acetone: water extracts were evaluated for procyanidin content, antioxidant activity and anti-glycation activity. The 6% metaphosphoric acid extracts were analyzed for ascorbic acid content.

HPLC Determinations of Phenolic Compounds

The determination of phenolic compounds was conducted following the methods of Tomas-Barberan et al (*28*). A model 600 HPLC pump coupled with a model 2996 photodiode array detector, operated by.Empower software (Waters, Vimodrone, Italy) was used. The separation was performed using a 250 x 4.6 mm i.d., 5 μm, Symmetry C18 reverse-phase column

(Waters, Vimodrone, Italy). For preparation of quaternary mobile phases, 5% formic acid was first added to stock solutions of methanol and water. Mobile phases were prepared from these solutions as follows: mobile phase A consisted of water/methanol (95:5, v/v), mobile phase B was water/methanol (88:12, v/v), mobile phase C was water/methanol (20:80, v/v), and mobile phase D was 100% methanol. The gradient elution system was as follows: 0-5 min, used 100% A; 5.1-10 min followed a linear gradient to reach 100%; 10.1-13 min used 100% B; 13.1-35 min followed a linear gradient to reach 75% B and 25% C; 35.1-50 min followed a linear gradient to reach 50% B and 50% C; 50.1-52 min followed a linear gradient to reach 100% C; 52.1-57 min used 100% C; 57.1-60 min used 100% D. Injection volume was 20 μL using a Rheodyne loop. All analyses were conducted at 22°C. The flow rate was 1.0 mL·min^{-1}. The eluent was monitored by a model 2996 photodiode array detector already specified: model 2996 set at λ = 280 and 330 nm.

Standards of chlorogenic acid, EC, phloridzin (phloretin 2'O-glucoside) and caffeine were used to identify peaks by retention times and UV-vis spectra, and to build calibration curves for quantification. EC, phloridzin, and caffeine were quantified at 280 nm; chlorogenic acid at 330 nm. Standards of EGCG, EGC, ECG, GCG and C were used to identify peaks by retention times and UV-vis spectra. These flavan-3-ols were quantified using the calibration curve built with EC and the relative response factors reported by Wang et al. (2003).

A peak was tentatively assigned to phloretin 2'O-xyloglucoside based on its UV-vis spectrum and literature data and quantified at 280 nm using the calibration curve built with phloridzin. Concentrations were expressed as milligrams per kilogram of dry product.

Ascorbic Acid Determination

The ascorbic acid content was determined by the Waters model HPLC following the methods of Mannino and Pagliarini (*29*). Sample extract was diluted with 6% metaphosphoric acid that contained 1 g·L^{-1} of sodium metabisulphite. A Bio-Rad Fruit Quality Analysis column (300 × 7.8 mm i.d.) was used. The mobile phase was 1 mM sulfuric acid run under isocratic conditions at 22°C at a flow rate of 1 mL·min^{-1}. Ascorbic acid was detected at $\lambda = 245$ nm and quantified based on a calibration curve made with pure standard. Analyte concentrations were reported as mg·kg^{-1} of dry product.

Determination of Procyanidin Content

To prepare samples for total procyanidin determination, 0.25 mL aliquots of sample from 70% aqueous acetone extracts were dried under nitrogen gas. Once dried, samples were reconstituted in 1 mL of 0.1 M phosphate buffer, pH 7.0. Samples were then filtered through a 500 mg Sep-pak C-18 cartridge (Waters, Vimodrone, Italy). This was eluted with 1 mL of methanol (*5*). For samples containing GT, chlorophyll was removed by mixing 1 mL of hexane with 1 mL of the filtered sample extract. The lower phase was used for analysis.

To conduct total procyanidin content analysis, the vanillin assay was followed using the methods of Sun et al (*30*). In brief, 0.5 mL of filtered sample or standard dissolved in methanol, 1.25 mL of 1% vanillin dissolved in methanol, and 1.25 mL of 9N sulfuric acid dissolved in methanol were added together. The mixture was incubated at 25°C until maximum absorbance was achieved at $\lambda = 500$ nm. Results were estimated using a standard curve made from catechin. Results are reported as mg of catechin equivalents·kg^{-1} of product, dry weight.

Folin-Ciocalteu Method

Analysis of the total polyphenolic content by the Folin-Ciocalteu method was conducted on methanol and 70% acetone extracts following the methods of Singleton et al (*31*) with modifications. In brief, to 6 mL of deionized water was added either 76 µL of standard or apple extract or 38 µL of apple and GT extract with 38 µL of solvent. This was followed by 380 µL of Folin-Ciocalteu reagent and 1,140 µL of 20% sodium carbonate. The reaction mixtures were then incubated at 22°C for 45 minutes. During this time, samples were transferred to plastic cuvettes and evaluated for absorbance on a Jasco UVDEC-610 spectrophotometer (Jasco Europe, Cremella, LC, Italy) at $\lambda = 760$ nm against a deionized water blank. A standard curve was created using catechin. All samples and standards were analyzed in triplicate. Results were reported as mg of catechin equiv·kg^{-1} of dry sample.

Determination of Ferric Reducing/Antioxidant Power (FRAP)

The Ferric Reducing/Antioxidant Power method (FRAP) was completed following the methods of Benzie and Strain (*32*) with modification. To each tube was added 1 mL of methanolic or 70% acetone extract from sample and 3 mL of FRAP reagent (25 mL of 300 mM acetate buffer, pH 3.6; 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-*s*-triazine; 40 mM HCl; 2.5 mL of 20 mM ferric chloride). Tubes were vortexed and incubated in a 37°C water bath for exactly 4 min. Absorbance of samples was then measured on a spectrophotometer at $\lambda = 593$ nm against a deionized water blank. Iron (II) sulfate heptahydrate was used as standard (0.1, 0.2, 0.4, 0.6, and 1.0 mM). All samples and standards were measured in triplicate. FRAP results were reported as mmol of ferrous sulfate equivalents·kg^{-1} of dry sample.

Determination of DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was completed following the methods of Lavelli (*33*). The reaction mixture consisted of 1 mL of 70% aqueous acetone extract from samples of various dilutions and 2.5 mL of 6.35×10^{-5} M DPPH solution (DPPH dissolved in methanol). The initial absorbance of the solution was measured at $\lambda = 515$ nm, and then through 30 min of incubation at 22°C. Constant absorbance values were achieved through 30 min of incubation. Trolox was used as standard. Four dilutions of duplicate samples were analyzed. Results were calculated as % decrease in DPPH concentration, which was determined based on decrease in initial and final absorbance. Results were reported as Trolox equivalents, which was the ratio of I_{50} of Trolox (nanomoles) per I_{50} of sample (mg, dry weight).

Determination of Enzymatic Activity

For determination of peroxidase and polyphenoloxidase enzymatic activity, 0.10 g of sample was added to 1 mL of buffer (0.03 M acetic acid, 0.14 M dipotassium phosphate, 1 M sodium chloride, and 5% (w/w) polyvinylpolypyrrolidone, pH 6.5) filtered through Whatmann no. 1 filter paper. The mixture was then centrifuged at $10,000 \times$ g at 20°C for 10 min.

Peroxidase activity was determined following the methods of Ahn et al (*34*). A 200 μL aliquot of filtered supernatant was added to 100 μL of 640 mM guaiacol, 100 μL of 400 mM hydrogen peroxide, and 3.6 mL of 100 mM phosphate buffer (pH 6.5).

For the PPO assay, 0.1 mL supernatant was added to 0.9 mL of 10 mM chlorogenic acid in filtered buffer (*34*). This was then incubated at 25°C, and the reaction rate was estimated according to an increase in absorbance at $\lambda = 400$ nm. Results were expressed according to change in absorbance at 400 nm·min^{-1}·g^{-1} of dry sample.

Determination of Fructose-Induced Glycation of Bovine Serum Albumin

The inhibition of glycation of bovine serum albumin was conducted with both the methanolic and the 70% acetone extracts according to the procedure described by McPherson et al. (*20, 29*) with modification. Using 1.5 mL microcentrifuge tubes, the reaction mixture consisted of 50 µL of sample or standard, 900 µL of phosphate buffer (200 mM potassium phosphate buffer, pH 7.4 with 0.02% sodium azide, treated with Chelex resin), 300 µL of albumin solution (50 mg·mL^{-1} bovine serum albumin in chelex-treated phosphate buffer), and 300 µL of fructose solution (1.25 M fructose in chelex-treated phosphate buffer). Tubes were capped and incubated at 38°C for 72 h. Following incubation, samples were analyzed for fluorescence on a Perkin-Elmer LS 55 Luminescence Spectrometer (Perkin-Elmer, United Kingdom) with an excitation/emission wavelength pair $\lambda = 370/440$ nm with 5 nm slit width, read against incubated phosphate buffer blank. Each standard or sample was evaluated at five dilutions in triplicate over two separate days. Methanolic extract of catechin was evaluated as an external standard. Results were reported as % inhibition of fructose-induced glycation of bovine serum albumin, where % inhibition = (fluorescence intensity of sample or standard − intensity of albumin standard) / (intensity of albumin and fructose standard − intensity of albumin standard). Data points of concentration versus % inhibition were plotted and fitted with linear regression models using Excel® 2003 software. An estimate of inhibition of protein glycation by 30% (IC$_{30}$) was selected, since this level of inhibition occurred within the linear range of all samples evaluated. Results were reported based on the 30% inhibition of AGE formation from the external standard catechin.

RESULTS

Physicochemical Characterization of Product

The titratable acidity, pH, and soluble solids of apple and GT-apple samples was performed on product after freeze-drying (**Table 5.1**). The moisture contents of samples was determined after freeze-drying and then after equilibration at $a_w = 0.751$ (**Table 5.1**). Samples reached equilibration within 5 d. The titratable acidity of GT-fortified apple product was significantly higher than the control apple product ($p \leq 0.05$), although this difference is considered minimal. Results were represented based on g of malic acid, which is the predominant organic acid in apples (*35*). The pH of both apple and apple & GT products was high-acid, but was not statistically different between products ($p \leq 0.05$). The soluble solids of both samples were similar. The moisture contents of apple and GT-apple products were significantly different immediately after freeze-drying ($p \leq 0.05$), but were similar when equilibration was reached, having adsorbed water to about 26.3 % moisture, d.w.

The L*, a*, and b* color evaluation of samples was performed through 52 d of storage (**Figure 5.1**). Changes in colorimetric parameters were modeled to pseudo-zero-order kinetic models, with changes occurring over time indicated by variation in the rate constant *k* (**Table 5.2**). Since samples equilibrated within 5 d of storage, color values are also indicated after 5 d of storage and then after 30 d of storage. After 5 d of storage, the L* (0 = blackness, 100 = whiteness) for GT-fortified apple product was slightly darker than apple, having values of 70 and 73, respectively. The color became increasingly darker at a similar rate for both treatments, which is evidenced by the similar values of *k*. The a* (- a = greenness, + a = redness) values for GT-fortified apple product and control apple were very similar but increased over storage, indicating the development of more red coloration. The apple control increased only marginally

144

faster than GT-fortified product. The b* (- b = blueness, + b = yellowness) values for both GT-fortified and unfortified apple products decreased during storage within the yellow range, with a greater rate of decrease occurring for apple products.

Degradation of Phenolic Compounds during Storage

The degradation of polyphenolic compounds extracted with methanol from GT-fortified and unfortified apple products was modeled following a pseudo-first-order kinetic model:

1) $Ln\ C = Ae\text{-}kt$

(Table 5.3). The degradation rate constant k is indicated. Since equilibration of samples occurred within 5 d of storage, antioxidant concentrations are shown after 5 d and then after 30 d of storage. The correlation coefficient (R) is also shown, providing an indication of the model fit for the degradation of each analyte.

The monomeric flavan 3-ols EC and C are the predominant catechins found in apples (*2*). EC and C degraded significantly for apple. The C content of apple degraded below the limit of detection by 30 d of storage. For GT-fortified apple, the EC content was significantly greater than apple, and the degradation rate k was lower. The C content remained stable through storage.

The monomeric flavanols EGC, EGCG, ECG, and GCG are found only in GT (*16*).   In the GT-fortified apple samples, the most to least rapid levels of degradation occurred from EGCG > ECG > EGC > GCG. The caffeine found in GT-fortified apple also remained constant through storage.

The rates of chlorogenic acid degradation for GT-fortified and unfortified apple products were similar and relatively slower compared to other analytes. The ascorbic acid content was analyzed daily, and levels existed below the limit of detection by 10 d of storage. Contents of

procyanidin B2 and total procyanidins also decreased for both GT-fortified and unfortified apple products. The procyanidin B2 content determined in methanol extracts of GT-fortified apple samples was initially similar to apple, but degraded at only half the rate as in apple. The total procyanidins determined in the acetone extracts by the Vanillin assay degraded at similar rates among both sample treatments.

Inhibition of Fructose-Induced Glycation of Bovine Serum Albumin

Inhibition of fructose-induced glycation of bovine serum albumin was evaluated on methanol and 70% acetone extracts of GT-fortified and unfortified apple products (**Table 5.4**). Methanol extracts were evaluated at times 0, 7, 24, and 45 d of storage. The acetone:water (70:30, v/v) extracts were evaluated at 0 and 45 d of storage. These were considered extreme endpoints, which would demonstrate the greatest effect of storage on inhibition of glycation.

Among all methanol and 70% acetone extracts tested, there was a decrease in inhibitory activity following storage. In the methanol extracts, GT-fortified apple products had about a 3-4 fold greater AGE inhibitory activity than apple. A 35% decrease in inhibitory activity occurred for unfortified apple samples over storage. In GT-fortified apple extract from methanol, a significant decrease occurred between samples from 0 and 7 d of storage. Inhibitory activity then increased with storage. In 70% acetone extracts, GT-fortified apple products showed at least a two-fold increase in inhibitory activity compared to apple. In unfortified apple products, a significant decrease in activity occurred during storage over 45 d. In GT-fortified apple products, the decrease observed was not significantly different ($p \leq 0.05$). Inhibitory activity was also compared to catechin, which served as an external standard. In evaluation, 0.216 mg·mL^{-1} of catechin was necessary to induce a 30% inhibitory effect on AGE formation.

***In Vitro* Antioxidant Activity**

Total polyphenolic content by the Folin-Ciocalteu method, *in vitro* antioxidant capacity analysis by FRAP and DPPH radical scavenging activity, and estimation of inhibition of AGE were correlated to the total polyphenolic content (**Figure 5.2**) of 70% acetone.   Strong correlations were made for FRAP values (R = 0.96), DPPH radical scavenging activity (R = 0.91), and anti-AGE activity (R = 0.98) with total polyphenolic content.

DISCUSSION

GT-fortified and unfortified apple products evaluated over storage showed changes in physicochemical properties, phenolic content, antioxidant activity, and anti-glycation activity. In the marketplace, intermediate moisture food products exist such as rolled oats, dried fruits, or grained nougats (*36*). During transport and processing, dry powder mixes may also be inadvertently exposed to higher moisture conditions (*37*). As new functional food formulations arise that include the fortification of conventional food items with antioxidant-rich extracts such as green tea extract, information is needed on issues concerning phytochemical stability and antioxidant and health properties.   It is also important to investigate how the functional ingredient will affect the physical or chemical properties of the food to which it is added. This study showed such effects on product L*, a*, and b* color, and changes in nutritional quality through decreases in phenolic content, antioxidant activity, and anti-glycation properties through ability of extracts to inhibit formation of AGE.

During storage, the GT-fortified and unfortified apple products became noticeably darker in color. The changes in L*, a*, and b* indicated that the products became darker and browner. This may have occurred due to the formation of Maillard reaction products (*36*), caramelization

or ascorbic acid degradation (*38*). The pH of both treatments was pH 3-4. In acidic

environments of pH < 5, reducing sugars may react with amines to produce glycosylamines.

These may then undergo an Amadori rearrangement to form products such as furan derivatives

(*36*), which may develop brown color pigmentation. These polymeric materials may also affect

antioxidant properties (*39*). 5-Hydroxymethylfurfural is a Maillard reaction product that forms

under acidic conditions, especially at pH 3-5 (*40*). It is an indicator of deterioration of apple

juice concentrate quality during storage (*38*).

In the production of GT-fortified and unfortified products, hot water GT extract was

added to apple homogenate to provide the equivalent serving of a cup of green tea per serving

(50 g) of freeze-dried apple product. During freeze-drying, the fortified product retained about

80% of its green tea catechin (GTC) content. The fortification level was based on recovery of

GTC after freeze-drying. A 50 g serving of apple product is based on a similar serving size of

equivalent consumer products found in the marketplace. A serving of GT-fortified apple product

included 330 mg of monomeric flavonoids and 120 mg of total procyanidins. This content was

estimated based on EGCG content of 120 mg, which is consistent with a typical cup of GT that

contains 55-110 mg of EGCG (*41*). The EGCG content was selected as the marker catechin,

since it is often present as the predominant flavan 3-ol among green tea varieties, and has

significant bioactive properties (*10, 18*).

During storage through 45 d, most phenolic compounds degraded significantly.

Degradation followed a pseudo-first-order kinetic model, which is consistent with other studies

following green tea catechin degradation over storage (*9*). The kinetic constant, *k*, is reported

along with the correlation coefficient of the regression equation fit to model the degradation

(Table 2). Since one portion of the experimental objective involved modeling degradation over

storage, an estimate of phenolic content after 30 d of storage is also provided, including 95%

confidence limits.

Several factors may have contributed to phenolic degradation, which included moisture

content and availability (*37*), pH and ascorbic acid content (*10*), temperature (*9*), interaction with

phenolic compounds (*42*), food matrix composition, and oxygen. Phenolic degradation occurred

among both methanol and acetone:water (70:30, v/v) extracts. Aqueous acetone was selected as

an extracting solvent, since it is known to also remove the procyanidin fraction from samples

better than methanol or ethanol (*43*). The availability of moisture for the IM-products with

equilibrium moisture contents at 26.3% may have accelerated reaction rates. Previous work in

our laboratories (data not shown) showed a proportional relation between increasing a_w with

increasing rate of phenolic compound degradation in both GT-fortified and unfortified apple

products. This phenomenon is thought to occur because of increased mobility of molecules in

response to greater water availability (*37*). In the Maillard reaction, the highest reaction rates

regarding substrate loss occur in the intermediate water activity range (*36*).

The stability of GTC is affected by pH. In pH > 6, GTC readily degrade. They show

increasing stability at lower pH, with maximal stability between pH 3-4 during thermal

processing and storage (*10*). Apples are then suitable vehicles for GT delivery, since the pH of

the apple products was about pH 3.6-3.7, which is suitable for maintaining GTC stability. When

degradation of GTC occurs, some catechin reactants may epimerize into an isomeric form.

Epimerization may occur as a result of temperature and holding time during thermal processing

(*44*). For example, when EGCG is thermally processed, it may epimerize into GCG (*10*).

The epimerization process may occur among all GTC. In degradation, the epimerized catechin may convert to a non-epicatechin form (*9*), which may help explain GTC degradation (**Table 5.3**). Degradation and epimerization of EGCG may also occur co-currently. Wang et al (*9*) found that at temperatures above 44°C, GCG epimerized faster to EGCG than the rate that EGCG degraded. At temperatures above 98°C, the epimerization from GCG to EGCG became the dominant reaction pathway.

The caffeine contents of GT-fortified and unfortified apple products was very stable through storage. A 50 g serving of product would provide about 70 mg of caffeine, which is consistent with a cup of green tea infusion (*41*). A typical 8-oz. cup of American-style brewed coffee may have 70 – 110 mg caffeine (*45*). Methods are also available to naturally-decaffeinate green tea extract while retaining the polyphenolic content. By following a basic hot-water steeping procedure, 83% of the caffeine from green tea infusion can be removed while retaining 95% of total catechin content (*46*).

Most studies addressing the pH stability of GTC used beverage models for experimentation. As functional foods are increasingly fortified with GT for its health benefits, the GTC are exposed to varying levels of pH and moisture content. These levels are different from freshly brewed hot tea or acidified, shelf-stable beverages. Very few studies have addressed these issues. Ortiz et al (*37*) evaluated GTC dry beverage mixes with added ingredients including ascorbic acid, citric acid, and sucrose in controlled environments ranging up to 85% relative humidity. They showed increasing rates of GTC degradation at higher water activities. They also showed possible interaction between additive ingredients affecting GTC stability.

Ascorbic acid was present in the GT-fortified and unfortified apple products, although the content degraded below the limit of detection by 10 d of storage. How organic acids such as ascorbic and citric acids affect GTC stability has been studied in green tea beverages. Chen et al (*10*) found that different beverage ingredients may interact and affect GTC stability. Chen et al (*42*) found that ascorbic acid added to pH 7.4 buffer significantly improved GTC stability, where a dose-response effect was observed. The ascorbic acid particularly affected levels of EGCG and EGC. Chen et al (*10*) found that ascorbic acid acted as an antioxidant during the first month of green tea storage, but then acted as a pro-oxidant in subsequent months. When GTC were added to solutions of just sucrose or sucrose with ascorbic or citric acids, the sucrose had little effect on GTC stability. Alternately, ascorbic and citric acids were detrimental to stability in ≤ 2 months, but where pH of systems tested was not reported (*47*). No improvement of GTC stability was observed when evaluating citric acid (*42*). Similar results were also observed using cocoa flavan 3-ols (*48*).

Antioxidant Activity

In vitro antioxidant activity was evaluated using the Folin-Ciocalteu reducing capacity method for total polyphenolic content, FRAP, and DPPH radical scavenging activity. Among all analyses, GT fortification increased antioxidant activity for apple products. For total polyphenolic content, little change in total activity occurred in samples over 45 d of storage. The Folin-Ciocalteu reducing capacity of approximately 7,000-8,000 mg catechin equiv·kg^{-1}, dry weight for methanol and acetone:water (70:30, v/v) extracts of freeze-dried apple flesh was in agreement with other sources (*3, 43, 49*).

Several chemical reaction mechanisms may have occurred during storage of samples, including degradation of monomeric flavan 3-ols and phenolic acids, and formation of Maillard reaction intermediates and products. Since the Folin-Ciocalteu method measures total oxidizable substances, the assay only provides a measure of total polyphenolic reducing capacity. Whereas the activity of monomeric flavonoids may decrease, the concentration of corresponding epimers may have increased, or Maillard reaction products may have contributed antioxidant activity. As tea is subjected to processing and storage, it may form brown-colored macromolecular products or intermediates that are products of oxidation and polymerization of monomeric flavan 3-ols, which can also exert antioxidant activity (*50*). The depolymerization of procyanidin fractions from green tea extract or apples may also occur during storage, contributing more reactive substances for detection by analysis.

Inhibition of AGE Formation

Extracts from methanol and acetone:water (70:30, v/v) of GT-fortified and unfortified apple products inhibited fructose-induced glycation of bovine serum albumin (**Table 5.4**). The oxidation of cells is always occurring, and it is associated with chronic disease risk such as Type II diabetes or atherosclerosis (*51-53*). People suffering from a disease such as Type II diabetes may suffer accelerated rates of cellular oxidation due to impaired physiologic states from insulin resistance or hyperglycemia, forming AGE (*54*). A common medical biomarker is the status of hemoglobin $A1_C$, which provides a measure of blood sugar control by evaluating the oxidation state of the human red blood cell (*55*). When elevated levels of blood sugar are present in the blood, then theoretically accelerated rates of oxidation may occur.

The glycation process can occur by an autoxidation mechanism. A reducing sugar reacts with a free amine group, creating a Schiff base. When catalyzed by a free metal ion, the Schiff base may then convert into an Amadori product and form AGE and cross-linking proteins (Nakagawa et al, 2002). In particular, fructose and glucose may catalyze the reaction of protein cross-linking at similar rates, but fructose is capable of catalyzing the formation of covalent, non-disulfide linkages at a ten-fold rate over glucose (*20*). Certain compounds are capable of chelating the free metal ion, such as ethylenediaminetetraacetic acid (EDTA) commonly used in food products, or polyphenolic compounds such as flavonoids (*56*) or tannins (*57*). This may involve a mechanism for explaining how flavonoids may inhibit formation of AGE. The cells of healthy individuals still undergo the glycation process, but the reaction occurring at an accelerated rate is associated with chronic disease risk.

Dearlove et al (*58*) demonstrated inhibition of AGE with extracts from spices and herbs. The polyphenols in these extracts were thought to inhibit the glycation reaction. Nakagawa et al (2002) found that GTC containing a gallate moiety at the C-3 position (e.g. EGCG, GCG, and ECG) showed significantly greater antioxidative activity in preventing formation of AGE following a dose-response relationship than EGC, GC, EC, and C. Similar more potent inhibitory activities occurred for catechins containing an α-hydroxyl moiety over a β-hydroxyl moiety at the C-3 position.

Following a typical Western diet is considered pro-inflammatory, since it is often deficient in antioxidant-rich fruits, vegetables, and whole-grains. Consuming a food product such as apples fortified with GTC may help increase daily antioxidant consumption, or may help provide a dietary intervention strategy for helping to manage the inflammatory risk associated with chronic disease.

153

Abbreviations Used

Ferric reducing/antioxidant power (FRAP); 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl radical scavenging activity (DPPH); Advanced glycation endproducts (AGE); Green tea (GT); (-)-epigallocatechin gallate (EGCG); (-)-epicatechin (EC); (-)-epigallocatechin (EGC); (-)-epicatechin gallate (ECG); (-)-gallocatechin gallate (GCG); (-)-gallocatechin (GC); (-)-catechin gallate (CG); (-)-catechin (C); Green tea catechins (GTC); Ethylenediaminetetraacetic acid (EDTA)

REFERENCES

1.   Kaur, C.; Kapoor, H. C., Antioxidants in fruits and vegetables -- millennium's health *International Journal of Food Science and Technology* **2001,** 36, 703-725.

2.   Boyer, J.; Liu, R. H., Apple phytochemicals and their health benefits. *Nutrition Journal* **2004,** 3.

3.   Khanizadeh, S.; Tsao, R.; Rekika, D.; Yang, R.; Charles, M. T.; Rupasinghe, H. P. V., Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *Journal of Food Composition and Analysis* **2008,** 21, 396-401.

4.   Van der Sluis, A. A.; Dekker, M.; Skrede, G.; Jongen, W. M. F., Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *Journal of Agricultural and Food Chemistry* **2002,** 50, 7211-7219.

5.   Vrhovsek, U.; Rigo, A.; Tonon, D.; Mattivi, F., Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry* **2004,** 52, 6532-6538.

6.   Gerhauser, C., Cancer chemopreventive potential of apples, apple juice, and apple components. *Planta Medica* **2008,** 74, (13), 1608-1624.

7.   Wang, H.; Provan, G. J.; Helliwell, K., HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry* **2003,** 81, 307-312.

8.   Unno, T.; Sagesaka, Y. M.; Kakuda, T., Analysis of tea catechins in human plasma by high-performance liquid chromatography with solid-phase extraction. *Journal of Agricultural and Food Chemistry* **2005,** 53, 9885-9889.

9.   Wang, R.; Zhou, W.; Jiang, X., Reaction kinetics of degradation and epimerization of epigallcatechin gallate (EGCG) in aqueous system over a wide temperature range. *Journal of Agricultural and Food Chemistry* **2008,** 56, (8), 2694-2701.

10.   Chen, Z.-Y.; Zhu, Q. Y.; Tsang, D.; Huang, Y., Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry* **2001,** 49, (1), 477-482.

11.   Kao, Y.-H.; Chang, H.-H.; Lee, M.-J.; Chen, C.-L., Tea, obesity, and diabetes. *Molecular Nutrition in Food Research* **2006,** 50, 188-210.

12.   Nagle, D. G.; Ferreira, D.; Zhou, Y.-D., Epigallocatechin-3-gallate (EGCG): Chemical and biomedical perspectives. *Phytochemistry* **2006,** 67, 1849-1855.

13.   Zaveri, N. T., Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life Sciences* **2006,** 78, 2073-2080.

14.   Tang, N.; Yuemin, W.; Zhou, B.; Wang, B.; Rongbin, Y., Green tea, black tea consumption and risk of lung cancer: A meta-analysis. *Lung Cancer* **2009**.

15.   Bassiouny, M. A.; Kuroda, S.; Yang, J., Topographic and radiographic profile assessment of dental erosion--Part III: Effect of green and black tea on human dentition. *General Dentistry* **2008,** July-August, 451-461.

16.   Boschmann, M.; Thielecke, F., The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: a pilot study. *Journal of the American College of Nutrition* **2007,** 26, (4), 389S-395S.

17.   Eckel, R. H.; Grundy, S. M.; Zimmet, P. Z., The metabolic syndrome. *Lancet* **2005,** 365, 1415-1428.

18.   Thielecke, F.; Boschmann, M., The potential role of green tea catechins in the prevention of the metabolic syndrome - A review. *Phytochemistry* **2009,** 70, (1), 11-24.

19.   Anderson, R. A.; Polansky, M. M., Tea enhances insulin activity. *Journal of Agricultural and Food Chemistry* **2002,** 50, (24), 7182-7186.

20.   McPherson, J. D.; Shilton, B. H.; Walton, D. J., Role of fructose in glycation and cross-linking of proteins. *Biochemistry* **1988,** 27, 1901-1907.

21.   Thornalley, P. J., Use of aminoguianidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Archives of Biochemistry and Biophysics* **2003,** 419, 31-40.

22.   Wu, C.-H.; Yen, G.-C., Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *Journal of Agricultural and Food Chemistry* **2005,** 53, 3167-3173.

23.   Nakagawa, T.; Yokozawa, T.; Terasawa, K.; Shu, S.; Juneja, L. R., Protective activity of green tea against free radical- and glucose-mediated protein damage. *Journal of Agricultural and Food Chemistry* **2002,** 50, 2418-2422.

24.   Greenspan, L., Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards -- A. Physics and Chemistry* **1977,** 81A, (1), 89-96.

25.   AOAC 1998 International. In *Official Methods of Analysis (Method 934.06).* Maryland, United States.

26.   AOAC 2000 International. In *Official Methods of Analysis (Method 942.15)*, Maryland, United States.

27.   Vanzani, P.; Rossetto, M.; Rigo, A.; Vrhovsek, U.; Mattivi, F.; D'Amato, E.; Scarpa, M., Major phytochemicals in apple cultivars: Contribution to peroxyl radical trapping efficiency. *Journal of Agricultural and Food Chemistry* **2005,** 53, 3377-3382.

28.   Tomas-Barberan, F. A.; Gil, M. I.; Cremin, P.; Waterhouse, A. L.; Hess-Pierce, B.; Kader, A. A., HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *Journal of Agricultural and Food Chemistry* **2001,** 49, 4748-4760.

29.   Mannino, S.; Pagliarini, E., A rapid HPLC method for the determination of vitamin C in milk. *Lebensmittel-Wissenchaft und-Technologie* **1988,** 21, 313-314.

30.   Sun, B.; Ricardo-da-Silva, J. M.; Spranger, I., Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry* **1998,** 46, 4267-4274.

31.   Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M., Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology* **1999,** 299, 152-178.

32.   Benzie, I. F. F.; Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. *Analytical Biochemistry* **1996,** 239, 70-76.

33.   Lavelli, V., Comparison of the antioxidant activities of extra virgin olive oils. *Journal of Agricultural and Food Chemistry* **2002,** 50, (26), 7704-7708.

34.   Alvarez-Parrilla, E.; de la Rosa, L. A.; Rodrigo-Garcia, J.; Escobedo-Gonzalez, R.; Mercado-Mercado, G.; Moyers-Montoya, E.; Vazquez-Flores, A.; Gonzalez-Aguilar, G. A., Dual effect of β-cyclodextrin (β-CD) on the inhibition of apple polyphenol oxidase by 4-hexylresorcinol (HR) and methyl jasmonate (MJ). *Food Chemistry* **2007,** 101, 1346-1356.

35.   Haard, N. F.; Chism, G. W., Ch. 16. Characteristics of edible plant tissues. In *Food Chemistry*, Third ed.; Fennema, O. R., Ed. Marcel Dekker, Inc.: New York, 1996; pp 943-1012.

36.   Fennema, O. R., In *Food Chemistry*, Third ed.; Marcel Dekker, Inc.: New York, 1996; p 1067.

37.   Ortiz, J.; Ferruzzi, M. G.; Taylor, L. S.; Mauer, L. J., Interaction of environmental moisture with powdered green tea formulations: effect on catechin chemical stability. *Journal of Agricultural and Food Chemistry* **2008,** 56, (11), 4068-4077.

38.   Burdurlu, H. S.; Karadeniz, F., Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry* **2003,** 80, 91-97.

39.   Yanagimoto, K.; Lee, K.-G.; Ochi, H.; Shibamoto, T., Antioxidative activity of heterocyclic compounds found in coffee volatiles produced by Maillard reaction. *Journal of Agricultural and Food Chemistry* **2002,** 50, (19), 5480-5484.

40.   Gentry, T. S.; Roberts, J. S., Formation kinetics and application of 5-hydroxymethylfurfural as a time-temperature indicator of lethality for continuous pasteurization of apple cider. *Innovative Food Science and Emerging Technologies* **2004,** 5, 327-333.

41.   Reto, M.; Figueira, M. E.; Filipe, H. M.; Almeida, C. M. M., Chemical composition of green tea (*Camellia sinensis*) infusions commercialized in Portugal. *Plant Foods in Human Nutrition* **2007,** 62, 139-144.

42.   Chen, Z.-Y.; Zhu, Q. Y.; Wong, Y. F.; Zhang, Z.; Chung, H. Y., Stabilizing effect of ascorbic acid on green tea catechins. *Journal of Agricultural and Food Chemistry* **1998,** 46, (7), 2512-2516.

43.   Hamauzu, Y.; Yasui, H.; Inno, T.; Kume, C.; Omanyuda, M., Phenolic profile, antioxidant property, and anti-influenza viral activity of chinese quince (*Pseudocydonia sinensis* Schneid.), Quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *Journal of Agricultural and Food Chemistry* **2005,** 53, (4), 928-934.

44.   Wang, H.; Helliwell, K., Epimerisation of catechins in green tea infusions. *Food Chemistry* **2000,** 70, 337-344.

45.   Fujioka, K.; Shibamoto, T., Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chemistry* **2008,** 106, 217-221.

46.   Liang, H.; Liang, Y.; Dong, J.; Lu, J.; Xu, H.; Wang, H., Decaffeination of fresh green tea leaf (*Camellia sinensis*) by hot water treatment. *Food Chemistry* **2007,** 101, 1451-1456.

47.   Su, Y. L.; Leung, L. K.; Huang, Y.; Chen, Z.-Y., Stability of tea theaflavins and catechins. *Food Chemistry* **2003,** 83, 189-195.

48.   Zhu, Q. Y.; Hammerstone, J. F.; Lazarus, S. A.; Schmitz, H. H.; Keen, C. L., Stabilizing effect of ascorbic acid on flavan-3-ols and dimeric procyanidins from cocoa. *Journal of Agricultural and Food Chemistry* **2003,** 51, (3), 828-833.

49.   Hagen, S. F.; Borge, G. I. A.; Bengtsson, G. B.; Bilger, W.; Berge, A.; Haffner, K.; Solhaug, K. A., Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica*) Borkh., cv. Aroma. *Postharvest Biology and Technology* **2007,** 45, 1-10.

50.   Manzocco, L.; Anese, M.; Nicoli, M. C., Antioxidant properties of tea extracts as affected by processing. *Lebensmittel-Wissenchaft und-Technologie* **1998,** 31, 694-698.

51.   Dehnavi, R. A.; Beishuizen, E. D.; Reema, V. D.; Cessie, S. L.; Huisman, M. V.; Kluft, C.; Princen, H. M. G.; Tamsma, J. T., The impact of metabolic syndrome and CRP on vascular phenotype in type 2 diabetes mellitus. *European Journal of Internal Medicine* **2008,** 19, 115-121.

52.   Ford, E. S.; Li, C., Metabolic syndrome and health-related quality of life among U.S. adults. *Annals of Epidemiology* **2008,** 18, (3), 165-171.

53.   Luca, C. D.; Olefsky, J. M., Inflammation and insulin resistance. *FEBS Letters* **2008,** 582, 97-105.

54.   Peyroux, J.; Sternberg, M., Advanced glycation endproducts (AGEs): pharmacological inhibition in diabetes. *Pathologie Biologie* **2006,** 54, 405-419.

55.   Boer, H. D.; Keizers, R.; Jansen, M.; Verschoor, L.; Ruineman-Koerts, J., Glycaemic control without weight gain in insulin requiring type 2 diabetes: 1-year results of the GAME regimen. *Diabetes, Obesity and Metabolism* **2006,** 8, 517-523.

56.   Nest, G. L.; Caille, O.; Woudstra, M.; Roche, S.; Burlat, B.; Belle, V.; Guigliarelli, B.; Lexa, D., Zn-polyphenol chelation: complexes with quercetin, (+)-catechin, and derivatives: II Electrochemical and EPR studies. *Inorganica Chimica Acta* **2004,** 357, 2027-2037.

57.   Zijp, I. M.; Korver, O.; Tijburg, L. B. M., Effect of tea and other dietary factors on iron absorption. *Critical Reviews in Food Science and Nutrition* **2000,** 40, (5), 371-398.

58.   Dearlove, R. P.; Greenspan, P.; Hartle, D. K.; Swanson, R. B.; Hargrove, J. L., Inhibition of protein glycation by extracts of culinary herbs and spices. *Journal of Medicinal Food* **2008,** 11, (2), 275-281.

TABLES

Table 5.1. Titratable acidity, pH, soluble solids content and moisture content of apple and apple and GT products

Quality index	Sample	
	Apple	Apple & GT
Titratable Acidity (g malic acid/100 g product, d.w.)	$2.52^a \pm 0.03$	$2.76^b \pm 0.01$
pH	3.58 ± 0.01	3.72 ± 0.06
Soluble solids (g sucrose/100 g product, d.w.)	74.08 ± 1.68	76.06 ± 1.21
Moisture Content (%)		
After freeze-drying	$3.71^b \pm 0.06$	$3.17^a \pm 0.17$
At equilibrium a_w 0.7509	26.30 ± 0.07	26.29 ± 0.11

Different letters within the same row indicate a significant difference using Tukey's HSD ($p \leq 0.05$)

Moisture content was evaluated both after freeze-drying and at the equilibrium a_w of 0.7509. All other evaluations were performed on freeze-dried products.

Table 5.2. Rate constants for color variation and estimates for colorimetric parameters at the equilibrium a_w of 0.7509 ($C_{5\ days}$) and after 30 days of storage at a_w of 0.7509, at 30°C, in the dark ($C_{30\ days}$) in IM apple and IM apple and GT.

Colorimetric parameter	Sample	k (colorimetric unit*days^{-1})	R	$C_{5\ days}$	$C_{30\ days}$
L^*	IM Apple	-0.178 ± 0.033	-0.72	73 ± 7	68 ± 6
	IM Apple & GT	-0.179 ± 0.021	-0.86	70 ± 4	65 ± 4
a^*	IM Apple	0.140 ± 0.004	0.99	-0.78 ± 0.71	2.70 ± 0.71
	IM Apple & GT	0.121 ± 0.004	0.99	-0.11 ± 0.78	3.14 ± 0.78
b^*	IM Apple	-0.147 ± 0.024	-0.78	25.1 ± 4.8	21.4 ± 4.8
	IM Apple & GT	-0.098 ± 0.01	-0.94	23.1 ± 1.4	20.6 ± 1.4

Data were fitted to pseudo-zero-order kinetics: $C = C_0 \pm k^*t$; $p < 0.01$.

Table 5.3. Rate constants for antioxidant degradation and estimates for antioxidant contents at the equilibrium a_w of 0.7509 ($C_{5\ days}$) and after 30 days of storage at a_w of 0.7509, at 30°C, in the dark ($C_{30\ days}$) in IM apple and IM apple and GT.

Analyte	Sample	k (days^{-1})	R	$C_{5\ days}$ (mg·kg^{-1}, d.w.)	$C_{30\ days}$ (mg·kg^{-1}, d.w.)
EC	Apple	0.034 ± 0.002	-0.99	234 ± 66	100 ± 30
	Apple & GT	0.014 ± 0.001	-0.97	770 ± 130	530 ± 90
C	Apple	0.072 ± 0.012	-0.96	38 ± 11	0
	Apple & GT	n.s.		300 ± 23	300 ± 23
EGC	Apple & GT	0.011 ± 0.001	-0.96	1900 ± 300	1500 ± 300
EGCG	Apple & GT	0.020 ± 0.001	-0.99	2270 ± 320	1400 ± 200
ECG	Apple & GT	0.013 ± 0.004	-0.79	473 ± 200	300 ± 159
GCG	Apple & GT	0.009 ± 0.001	-0.96	510 ± 60	406 ± 40
Caffeine	Apple & GT	n.s.		1273 ± 103	1273 ± 103
Chlorogenic acid	Apple	0.006 ± 0.001	-0.86	1130 ± 150	900 ± 150
	Apple & GT	0.007 ± 0.001	-0.94	1130 ± 90	873 ± 90
Ascorbic acid	Apple	0.471 ± 0.03	-0.99	12 ± 7	n.d.
	Apple & GT	0.542 ± 0.04	-0.99	8 ± 3	n.d.
Procyanidin B$_2$	Apple	0.055 ± 0.004	-0.97	322 ± 140	83 ± 40
	Apple & GT	0.021 ± 0.007	-0.82	372 ± 170	220 ± 100
Total Procyanidins	Apple	0.011 ± 0.002	-0.93	1340 ± 370	1021 ± 270
	Apple & GT	0.011 ± 0.001	-0.99	2300 ± 200	1730 ± 200

n.s. = No significant degradation occurred during storage; n.d. = Concentration below limit of detection; reported values are average content ± SD over 45 days of storage
Total procyanidins were determined by the vanillin assay
Epicatechin (EC), catechin (C), epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and gallocatechin gallate (GCG)

Table 5.4. Anti-AGE formation properties of IM apple and IM apple and GT

Sample	Storage time (days)	Catechin equiv (mmol/kg d.w.)	
		Extraction solvent	
		Methanol	Acetone:water, 70:30
Apple			
	0	17.2^b	31.3^d
	7	13.2^a	
	24	11.4^a	
	45	11.2^a	23.4^c
Apple and GT			
	0	50.7^f	75.2^g
	7	44.8^e	
	24	46.8^{ef}	
	45	49.5^{ef}	66.2^g

Different letters within the same column and sample indicate a significant difference using Tukey's HSD ($p \leq 0.05$)

Figure Captions

Figure 5.1. *L**, *a** and *b** colorimetric parameters for IM apple (◆) and IM apple and GT (◊) as a function of storage time. Regression relationships are reported in Table 5.2.

Figure 5.2. Correlation between total phenolics (evaluated by the Folin Ciocalteu reagent, mmol catechin equiv/kg d.w.), FRAP (mmol FeII/kg d.w., ■, R = 0.96), DPPH radical scavenging activity (mmol trolox/kg d.w., ▲, R = 0.91) and anti-AGE activity (mmol catechin equiv/kg, ◆, R = 0.98).
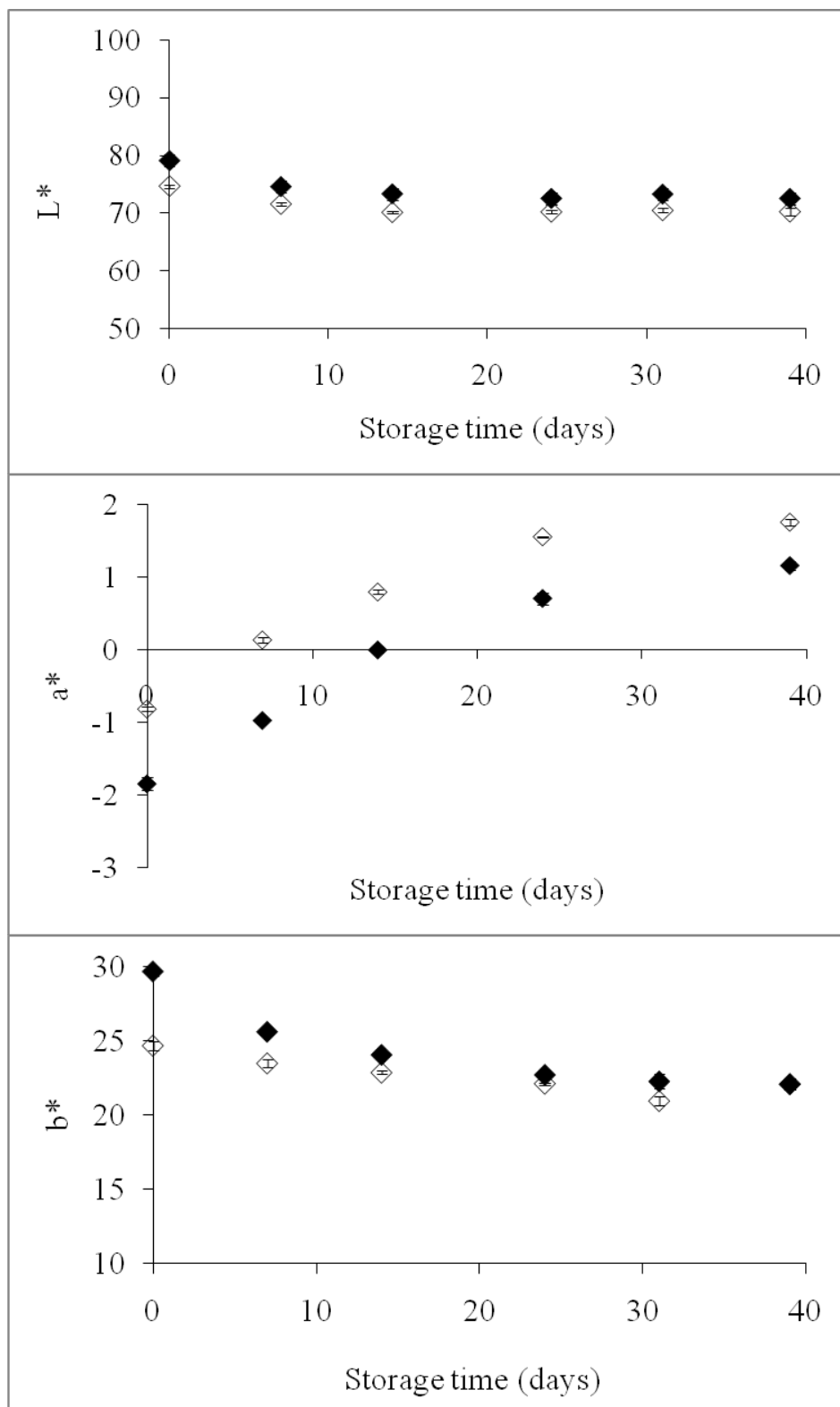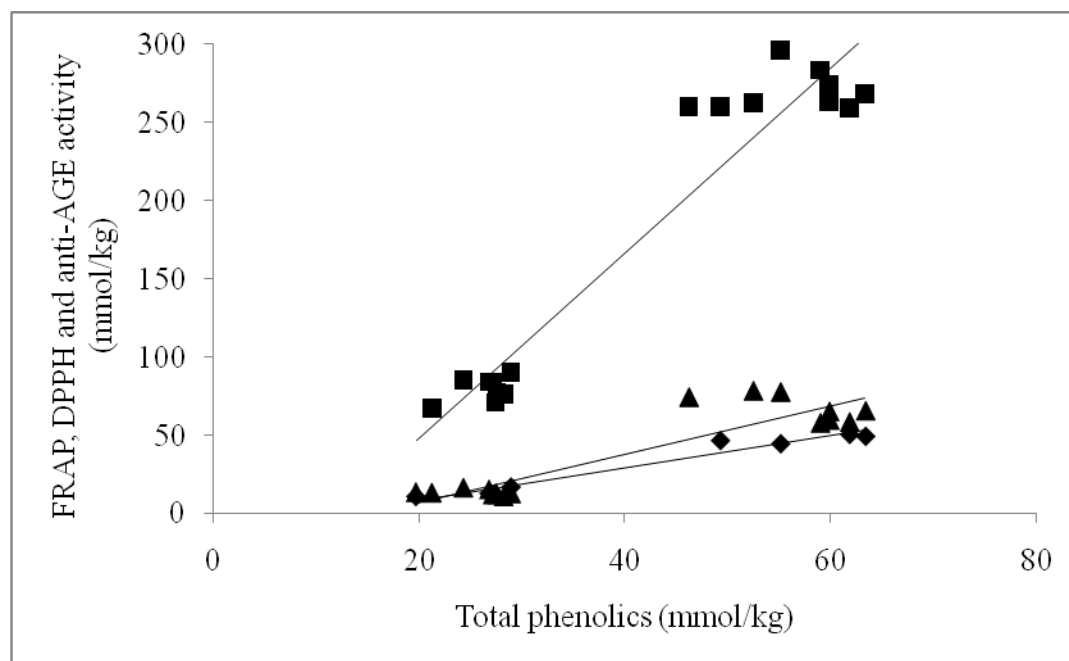
Figure 5.1.

Figure 5.2.

CHAPTER 6. PHYTOCHEMICAL STABILITY IN DRIED APPLE AND GREEN TEA FUNCTIONAL PRODUCTS AS RELATED TO MOISTURE PROPERTIES

ABSTRACT

In this study, apple products with and without added green tea extracts were freeze-dried and stored for up to 45 days at 30°C in low and intermediate moisture environments (a_w 0.11, 0.22, 0.32, 0.57, and 0.75). Kinetic models were developed for the decrease in select green tea and apple monomeric and polymeric flavanols, ascorbic acid, caffeine and total phenolics. Product moisture isotherms were made for each product. Glass transition temperature (T_g) at various moisture levels were measured by differential scanning calorimetry and water mobility by nuclear magnetic resonance (NMR). Chemical changes were related to T_g, a_w, and water mobility in the products.

In general, phytochemical degradation occurred more rapidly at higher moisture contents, except for caffeine, total dihydrochalcones, and chlorogenic acid. In the product containing apple with green tea, the content of epigallocatechin gallate, the predominant green tea catechin, decreased by 44% and 60% after 45 days of storage at a_w of 0.57 and 0.75, respectively. Phytochemical degradation correlated with increasing a_w, T_g, and NMR evaluation. This study showed that, in general, storage at a_w 0.75 most affected phytochemical stability and antioxidant properties.

Keywords: apple, green tea, differential scanning calorimetry, nuclear magnetic resonance, water activity

1. Introduction

Consumption of green tea (GT) from the leaves and buds of *Camellia sinensis* originated in China over 2000 years ago. Its components have been found to have anti-oxidative, anti-mutagenic, anti-inflammatory, anti-cariogenic, anti-diabetic, anti-bacterial, and anti-viral properties, and may reduce the risk of coronary heart disease or cancer (Cabrera, Artacho & Gimenez, 2006; Zaveri, 2006). Green tea is high in polyphenolic compounds, which are comprised mostly of monomeric and polymeric flavan-3-ols. The predominant monomeric flavan-3-ols of green tea catechins include catechin (C), epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG). The antioxidant and processing stability of green tea catechins in beverages has been extensively studied (Chen, Zhu, Wong, Zhang & Chung, 1998; Chen, Zhu, Tsang & Huang, 2001; Yang, Hwang & Lin, 2007; Labbe, Tetu, Trudel & Bazinet, 2008; Wang, Zhou & Jiang, 2008). Ortiz et al (2008) evaluated the storage stability of GTC in dry beverage powder at different relative humidity. In the marketplace, there is growing interest for utilization of green tea extract put into other types of food products.

Apples are also another important dietary source of nutrients and phytochemicals. They are consumed fresh, dried, pureed, or in juice (Van der Sluis, Dekker, Skrede & Jongen, 2002; Guyot, Marnet, Sanoner & Drilleau, 2003; Deng & Zhao, 2008; Oszmianski, Wolniak, Wojdylo & Wawer, 2008). The consumption of apples is associated with anti-oxidative, anti-hypercholesterolemic, and anti-carcinogenic properties, and may reduce the risk of developing coronary heart disease, diabetes, or asthma (Boyer & Liu, 2004). The polyphenolic content of apples includes procyanidins, hydroxycinnamic acids, dihydrochalcones, flavonols, flavan-3-ols,

ascorbic acid, and anthocyanins in the peel of red varieties (Guyot et al., 2003; Khanizadeh, Tsao, Rekika, Yang, Charles & Rupasinghe, 2008).

Moisture levels and moisture properties determine antioxidant stability in food systems, and also effect the color, flavor, or nutritional content (Bell, 2007). For research studies, dried products are typically stored at different relative humidity in order to specify particular water activity (a_w) levels in the product. This has allowed the development of mathematical models that predict changes in product chemical or physical properties over time with respect to moisture content (Welti-Chanes, Guerrero, Barcenas, Aguilera, Vergara & Barbosa-Canovas, 1999; Venir, Munari, Tonizzo & Maltini, 2007). Differences in moisture content determines water mobility and the degree of plasticization of larger food molecules, which also affects rates of chemical reactions (Bell, 2007; Labuza & Altunakar, 2007).

Differential scanning calorimetry (DSC) allows for analysis of parameters such as the glass transition temperature (T_g), which can then be related to moisture properties. This helps in understanding how water affects the physical state of a material, which can then be related to other physicochemical attributes (Deng et al., 2008). Dried products are often amorphous materials that exist in a glassy state below their T_g and in a rubbery, less solid-like state at temperatures greater than T_g. At storage temperatures that are greater than the T_g, there is also greater molecular mobility and lower viscosity. A plasticizer such as water can decrease the T_g (Welti-Chanes et al., 1999). Moisture properties can thus influence phytochemical stability and antioxidant properties.

The state of water in a system may also be evaluated using nuclear magnetic resonance (NMR) relaxation techniques based on spin magnetization relaxation. NMR may differentiate water as low- or high-molecular mobility based on relaxation properties (Choi & Kerr, 2003a;

Choi, Kim, Hanna, Weller & Kerr, 2003b). This method of analysis allows for the evaluation of moisture mobility as related to chemical or physical changes in a food product, such as in bread staling (Van Nieuwenhuijzen, Primo-Martin, Meinders, Tromp, Hamer & Van Vliet, 2008) or in pharmaceuticals (Yoshioka & Aso, 2007).

Previous studies have shown the benefit of apple products fortified with green tea (GT-apple) as a novel functional product with a more complete antioxidant profile. The objectives of this study were to evaluate properties related to water dynamics in dried apple and GT-apple samples stored at different relative humidities, and to determine how these are related to phytochemical stability.

2.   Materials and Methods

2.1 Materials

Reagents for moisture isotherm development included lithium chloride, potassium acetate, magnesium chloride, sodium bromide, and sodium chloride, and were purchased from Sigma Aldrich (Milan, Italy). Analytical standards of ascorbic acid, chlorogenic acid, caffeine, and phloridzin were purchased from Sigma Aldrich (Milan, Italy). The standards of procyanidin B2, EC, C, EGC, ECG, GCG, and EGCG were purchased from Extrasynthese (Lyon, France).

2.2 Preparation of Samples

Hot water green tea extract (GT) was prepared by extracting 25 g of dried tea leaves (Java Green Tea, Twinings, London, UK) in 500 mL of pre-heated 85°C deionized water for 5 min. The extract was then immediately cooled in an ice water slurry and filtered through

Whatmann no. 4 filter paper. A portion of the GT extract was removed for HPLC analysis. It was diluted 1:20 (v/v) with water:methanol (95:5, v/v) and acidified with 5% formic acid.

Fresh apples (Melinda, Revo, Italy) of the Golden Delicious variety were purchased at a supermarket. The apples were peeled, cored, and quartered. Apple quarters were randomly designated for either the control or experimental batches. Each batch consisting of 2 kg of quartered apple pieces was then blanched for 4 min in 100°C deionized water. Apples were then immersed in an ice water slurry and drained over paper towels. Apple pieces were then pureed in a K 3000 Braun Multisystem blender (Braun, Kronberg, Germany) for 3 min. After blending, 384 mL of GT extract was added to the experimental apple batch and mixed thoroughly. The apple and GT-apple samples were then spread onto stainless steel trays and freeze-dried (Lyoflex Edwards, Crawley, UK).

2.3 Determination of Moisture Sorption Isotherms

Freeze-dried materials were ground in the food processor and sieved (800 μm). Powders were then weighed into Petri dishes (0.141 g of powder/cm^2) in duplicate and stored in desiccators at 30°C suspended over saturated salt solutions having different water activity (a_w): lithium chloride ($a_w = 0.113$), potassium acetate ($a_w = 0.216$), magnesium chloride ($a_w = 0.324$), sodium bromide ($a_w = 0.560$), and sodium chloride ($a_w = 0.751$). The a_w of saturated salt solutions were checked using an Aqualab water activity meter (Decagon Devices, WA, USA). The a_w values were in agreement with those published by Greenspan (1977). After samples reached equilibrium, their moisture content was determined after drying in a vacuum oven at 70°C and 50 torr for 18 h (AOAC, 1998).

Plots of moisture content versus a_w were fit to the Guggenheim-Anderson-de Boer (GAB) model:

$$m_o = \frac{m_o k c \, a_w}{(1 - k \, a_w)(1 - k \, a_w + c \, k \, a_w)}$$ (1)

where m_o is the monolayer moisture content, c is a factor associated with surface enthalpy, and k represents a multilayered moisture component (Labuza et al., 2007).

2.4 Differential Scanning Calorimetry and Glass Transitions

About 10-15 mg of equilibrated apple or GT-apple was hermetically sealed into aluminum DSC pans. Samples were then analyzed by a DSC 1 differential scanning calorimeter (Mettler-Toledo, Inc., Columbus, OH, United States). This was equipped with StarE software, which uses first-derivative analysis to calculate the glass transition temperature including onset (T_{go}) and midpoint temperatures (T_{gm}). The thermal scanning profile consisted of equilibration of sample pans at 30°C for 2 min, cooling to -50°C at 5°C per min, holding at -50°C for 5 min, and then heating to 80°C at 5°C per min. Scans were conducted under a nitrogen gas flush.

The Gordon and Taylor equation (Gordon & Taylor, 1952) was used to model the relationship between glass transition temperatures and moisture properties of samples:

$$T_{go} = \frac{w_s T_{gs} + k w_w T_{gw}}{w_s + k w_w}$$ (2)

where T_{go} is the observed glass transition temperature, T_{gs} the glass transition temperature of amorphous dry solid, T_{gw} the glass transition temperature of amorphous water, k a constant, w_s is the weight fraction of solids, and w_w is the weight fraction of water (Welti-Chanes et al., 1999). For water, T_{gw} was taken as -135°C (Johari, Hallbrucker & Mayer, 1987; Welti-Chanes et al., 1999).

2.5 Nuclear Magnetic Resonance Studies

H^1 NMR analysis was conducted using a 20 mHz H^1 NMR spectrometer (Resonance Instruments, Whitney, U.K.). Each equilibrated sample was removed from its relative humidity chamber and transferred into 18 mm diameter NMR glass tubes to a height of less than 25 mm and then immediately sealed.

The methods of Choi, Kim et al (2003b) were followed with modifications. The transverse spin-spin relaxation (T$_2$) of protons was analyzed using the free induction decay (FID) and the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences (Ruan & Chen, 2001). The acquisition parameters consisted of a 90° pulse set at 4.1 µs and a 2 s recycle delay. The pulse length was 60 µs for the spacing at 90° to 180° (τ). The analysis was conducted at 22°C. FID curves were fit to a Gaussian model, while those from CPMG studies were fit with a multiple-exponential model.

2.6 Extraction of Samples for Chemical Analysis

For subsequent chemical analysis, the extraction of samples was performed in 25 mL centrifuge tubes using 0.5 g of sample in 10 mL of methanol (Van der Sluis et al., 2002), 10 mL of acetone: water (70:30, v/v) (Vanzani et al., 2005), or 5 mL of 6% metaphosphoric acid (containing 1 g/L of sodium metabisulphite) (Vrhovsek, Rigo, Tonon & Mattivi, 2004). The tubes were then vortexed for 2 min and subjected to centrifugation (10,000 $\times$ *g* for 10 min). The supernatants were removed and filtered through Whatmann no. 4 filter paper.

2.8 HPLC Determination of Phenolic Compounds

The method of Tomas-Barberan et al (2001) was used to determine the concentrations of phenolic compounds. Methanolic extracts were injected on a 250×4.6 mm i.d., 5 µ, Symmetry C18 reverse-phase column (Waters, Vimodrone, Italy). The HPLC was coupled to a model 600 HPLC pump and a model 2996 photodiode array detector, and online analysis was done with Empower software (Waters, Vimodrone, Italy). Mobile phase stock solutions of water and methanol were acidified to 5% of formic acid. A gradient elution mobile phase separation consisted of the following: phase A of water:methanol (95:5, v/v), phase B of water:methanol (88:12, v/v), phase C of water:methanol (20:80, v/v), and phase D of 100% methanol. The gradient elution was adjusted as follows: (1)100% of A for 0-5 min, (2) a linear gradient to reach 100% of B from 5-10 min, (3) 100% of B from 10-13 min, (4) a linear gradient to reach 75% of B and 25% of C from 13-35 min, (5) a linear gradient to reach 50% of B and 50% of C from 35-50 min, (6) a linear gradient to reach 100% of C from 50-52 min, (7) 100% of C from 52-57 min, and (8) 100% of D from 57-60 min. The flow rate was 1.0 mL/min. The injection volume was 20 µL using a Rheodyne loop. The analysis was conducted at 22°C. The eluent was monitored at 280 and 330 nm for detection.

The standards of EC, chlorogenic acid, phloridzin (phloretin 2'*O*-glucoside), and caffeine were used for identification of peaks by retention times and UV-vis spectra, and were used to build calibration curves. EC, phloridzin, and caffeine were detected at 280 nm, while chlorogenic acid was detected at 330 nm. The standards of C, EGC, GCG, ECG, and EGCG were used for identification of peaks by retention times and UV-vis spectra. All peaks were detected at 280 nm. Quantification was based on the calibration curve built from EC and relative response factors (Wang, Provan & Helliwell, 2003). The peak for phloretin 2'*O*-xyloglucoside

was based on UV-vis spectra and literature data. Detection and quantification occurred at 280 nm using a calibration curve built from phloridzin. The samples were analyzed in at least duplicate.

Peak concentrations for each analyte were fit to a pseudo-first-order kinetic model:

$$\ln = Ae^{-kt} \tag{3}$$

The reaction rate coefficient (k) was determined for each analyte at each storage a_w.

2.9 Determination of ascorbic acid content

The determination of the ascorbic acid content of extracts containing 6% metaphosphoric acid was conducted following the methods of Mannino and Pagliarini (1988). The sample extracts were diluted as necessary by 6% metaphosphoric acid (containing 1 g/L of sodium metabisulphite). The analysis occurred in a Waters model HPLC using a 300 × 7.8 mm i.d. Bio-Rad Fruit Quality Analysis column. An isocratic mobile phase of 1 mM sulfuric acid at 1 mL/min flow rate was used. Analyses were conducted at 22°C. Detection of ascorbic acid occurred at 245 nm. A calibration curve of ascorbic acid was made with pure standard. Ascorbic acid concentrations were also fit to pseudo-first-order kinetic models.

2.10 Determination of Total Procyanidin Content

The determination of the total procyanidin content of samples required the following sample preparation steps. Aliquots of 0.25 mL of 70:30 acetone: water (v/v) extracts were dried under nitrogen gas. Samples were then reconstituted in 1 mL of 0.1 M phosphate buffer, pH 7.0 and filtered with 500 mg Sep-pak C-18 cartridges (Waters, Vimodrone, Italy). The retained components were eluted with 1 mL of methanol (Vrhovsek et al., 2004). For extracts containing

green tea polyphenols, the removal of chlorophyll was also necessary. GT-containing extract was mixed with hexane (1:1, v/v). Once two phases had separated, the upper phase was discarded and the lower phase analyzed for procyanidin content.

The determination of the total procyanidin content of samples was conducted following the vanillin assay (Sun, Ricardo-da-Silva & Spranger, 1998). The reaction mixture consisted of 0.5 mL of filtered extracts from 70:30 acetone:water (v/v) or standard dissolved in methanol, 1.25 mL of 1% vanillin in methanol, and 1.25 mL of 9 N sulfuric acid dissolved in methanol. The reaction mixture was held at 25°C until the maximum absorbance occurred at 500 nm. The results were reported as mg of catechin eq per kg of dry product, based on the development of a calibration curve of catechin standard.

2.11 Determination of Enzymatic Activity

Prior to determining polyphenoloxidase (PPO) activity, 100 mg of sample was added to 1 mL of buffer, which consisted of 0.03 M acetic acid, 0.14 M dipotassium phosphate, 1 M sodium chloride, and 5% (w/w) polyvinylpolypyrrolidone, pH 6.5. Extracts were then centrifuged (10,000 $\times$ *g* for 10 min) and filtered through Whatmann no. 1 filter paper.

Polyphenoloxidase activity was determined following the methods of Alvarez-Parilla et al (2007). The reaction mixture consisted of 100 µL of supernatant that was transferred into 900 µL of 10 mM chlorogenic acid in filtered buffer. The reaction mixture was then incubated at 25°C. The absorbance was measured and monitored at 400 nm. A linear increase in absorbance was equated to the reaction rate.

The determination of peroxidase activity (POD) followed the methods of Ahn et al (2007). Filtered supernatant (200 µL) was added to a reaction mixture containing 3.6 mL of 100 mM phosphate buffer (pH 6.5), 100 µL of 640 mM guaiacol, and 100 µL of 400 mM hydrogen peroxide. The reaction mixture was then incubated at 25°C, and the absorbance was continuously measured at 470 nm. Reaction rate was measured from the linear increase in absorbance.

3. Results and Discussion

3.1 Moisture Content, a_w and Glass Transition Temperatures

The moisture content, a_w and glass transition temperature (T_{go}) of apple and GT-apple samples are shown in Table 6.1. A moisture adsorption isotherm was developed for apple and GT-apple products, and the data fit with the GAB model (Fig. 6.1). Moisture content increased with a_w, and the curve was closest to a Type II isotherm, suggesting only modest hygroscopicity. From the GAB model, the factors m_o, c and k for the apple and GT-apple were m_o = 10.7 and 10.2 g of water/100 g of dry product, c = 2.28 and 2.45, respectively, and k = 0.89 and 0.90, respectively. The a_w values corresponding to the monolayer moisture (m_o) contents were a_w = 0.437 and 0.423 for apple and GT-apple, respectively. The m_o for fresh apples equilibrated at 50°-70°C ranged from 16.8-10.7 g of water/100 g of solids (Moraes, Rosa & Pinto, 2008); typical values for c range from 1 to 20 and for k 0.7 to 1.0 (Labuza et al., 2007).

Labuza and Altunacar (2007) provided a detailed discussion on moisture sorption isotherms. When the equilibrium moisture content is below the m_o, then the bulk of water present is bound or adsorbed to solids. The rate of chemical reactions is often the lowest at moisture contents near the m_o. At equilibrium moisture contents greater than m_o, additional bulk

phase water is present. This provides a flowing solvent medium in which chemical reactants can interact. For reactions that involve water, the reactant solubility may also increase, affecting the reaction rate. Greater solubility is attained until a critical moisture content is reached above which the reactants are completely hydrated (Labuza et al., 2007). At higher moisture contents, dilution of the reactants occurs and the reaction rate decreases (Bell, 2007). As equilibrium moisture contents change at different a_w, the effects of moisture mobility on plasticization of components in a material may also affect the physical state of the material.

Examples of DSC curves obtained for GT-apple products equilibrated at different a_w are shown in Fig. 6.2. Evaluation of the T_g provides an indication at what range of temperature the phase transition occurs. This is indicated by the onset (T_{go}) and midpoint (T_{gm}) temperatures, as reported for the T_g at each a_w (Table 6.1). Values for apple and GT-apple were not substantially different at a given a_w. As moisture content and a_w increased, T_g decreased from 8.72-10.28°C at 0.113 a_w to -38.76 to -39.15°C at 0.560 a_w. Decreasing T_g is often associated with increasing water content as the water serves to increase the relative free volume for motions of food molecules (Roos, 2007). At the highest a_w (0.751), a distinct T_g was not detected most likely because the transition was below -50°C. There were no significant differences between apple and GT-apple treatments at the same a_w for T_{go} or T_{gm}. Acevedo et al (2006) found comparable T_g values for freeze-dried apple products stored at 70°C and equilibrated to similar relative humidity levels.

All T_g values were below the storage temperature of 30°C, indicating that none of the samples were in the glassy state. However, the difference in temperatures (30°C-T_g) is a measure of how much the material exhibits solid-like behavior. Mechanical properties including

viscosity (η) have been related to the difference between temperature T and the glass transition temperature T_g, through for example, the Williams-Landel-Ferry model:

$$\ln \frac{\eta}{\eta_g} = -\frac{17.44(T - T_g)}{51.6 + (T - T_g)} \qquad (4)$$

where η_g is the viscosity at the glass transition, typically on the order of 10^{12} Pas.

The T_{go} values were also fit to the Gordon-Taylor equation (Eqn 3). T_g values were well-fit by the model with k = 4.49 and 4.77 for the apple and GT-apple, and intercept = 24.58 and 27.72°C, respectively (Fig. 6.3). The slope and intercept values were similar for GT-fortified and control apple products, and the model produced high correlation coefficients (0.98-0.99).

3.2 Nuclear magnetic resonance

Equilibrated samples were analyzed by H^1NMR using FID and CPMG pulse sequences. Curves for the FID runs are shown in Figure 6.4. CPMG pulse sequences were only successful for samples at a_w of 0.560 or 0.751. At $a_w \leq 0.33$, the signal decayed rapidly in under 50-100 μs. The curves were fit to Gaussian and exponential models. Samples at $a_w \leq 0.324$ were well fit with a single component (Table 6.2). For apple, the single T_2^* values were 16.3, 23.6 and 31.6 μs at a_w= 0.113, 0.216 and 0.324. For GT-apple, T_2^* values were 12.7, 25.7 and 32.3 μs. While the very short decay times are indicative of solid relaxation, the increase in T_2^* does suggest that those solid components were plasticized by the increasing presence of water.

For samples held at a_w of 0.560 and 0.751, the FID curves were better fit by a 2 component model. The fast decaying component (T_{2a}^*) was 44.2 and 60.4 μs for apple (at 0.560 and 0.751 a_w), and 45.1 and 51.9 μs for GT-apple. Again, this can be attributed to relaxation processes in the solid component with enhanced mobility due to the plasticization of water. The

slower decaying component (T_{2b}^*) was 305.6 and 526.1 μs for apple (at 0.560 and 0.751 a_w), and 255.2 and 834.2 μs for GT-apple. This second component can be attributed to more mobile water not closely associated with larger molecules. However, this water is still close enough to diffuse to and exchange molecular spins with those molecules within the relaxation time. Thus, the relaxation times, in the range of 255-834 μs, are still much shorter than the T_2^* times of 1-2 s typical of bulk water.

Results for the transverse relaxation times can be related to the moisture isotherm data. That is, at $a_w \le 0.33$ the NMR data showed only solid-like behavior in which water enhances the mobility of food components. At higher a_w, a second component was present indicative of a region of water with greater mobility. Interestingly, the GAB models of the moisture isotherms showed that the a_w values corresponding to monolayer moisture (m_o) were 0.437 and 0.423 for apple and GT-apple, respectively. According to the GAB theory, at $m < m_o$, no more than a single layer of water is sorbed at molecular surfaces. Above m_o, multiple layers of water exist that are not sorbed. Above the m_o, greater molecular mobility and solubility of analytes can be expected due to the presence of excess water as solvent, until a dilution effect prevails once analytes are completely solubilized (Bell, 2007).

3.3 Phytochemical degradation

Table 6.3 shows the initial levels of antioxidants in apple and GT-apple. Apples had substantial levels of EC, C and procyanidins, as well as ascorbic acid and chlorogenic acid. GT-apple also had substantial levels of EGC, EGCG, ECG and GCG, as well as caffeine. Concentrations of these molecules were measured over 45 days of storage and fitted to a pseudo-first-order kinetic model to determine the rate constant k (Table 6.4). Wang et al. (2008) also

found that green tea catechins followed a pseudo-first-order kinetic model. The antioxidants in apple and GT-apple showed varying levels of stability over storage with respect to a_w.

For EC, k increased with increasing a_w, both for apple (0.008-0.034 d^{-1}) and GT-apple (0.009-0.014 d^{-1}). In general, the rate of EC degradation was more rapid in the apple samples. Addition of GT also significantly increased the concentration of EC in samples (Table 3). For catechin (C), the GT- apple products had greater stability throughout 45 d of storage than apple (0.006-0.072 d^{-1}). Indeed, no significant change in C was measured in the GT-apple system. In apple products, degradation increased with higher a_w. The content of C was also initially lower in control apple products compared to GT-fortified apple products (56 versus 301 mg/kg).

EC and C have been found in both apples and green tea (Wang et al., 2003; Hagen et al., 2007). When tea extracts were held at relatively high storage time and temperatures, EC decreased whereas C increased (Khokhar & Magnusdottir, 2002). In this instance, a portion of EC may have epimerized to C. Similar isomerizations may occur for other catechins, which may convert from the epi- to non-epimerized forms in response to exposure to sub-optimal temperature (Wang et al., 2008) or pH $\geq$ 5 (Chen et al., 2001; Ito et al., 2003).

The compounds EGC, EGCG, ECG, and GCG are found primarily in green tea (Boschmann & Thielecke, 2007), and were added here in the GT–apple products. Initial levels were 2066 (EGC), 2317 (EGCG), 548 (ECG) and 542 mg/kg (Table 6.3). In general, the reaction rate constant increased with a_w (Table 6.4). EGCG and ECG generally degraded faster than EGC or GCG. EGCG may have epimerized to GCG. The EGCG-GCG epimerization pathway has shown temperature dependence. In a green tea storage study conducted at 25°C for 16 d, EGCG content decreased to 15% of initial levels, while GCG content increased to 0.5% of total monomeric flavan-3-ol compounds after 8 d, before decreasing thereafter (Wang et al.,

2008). GCG may also isomerize to EGCG, although this generally accounts for only a small fraction of EGCG content (Wang et al., 2008).

EGCG represents approximately 60% of the total monomeric flavan-3-ol compounds, and thus is an important determinant of green tea quality (Cabrera et al., 2006). At lower rates of degradation, especially at values less than $k = 0.008$, the pseudo-first order model did not fit well. Thus at a_w=0.113, little change in EGCG occurred during storage. Ortiz et al (2008) found that monomeric flavan-3-ol compound stability was maintained in dried GT beverage powders stored at ≤ 43% relative humidity at 22°C for 3 months. Stability varied with the presence of sugars, ascorbic acid, or citric acid. Interaction with other phytochemicals or food additives may also affect chemical stability of flavan-3-ols (Chen et al., 1998; Chen et al., 2001; Ortiz et al., 2008). When ascorbic acid is also present, it can exert anti-oxidative properties (Chen et al., 1998), but then become a pro-oxidant during storage (Zhu, Hammerstone, Lazarus, Schmitz & Keen, 2003). In general, the stability of monomeric flavan-3-ols from green tea can also be affected by moisture content and the availability of moisture. Degradation may proceed at higher moisture conditions when there is also greater mobility of reactants (Ortiz et al., 2008).

Polymerized flavan-3-ol compounds include procyanidins (Hagen et al., 2007). In this study, both the dimeric procyanidin B2 present in methanolic extracts and total procyanidin content present in the acetone:water (70:30, v/v) extracts were measured initially (Table 6.3). Green tea fortification significantly increased the content of total procyanidins over control apple products, but procyanidin B2 contents were similar. Apple contained 498 mg/kg procyanidin B2 and 1505 mg/kg total procyanidins, while GT-apple contained 507 mg/kg procyanidin B2 and 2438 mg/kg total procyanidins. The rate of procyanidin B2 degradation was 0.007- 0.053 mg/kg in apple and 0.011-0.023 mg/kg in GT-apple (Table 6.4). For apples, no change in procyanidin

B2 was measured at 0.113 a_w and for GT-apple at either 0.113 or 0.216 a_w. In general, k increased with increasing a_w. Degradation rates were higher for apple than GT-fortified apple products.

For total procyanidins, there was similar stability maintained through storage at a_w = 0.113 and 0.216 for both GT-apple and control apple products. In GT-apple products, rates of degradation increased with a_w. In apple products, no degradation occurred at a_w below 0.33, but then increased to equivalent levels as in GT-fortified products at a_w = 0.560 and 0.751. Procyanidins from apples include dimers such as procyanidin B2 (Hagen et al., 2007), and larger complexes with greater degree of polymerization (Hamauzu, Yasui, Inno, Kume & Omanyuda, 2005).

In this study, the stability of procyandins composed of polymeric or oligomeric flavan-3-ol compounds was greater than monomeric flavan-3-ol compounds comprised of the catechins (Table 6.4). Guyot et al (2003) postulated that the procyanidins are able to outcompete the catechins for electrophilic attack by *o*-quinones, thus participating to a greater extent in oxidation reactions. Among the green tea catechins, EC has also been studied as an inhibitor to the Maillard reaction, where it may bind to sugar fragments thereby reducing concentrations of reactant sugars (Totlani & Peterson, 2005). The Maillard reaction contributes to non-enzymatic browning and is comprised of a series of reactions that involve reaction of reducing sugars with free amino groups to produce a glycosylamine, which then reacts to form a Schiff base or Amadori product. At pH < 5, polymerization of reactants may occur, forming melanoidins that possess a brown color (BeMiller & Whistler, 1996; Martins, Jongen & van Boekel, 2001). In this study, both the apple and GT-apple products developed a browner color during storage (data not shown). 5-Hydroxymethylfurfural is a potential product of the Maillard reaction, which may

form at acidic pH < 4 (Gentry & Roberts, 2004) such as in Golden Delicious apple juice concentrate. Caramelization and ascorbic acid degradation may also contribute to non-enzymatic browning (Burdurlu & Karadeniz, 2003).

Before the addition of green tea extract, the apple portions had undergone a blanching step. This resulted in the denaturation of polyphenoloxidase and peroxidase enzyme, and no activity for either enzyme was found (data not shown). These enzymes may otherwise contribute to enzymatic browning in fruit and vegetable products (Alvarez-Parrilla et al., 2007). Since apples have a high concentration of reducing sugars (50.8%) (Acevedo et al., 2006), non-enzymatic browning reactions may also occur. In addition to availability and concentration of reactants, the Maillard reaction is also catalyzed by free metal ions. Monomeric and polymeric flavan-3-ol compounds, such as those found in apples and green tea may also chelate and inhibit this reaction. This occurs as flavan-3-ols bind to sugar fragments (Totlani et al., 2005), or to cations such as iron (Reznichenko et al., 2006).

At low moisture conditions (a_w ~ 0.1-0.4), the rate of the Maillard reaction proceeds at a relatively slow rate, but proceeds more rapidly at intermediate moisture conditions (~a_w 0.6-0.8) (Fennema, 1996). At moisture levels below m_o, the concentration of reactants is very high, but solute mobility is limited due to limited hydration and low mobility. At moisture levels above m_o, the availability of water for hydration of reactants is sufficient to increase reaction rates. This also increases diffusion rates for reactants, and may enhance reactant solubility. At a higher moisture level, a dilution effect on reactants results (Bell, 2007). For freeze-dried apples stored at 70°C, rates of non-enzymatic browning peaked at 52% equilibrium relative humidity (Acevedo et al., 2006). As non-enzymatic browning is a diffusion-controlled reaction, moisture was thought to enhance the ability of reactants to diffuse and interact. At points above the m_o,

184

structural collapse of samples can also occur, which reduces the number of pores and reaction sites available (White & Bell, 1999; Acevedo et al., 2006). In this study, since samples were blanched, pureed, and freeze-dried, this is not considered to be as significant a factor in antioxidant degradation.

The addition of GT extract to apples also contributed caffeine (1,3,7-trimethylxanthine) to the products (Table 6.3). The caffeine in GT-apple products showed better stability than most other analytes evaluated (Table 6.4). Greatest changes in caffeine content were observed at low moisture. For example, the reaction rate constants were k = 0.006, 0.003 and 0.004 d^{-1} at a_w= 0.113, 0.216 and 0.324, respectively. At a_w = 0.560 and 0.751, caffeine levels did not change through storage. In a storage study of green tea powder beverage mix, Ortiz et al. (2008) found that caffeine remained stable through three months of storage at 22°C.

The initial contents of ascorbic acid in both apple and GT-apple products were similar, that is 118 and 120 mg/kg (Table 6.3). The rate of ascorbic acid loss increased with a_w for both products, particularly at a_w above 0.324 (Table 6.4). For apple, k ranged from 0.007-0.009 d^{-1} at a_w 0.113-0.324, then increased to 0.058-0.471 d^{-1} at a_w 0.560 and 0.751. Similar trends were found for GT-apple, with k ranging from 0.005-0.009 d^{-1} at lower moisture, and 0.049-0.542 d^{-1} at a_w 0.560 and 0.751. Factors that contribute to ascorbic acid degradation include oxidizers, free metal ions as catalysts, and higher moisture contents (Bell, 2007). Temperature is also a factor, where it may follow first-order degradation kinetics (Van den Broeck, Ludikhuyze, Weemaes, Van Loey & Hendrickx, 1998).

Fresh apples are an abundant source of chlorogenic acid (3-caffeoylquinic acid), which is of the trans-cinnamic acid family of compounds (Guyot et al., 2003). The initial contents of chlorogenic acid in extracts of apple and GT-apple were 1061 and 1110 mg/kg (table 6.3).

185

Chlorogenic acid levels were quite stable for both apple and GT-apple at a_w 0.113-0.324, with no significant changes detected (Table 6.4). At intermediate moisture conditions of $a_w = 0.560$ and 0.751, values of k increased, but were still relatively low (0.002-0.006 d^{-1} for apple, and 0.004-0.006 for GT-apple). Chlorogenic acid is present predominantly in the flesh of apples and not in the skins (Hagen et al., 2007). During 9-month storage at 25°C of apple concentrate processed by HTST, total hydroxycinnamate contents decreased by 36%, where chlorogenic acid was the predominant compound present (Spanos, Wrolstad & Heatherbell, 1990). Guyot et al (2003) found that in the production of apple juice, oxidation of polyphenols was significantly more detrimental to stability of monomeric and polymeric flavan-3-ol compounds than to hydroxycinnamates. One possible mechanism for the relative stability of chlorogenic acid, as compared to catechins, is that chlorogenic acid may react with other polyphenols, oxidizing to an *o*-quinone form. This may then reversibly convert to its original state, whereas monomeric and polymeric flavan-3-ols may oxidize completely to form quinones (Cheynier & Ricardo da Silva, 1991).

The total dihydrochalcones include compounds such as phloridzin and phloretin xyloglucoside (Guyot et al., 2003). Initial contents of total dihydrochalcones apple and GT-apple were 135 and 142 mg/kg, respectively (Table 6.3). Rates of total dihydrochalcone degradation were generally higher for GT-apple than apple products (Table 6.4). With respect to moisture level, rates of degradation for GT- apple products were lowest at a_w 0.216 and 0.324 (0.007-0.009 d^{-1}) and highest at a_w 0.113 and 0.560-0.751 (0.012 d^{-1}). In apple products, where degradation rates were generally low (0.003-0.007 d^{-1}), the reaction rate decreased with increasing a_w. In both sample treatments, k had greater magnitude at the lowest $a_w = 0.113$. When evaluated in apple juice, Guyot et al. (2003) found that total dihydrochalcones were fairly

stable during storage. In apple juice, phloridzin and epicatechin react to form orange-colored oxidation products. These can contribute up to half of the juice color (Lea, 1984; Oszmianski & Lee, 1991).

4. **CONCLUSIONS**

This study showed that changes in moisture content or mobility affected phytochemical degradation, and that delineation between low and intermediate moisture conditions around the monolayer moisture value had variable effects on phytochemical stability. In a complex product such as apples fortified with green tea extract, multiple factors may affect the antioxidant status. Evaluation of product stability during storage is important when considering the production of consumer food products, as the antioxidant status may be affected. Fortification of apples with green tea extract provided a new type of value-added product, which would provide complementary health benefits from apples and green tea phenolics.

REFERENCES

Aoac 1998 international. *Official Methods of Analysis (Method 934.06).* Maryland, United
States.

Acevedo, N., Schebor, C. & Buera, M.P. (2006). Water-solids interactions, matrix structural
properties and the rate of non-enzymatic browning. *Journal of Food Engineering*, 77,
1108-1115.

Ahn, T., Paliyath, G. & Murr, P.D. (2007). Antioxidant enzyme activities in apple varieties and
resistance to superficial scald development. *Food Research International*, 40, 1012-
1019.

Alvarez-Parrilla, E., De La Rosa, L.A., Rodrigo-Garcia, J., Escobedo-Gonzalez, R., Mercado-Mercado, G., Moyers-Montoya, E., Vazquez-Flores, A. & Gonzalez-Aguilar, G.A. (2007). Dual effect of β-cyclodextrin (β-cd) on the inhibition of apple polyphenol oxidase by 4-hexylresorcinol (hr) and methyl jasmonate (mj). *Food Chemistry*, 101, 1346-1356.

Bell, L.N. (2007). Moisture effects on food's chemical stability. In G. V. Barbosa-Canovas, J. Anthony J. Fontana, S. J. Schmidt & T. P. Labuza. Water activity in foods: Fundamentals and applications (pp. 173-198). Ames, Iowa: IFT Press, Blackwell Publishing.

Bemiller, J.N. & Whistler, R.L. (1996). Carbohydrates. In O. R. Fennema. Food chemistry (pp. 157-224). New York: Marcel Dekker, Inc.

Boschmann, M. & Thielecke, F. (2007). The effects of epigallocatechin-3-gallate on thermogenesis and fat oxidation in obese men: A pilot study. *Journal of the American College of Nutrition*, 26, 389S-395S.

Boyer, J. & Liu, R.H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal*, 3.

Burdurlu, H.S. & Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, 80, 91-97.

Cabrera, C., Artacho, R. & Gimenez, R. (2006). Beneficial effects of green tea--a review. *Journal of the American College of Nutrition*, 25, 79-99.

Chen, Z.-Y., Zhu, Q.Y., Wong, Y.F., Zhang, Z. & Chung, H.Y. (1998). Stabilizing effect of ascorbic acid on green tea catechins. *Journal of Agricultural and Food Chemistry*, 46, 2512-2516.

Chen, Z.-Y., Zhu, Q.Y., Tsang, D. & Huang, Y. (2001). Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry*, 49, 477-482.

Cheynier, V. & Ricardo Da Silva, J.M. (1991). Oxidation of grape procyanidins in model solutions containing *trans*-caffeoyltartaric acid and polyphenol oxidase. *Journal of Agricultural and Food Chemistry*, 39, 1047-1049.

Choi, S.-G. & Kerr, W.L. (2003a). 1h nmr studies of molecular mobility in wheat starch. *Food Research International*, 36, 341-348.

Choi, S.-G., Kim, K.M., Hanna, M.A., Weller, C.L. & Kerr, W.L. (2003b). Molecular dynamics of soy-protein isolate films plasticized by water and glycerol. *Journal of Food Science*, 68, 2516-2522.

Deng, Y. & Zhao, Y. (2008). Effect of pulsed vacuum and ultrasound osmopretreatments on glass transition temperature, texture, microstructure and calcium penetration of dried apples (fuji). *LWT - Food Science and Technology*, 41, 1575-1585.

Fennema, O.R. (1996). In Food chemistry (pp. 1067). New York: Marcel Dekker, Inc.

Gentry, T.S. & Roberts, J.S. (2004). Formation kinetics and application of 5-hydroxymethylfurfural as a time-temperature indicator of lethality for continuous pasteurization of apple cider. *Innovative Food Science and Emerging Technologies*, 5, 327-333.

Gordon, M. & Taylor, J.S. (1952). Ideal copolymers and the second order transitions of synthetic rubbers. I. Noncrystalline copolymers. *Journal of Applied Chemistry*, 2, 493-500.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards -- A. Physics and Chemistry*, 81A, 89-96.

Guyot, S., Marnet, N., Sanoner, P. & Drilleau, J.-F. (2003). Variability of the polyphenolic composition of cider apple (*malus domestica*) fruits and juices. *Journal of Agricultural and Food Chemistry*, 51, 6240-6247.

Hagen, S.F., Borge, G.I.A., Bengtsson, G.B., Bilger, W., Berge, A., Haffner, K. & Solhaug, K.A. (2007). Phenolic contents and other health and sensory related properties of apple fruit (*malus domestica*) borkh., cv. Aroma. *Postharvest Biology and Technology*, 45, 1-10.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C. & Omanyuda, M. (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of chinese quince (*pseudocydonia sinensis* schneid.), quince (*cydonia oblonga* mill.), and apple (*malus domestica* mill.) fruits. *Journal of Agricultural and Food Chemistry*, 53, 928-934.

Ito, R., Yamamoto, A., Kodama, S., Kato, K., Yoshimura, Y., Matsunaga, A. & Nakazawa, H. (2003). A study on the change of enantiomeric purity of catechins in green tea infusion. *Food Chemistry*, 83, 563-568.

Johari, G.P., Hallbrucker, A. & Mayer, E. (1987). The glass-liquid transition of hyperquenced water. *Nature*, 330, 552-553.

Khanizadeh, S., Tsao, R., Rekika, D., Yang, R., Charles, M.T. & Rupasinghe, H.P.V. (2008). Polyphenol composition and total antioxidant capacity of selected apple genotypes for processing. *Journal of Food Composition and Analysis*, 21, 396-401.

Khokhar, S. & Magnusdottir, S.G.M. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the united kingdom. *Journal of Agricultural and Food Chemistry*, 50, 565-570.

Labbe, D., Tetu, B., Trudel, D. & Bazinet, L. (2008). Catechin stability of egc- and egcg-enriched tea drinks produced by a two-step extraction procedure. *Food Chemistry*, 111, 139-143.

Labuza, T.P. & Altunakar, B. (2007). Water activity prediction and moisture sorption isotherms. In G. V. Barbosa-Canovas, J. Anthony J. Fontana, S. J. Schmidt & T. P. Labuza. Water activity in foods: Fundamentals and applications (pp. 109-154). Ames, Iowa: IFT Press; Blackwell Publishing.

Lea, A.G.H. (1984). Farb- und gerbstoffe in englischen mostapfeln. *Fluess. Obst.*, 8, 356-361.

Mannino, S. & Pagliarini, E. (1988). A rapid hplc method for the determination of vitamin c in milk. *Lebensmittel-Wissenchaft und-Technologie*, 21, 313-314.

Martins, S.I.F.S., Jongen, W.M.F. & Van Boekel, M.A.J.S. (2001). A review of maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*, 11, 364-373.

Moraes, M.A., Rosa, G.S. & Pinto, L.A.A. (2008). Moisture sorption isotherms and thermodynamic properties of apple fuji and garlic. *International Journal of Food Science and Technology*, 43, 1824-1831.

Ortiz, J., Ferruzzi, M.G., Taylor, L.S. & Mauer, L.J. (2008). Interaction of environmental moisture with powdered green tea formulations: Effect on catechin chemical stability. *Journal of Agricultural and Food Chemistry*, 56, 4068-4077.

Oszmianski, J. & Lee, C.Y. (1991). Enzymatic oxidation of phloretin glucoside in model system. *Journal of Agricultural and Food Chemistry*, 39, 1050-1052.

Oszmianski, J., Wolniak, M., Wojdylo, A. & Wawer, I. (2008). Influence of apple puree preparation and storage on polyphenol contents and antioxidant activity. *Food Chemistry*, 107, 1473-1484.

Reznichenko, L., Amit, T., Zheng, H., Avramovich-Tirosh, Y., Youdim, M.B.H., Weinreb, O. & Mandel, S. (2006). Reduction of iron-regulated amyloid precursor protein and [beta]-amyloid peptide by (-)-epigallocatechin-3-gallate in cell cultures: Implications for iron chelation in alzheimer's disease. *Journal of Neurochemistry*, 97, 527-536.

Roos, Y.H. (2007). Water activity and glass transition. In G. V. Barbosa-Cánovas, J. Anthony J. Fontana, S. J. Schmidt & T. P. Labuza. Water activity in foods: Fundamentals and applications (pp. 29-46). Ames, Iowa: IFT Press/Blackwell Publishing.

Ruan, R.R. & Chen, P.L. (2001). Nuclear magnetic resonance techniques. In P. Chinachoti & Y. Vodovotz. Bread staling (pp. 113-128). Boca Raton: CRC Press.

Spanos, G.A., Wrolstad, R.E. & Heatherbell, D.A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural and Food Chemistry*, 38, 1572-1579.

Sun, B., Ricardo-Da-Silva, J.M. & Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. *Journal of Agricultural and Food Chemistry*, 46, 4267-4274.

Tomas-Barberan, F.A., Gil, M.I., Cremin, P., Waterhouse, A.L., Hess-Pierce, B. & Kader, A.A. (2001). Hplc-dad-esims analysis of phenolic compounds in nectarines, peaches, and plums. *Journal of Agricultural and Food Chemistry*, 49, 4748-4760.

Totlani, V.M. & Peterson, D.G. (2005). Reactivity of epicatechin in aqueous glycine and glucose maillard reaction models: Quenching of c2, c3, and c4 sugar fragments. *Journal of Agricultural and Food Chemistry*, 53, 4130-4135.

Van Den Broeck, I., Ludikhuyze, L., Weemaes, C., Van Loey, A. & Hendrickx, M. (1998). Kinetics for isobaric--isothermal degradation of $_l$-ascorbic acid. *Journal of Agricultural and Food Chemistry*, 46, 2001-2006.

Van Der Sluis, A.A., Dekker, M., Skrede, G. & Jongen, W.M.F. (2002). Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *Journal of Agricultural and Food Chemistry*, 50, 7211-7219.

Van Nieuwenhuijzen, N.H., Primo-Martin, C., Meinders, M.B.J., Tromp, R.H., Hamer, R.J. & Van Vliet, T. (2008). Water content or water activity: What rules crispy behavior in bread crust? *Journal of Agricultural and Food Chemistry*, 56, 6432-6438.

Vanzani, P., Rossetto, M., Rigo, A., Vrhovsek, U., Mattivi, F., D'amato, E. & Scarpa, M. (2005). Major phytochemicals in apple cultivars: Contribution to peroxyl radical trapping efficiency. *Journal of Agricultural and Food Chemistry*, 53, 3377-3382.

Venir, E., Munari, M., Tonizzo, A. & Maltini, E. (2007). Structure related changes during moistening of freeze dried apple tissue. *Journal of Food Engineering*, 81, 27-32.

Vrhovsek, U., Rigo, A., Tonon, D. & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry*, 52, 6532-6538.

Wang, H., Provan, G.J. & Helliwell, K. (2003). Hplc determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry*, 81, 307-312.

Wang, R., Zhou, W. & Jiang, X. (2008). Reaction kinetics of degradation and epimerization of epigallcatechin gallate (egcg) in aqueous system over a wide temperature range. *Journal of Agricultural and Food Chemistry*, 56, 2694-2701.

Welti-Chanes, J., Guerrero, J.A., Barcenas, M.E., Aguilera, J.M., Vergara, F. & Barbosa-Canovas, G.V. (1999). Glass transition temperature (t_g) and water activity (a_w) of dehydrated apple products. *Journal of Food Process Engineering*, 22, 91-101.

White, K. & Bell, L. (1999). Glucose loss and maillard browning in solids as affected by porosity and collapse. *Journal of Food Science*, 64, 1010-1014.

Yang, D.-J., Hwang, L.S. & Lin, J.-T. (2007). Effects of different steeping methods and storage on caffeine, catechins and gallic acid in bag tea infusions. *Journal of Chromatography A*, 1156, 312-320.

Yoshioka, S. & Aso, Y. (2007). Correlations between molecular mobility and chemical stability during storage of amorphous pharmaceuticals. *Journal of Pharmaceutical Sciences*, 96, 960-981.

Zaveri, N.T. (2006). Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. *Life Sciences*, 78, 2073-2080.

Zhu, Q.Y., Hammerstone, J.F., Lazarus, S.A., Schmitz, H.H. & Keen, C.L. (2003). Stabilizing effect of ascorbic acid on flavan-3-ols and dimeric procyanidins from cocoa. *Journal of Agricultural and Food Chemistry*, 51, 828-833.

Figure Legend

Fig. 6.1. Adsorption isotherm for (◆) apple and GT-apple (◊) products at 30°C

Fig. 6.2. DSC thermogram of GT-apple products equilibrated at different a_w.

Fig. 6.3. Glass transition onset temperature (T_{go}) versus moisture content for (◆) apple and GT-apple (◊) products. GT-apple data fit by the Gordon-Taylor model (Eqn. 3).

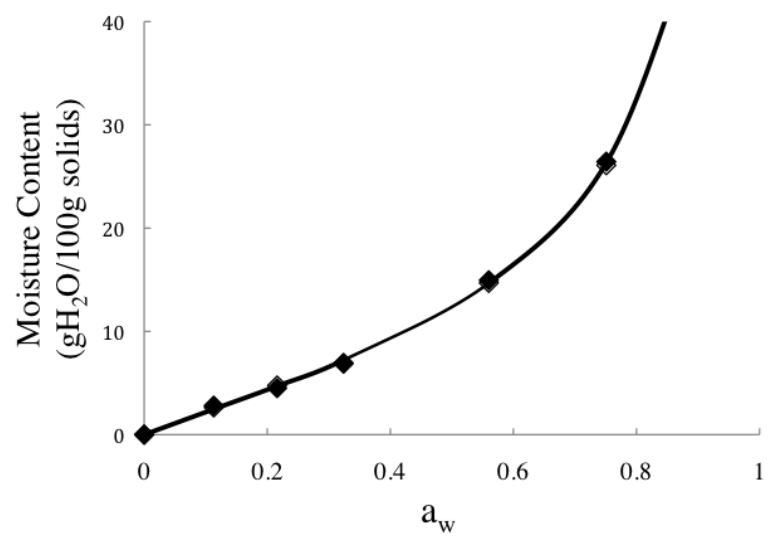**Fig. 6.4.** Free induction decay (FID) curves for GT-apple products at various a_w.
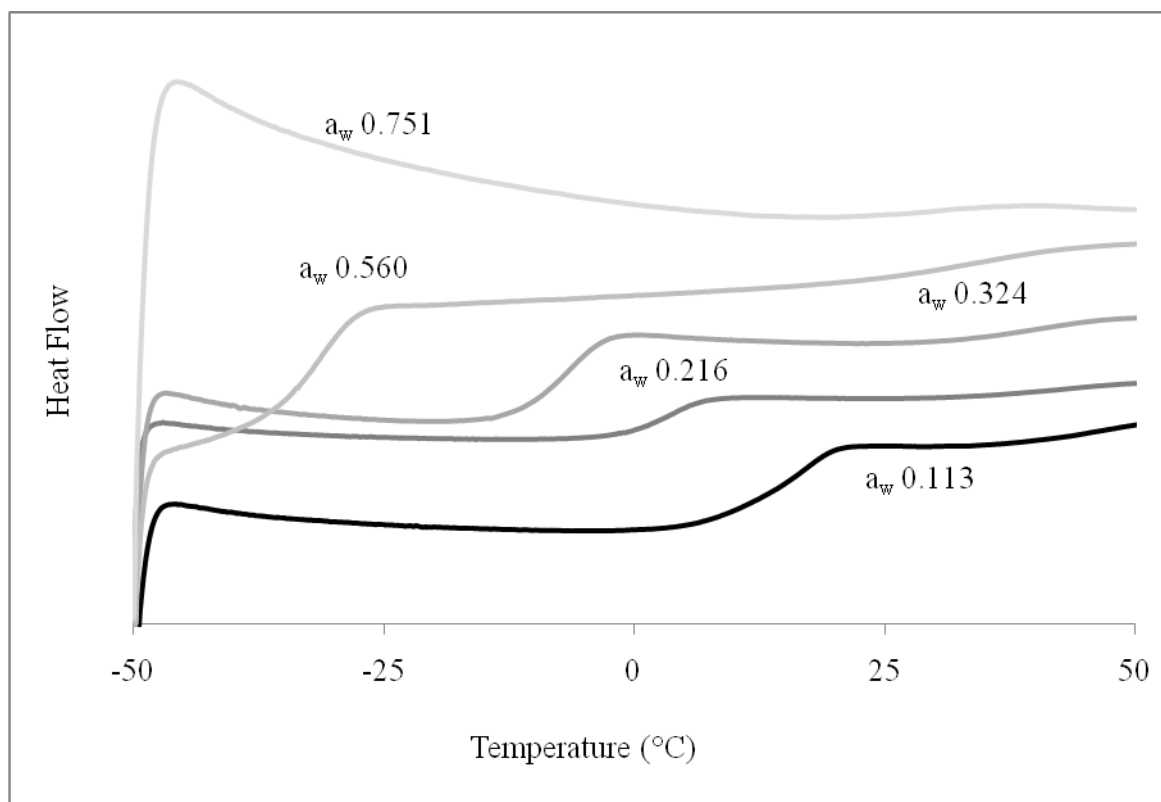
Figure 6.1

Figure 6.2
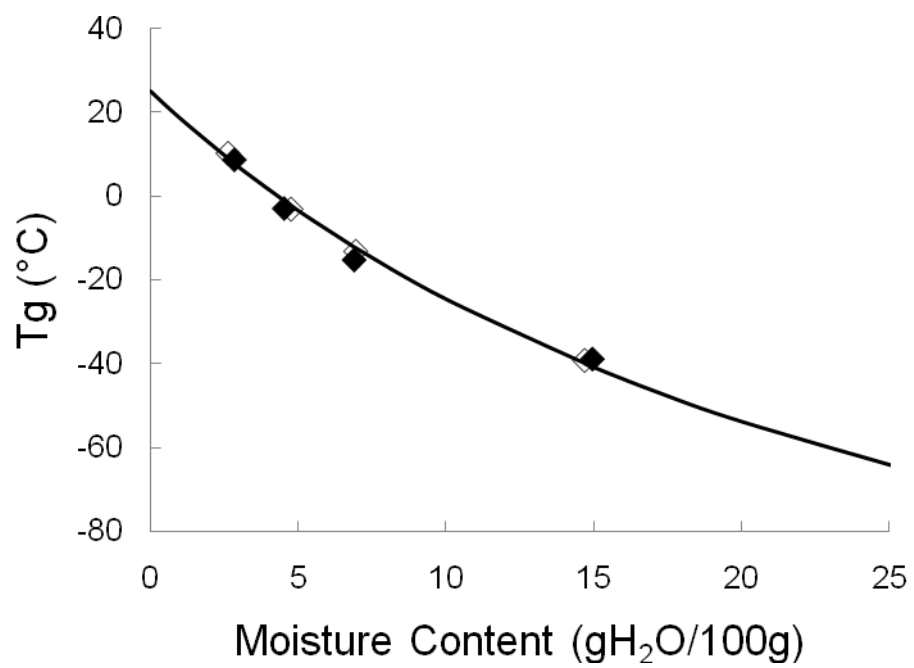
Figure 6.3
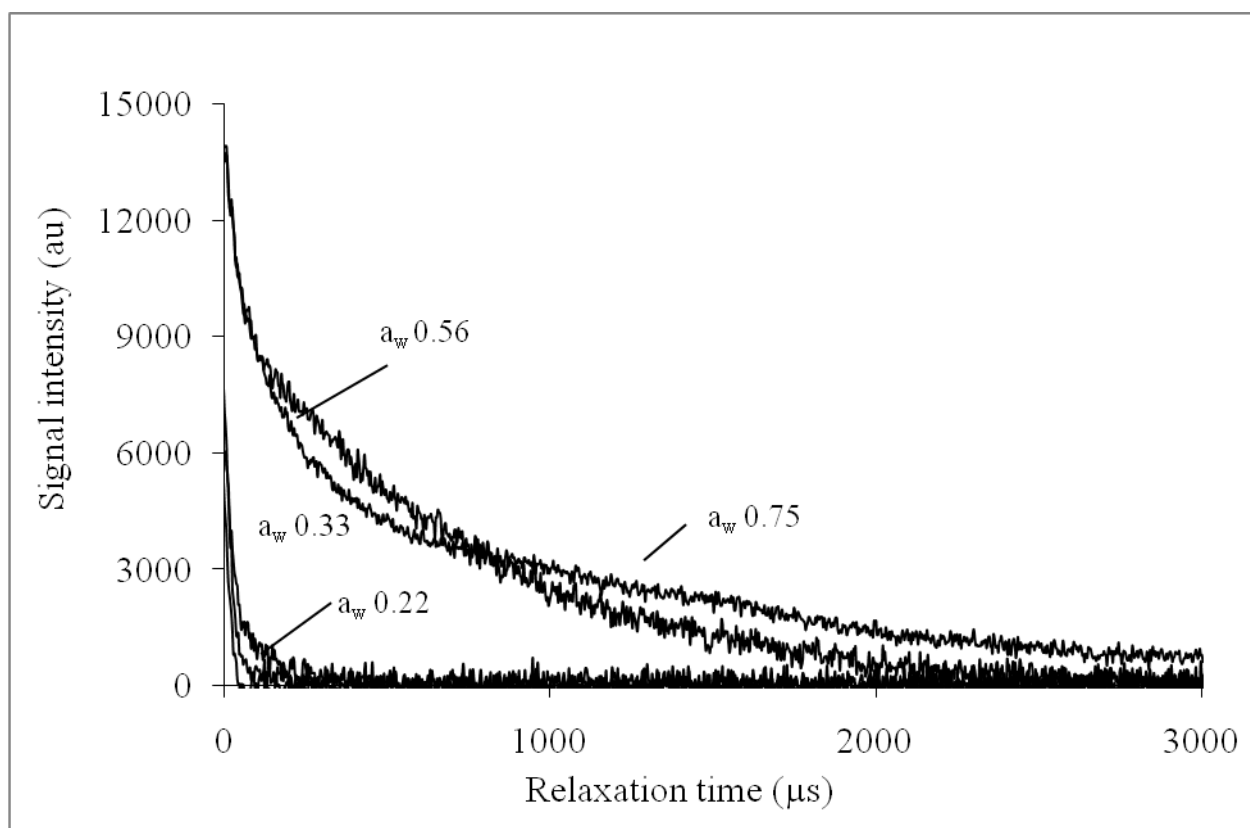
Figure 6.4

TABLES

Table 6.1. a_w, moisture content, and glass transition temperatures of apple and GT-apple products

a_w	Sample	Moisture Content (g water/100 g product, d.w.)	Onset Temp T_{go}	Midpoint Temp T_{gm}
0.113	Apple	2.81 ± 0.03	8.72 ± 1.64	12.85 ± 0.70
	GT-Apple	2.63 ± 0.26	10.28 ± 1.39	13.67 ± 0.09
0.216	Apple	4.50 ± 0.07	-2.89 ± 0.36	-0.295 ± 2.15
	GT-Apple	4.76 ± 0.12	-3.05 ± 1.49	0.17 ± 0.10
0.324	Apple	6.87 ± 0.25	-15.15 ± 1.90	-11.77 ± 1.71
	GT-Apple	6.95 ± 0.23	-13.12 ± 0.76	-9.23 ± 0.57
0.560	Apple	14.95 ± 0.42	-38.76 ± 0.87	-34.67 ± 0.46
	GT-Apple	14.69 ± 0.18	-39.15 ± 1.67	-34.41 ± 0.19
0.751	Apple	26.44 ± 0.07	n.d.	n.d.
	GT-Apple	26.09 ± 0.19	n.d.	n.d.

n.d. = not detectable;
No significant differences ($p \leq 0.05$) existed between apple and GT-apple products within a_w for moisture content, T_o, or T_m when analyzed using Tukey's HSD.

Table 6.2. One and two component fits to H^1 NMR
FID curves for dried apple and GT-apple products

a_w	%	T^*_{2a} (μs)	%	T^*_{2b} (μs)
Apple				
0.113	100	16.3		
0.216	100	23.6		
0.324	100	31.6		
0.560	48	44.2	52	305.6
0.751	33	60.4	67	526.1
GT-Apple				
0.113	100	12.7		
0.216	100	25.7		
0.324	100	32.3		
0.560	55	45.1	45	255.2
0.751	48	51.9	52	834.2

Table 6.3. Initial concentrations of antioxidants in apple and GT-apple products immediately after lyophilization

Antioxidant	Apple (mg/kg, d.w.)	GT-fortified apple (mg/kg, d.w.)
EC	$277^a \pm 18$	$860^b \pm 42$
C	$56^c \pm 4$	$301^d \pm 17$
EGC	n.d.	$2066^e \pm 157$
EGCG	n.d.	$2317^f \pm 63$
ECG	n.d.	$548^g \pm 28$
GCG	n.d.	$542^h \pm 32$
Procyanidin B2	$498^i \pm 5$	$507^i \pm 6$
Total Procyanidins	$1505^j \pm 257$	$2438^k \pm 89$
Caffeine	n.d.	$1429^l \pm 61$
Ascorbic acid	$118^m \pm 4$	$120^m \pm 3$
Chlorogenic acid	$1061^n \pm 18$	$1110^n \pm 41$
Total dihydrochalcones	$135^o \pm 7$	$142^o \pm 7$

Different letters within the same row indicate a significant difference ($p \leq 0.05$) using Tukey's HSD.

Table 6.4. Reaction rate constants (k) for changes in bioactive compounds from apple and GT-apple products

Antioxidant	a_w	Apple k (d^{-1})	R^2	GT-apple k (d^{-1})	R^2
EC	0.113	-0.008	0.94	-0.009	0.88
	0.216	-0.009	0.91	-0.010	0.96
	0.324	-0.013	0.98	-0.010	0.84
	0.560	-0.022	0.97	-0.013	0.90
	0.751	-0.034	0.99	-0.014	0.89
C	0.113	-0.006	0.83	n.c.	
	0.216	-0.008	0.87	n.c.	
	0.324	-0.022	0.87	n.c.	
	0.560	-0.045	0.96	n.c.	
	0.751	-0.072	0.99	n.c.	
EGC	0.113	n.d.		-0.006	0.55
	0.216	n.d.		-0.007	0.67
	0.324	n.d.		-0.007	0.74
	0.560	n.d.		-0.010	0.87
	0.751	n.d.		-0.009	0.86
EGCG	0.113	n.d.		-0.006	0.47
	0.216	n.d.		-0.009	0.83
	0.324	n.d.		-0.012	0.89
	0.560	n.d.		-0.013	0.92
	0.751	n.d.		-0.017	0.96
ECG	0.113	n.d.		-0.011	0.79
	0.216	n.d.		-0.012	0.81
	0.324	n.d.		-0.016	0.95
	0.560	n.d.		-0.017	0.98
	0.751	n.d.		-0.014	0.83
GCG	0.113	n.d.		-0.005	0.54
	0.216	n.d.		-0.004	0.33
	0.324	n.d.		-0.003	0.41
	0.560	n.d.		-0.004	0.33
	0.751	n.d.		-0.009	0.96
Procyanidin B2	0.113	n.c.		n.c.	
	0.216	-0.007	1.00	n.c.	
	0.324	-0.016	0.92	-0.011	0.85
	0.560	-0.039	0.99	-0.014	0.94
	0.751	-0.053	0.97	-0.023	0.67

Total Procyanidins	0.113	n.c.		n.c.	
	0.216	n.c.		-0.007	0.93
	0.324	-0.007	0.38	-0.010	0.97
	0.560	-0.014	0.54	-0.013	0.98
	0.751	-0.013	0.85	-0.013	0.70
Caffeine	0.113	n.d.		-0.006	0.45
	0.216	n.d.		-0.003	0.71
	0.324	n.d.		-0.004	0.68
	0.560	n.d.		n.c.	
	0.751	n.d.		n.c.	
Ascorbic acid	0.113	-0.007	0.94	-0.005	0.85
	0.216	-0.008	0.84	-0.006	0.97
	0.324	-0.009	0.96	-0.009	0.99
	0.560	-0.058	1.00	-0.049	0.97
	0.751	-0.471	0.97	-0.542	0.98
Chlorogenic acid	0.113	n.c.		n.c.	
	0.216	n.c.		n.c.	
	0.324	n.c.		n.c.	
	0.560	-0.002	0.91	-0.004	0.72
	0.751	-0.006	0.99	-0.006	0.84
Total dihydrochal-cones	0.113	-0.007	0.87	-0.012	0.83
	0.216	-0.006	0.49	-0.007	0.62
	0.324	-0.005	0.90	-0.009	0.69
	0.560	-0.003	0.95	-0.012	0.81
	0.751	-0.005	0.78	-0.012	0.84

n.c. = no significant change over time

n.d. = below limit of detection

CHAPTER 7. CONCLUSIONS

This dissertation focused on increasing the availability of functional food products for consumers, and evaluating the processing or storage stability, antioxidant activity, and health properties of polyphenolic compounds in functional products. Functional ingredients were paired with basic, staple food products. This was to provide value-added benefits by increasing the health-promoting properties of the food product, or to address certain dietary needs by delivering anti-metabolic syndrome or anti-diabetic properties. This occurred through addition of complementary polyphenolic compounds. The functional products were made from high-proanthocyanidin sorghum bran extract and white grape juice formulated with spice bioactive compounds, and green tea extract added to apple products.

In the production of functional food products, variables including microbial reductions of liquid product using continuous-flow high-pressure throttling and the stability and antioxidant activity of phytochemicals in green tea-supplemented apple products were also evaluated. This included analysis of how moisture mobility affected rates of phytochemical degradation. Thoroughly analyzing these aspects of food production are imperative to produce wholesome, safe food products that are stable through a specified time of storage. Elucidation of possible mechanisms for product failure, such as loss of documented nutrients or phytochemicals with affects from moisture content or mobility, will aid in steps to recognize or mitigate these potential pitfalls.