PIGMENTED POTATOES ON HEALTH: EFFECT ON OXIDATIVE STRESS,

INFLAMMATORY DAMAGE AND IMMUNE RESPONSE IN HUMANS, SENSORY

ATTRIBUTES, AND NUTRIENT RETENTION DURING PROCESSING

By

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Abstract

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The possible health benefits of pigmented potatoes are unknown. In experiment 1, healthy male participants were fed 150 g/d of one of the following for 6 wk: 1) white (WP), 2) yellow (YP), or 3) purple-fleshed (PP) potatoes for 6 wk. Blood samples were analyzed for oxidative stress, inflammatory damage and immune biomarkers. The DNA damage was lower ($P < 0.05$) in YP or PP compared to WP group. Blood C - reactive protein (CRP) and IL-6 were lower ($P < 0.08$) in PP than WP. Subjects in YP had lower ($P < 0.08$) IL-6 and tended to have lower ($P > 0.1$) CRP than WP. Percent of total Tc cells were lower ($P < 0.04$) while B cells were higher in PP than in WP ($P < 0.05$). Experiment 2 compared drum drying (DD), freeze drying (FD) and Refractance Window™ drying (RW) on the retention of bioactive compounds in white (WP), yellow (YP), red (RP) and purple potatoes (PP). The DD increased ($P <0.001$) the amount of phenolics in WP. All drying methods decreased ($P <0.001$) anthocyanins by 29-67% in RP and PP; FD retained more ($P <0.001$) anthocyanins compared to the other methods. Drying decreased ($P <0.01$) carotenoids by 31-52% in YP; no significant differences were found among

processing methods. While drying decreased (P <0.001) total antioxidant activity of YP, RP and PP by 50-71%, drying increased (P <0.001) antioxidant activity in WP by 58-108%. Overall, FD and RW methods resulted in the least destruction of bioactive compounds in potatoes. In experiment 3, rank-sum sensory acceptance was performed by 60 untrained panelists for aroma, appearance, flavor and overall perception of WP, YP and PP. The PP was the least favorable (P < 0.05) for aroma and appearance attributes. However, no significant differences were seen for overall perception. In conclusion, consumption of yellow and purple pigmented potatoes reduced inflammation, DNA damage, and modulates immune cell response in males. No significant differences were observed among potatoes for overall acceptance. Drying significantly affected the concentration of bioactive molecules and should be considered with reference to the antioxidant class intended to be optimized.

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CHAPTER 1

LITERATURE REVIEW

1. Biological role of oxidants and antioxidants

Free radicals

Free radicals are important in biological systems because they are involved in many reactions. A free radical is defined as any atom or molecule capable of independent existence and contains at least one unpaired electron (Morello et al., 2002). The formation of a free radical occurs when a non-radical species loses one electron, leaving one unpaired electron. A series of chain reactions result in the formation of radicals; these include initiation, propagation and termination (Morello et al., 2002). Initiation refers to the start of the reaction, propagation is the generation of multiple radicals and reaction products, and termination is the step where radical chemistry is stop by the formation of stable products. The most important radicals capable of chain reactions in biological systems include nitric oxide, dioxygen, superoxide and lipid hydroperoxyl radicals (Thomas, 2000).

Reactive oxygen species (ROS) is a term often used to describe reactive molecules containing oxygen that are capable of oxidizing other molecules (Halliwell, 2001). ROS are radical and non-radical derivatives of oxygen. For example, hydrogen peroxide, a non-radical ROS, is a cellular by-product that can undergo degradation. Under certain conditions, cellular hydrogen peroxide degradation can fail, leaving highly reactive hydrogen peroxide that can form other radicals (Frei, 1994).

Oxidative stress

Free radicals are often implicated in the development of many chronic diseases. Essential molecules in the body such as proteins, lipids and DNA are necessary for cellular processes. Reactive free radicals can damage these macromolecules by oxidation, potentially leading to disruption in cellular function. Oxidation of a biological molecule is defined as an increase in the positive valence state, by the removal of one or more electrons from the molecule (Kehrer & Smith, 1994). Once a cell is incapable of normal function, stress occurs, leading to disease (Frei, 1994; Roberfroid & Calderon, 1995).

Oxidative stress is a disturbance in the prooxidant-antioxidant balance (Sies, 1985), and can potentially lead to cellular damage. ROS are believed to be a major contributor to the onset of oxidative stress in biological systems (Halliwell, 2001). Increasing evidence has demonstrated that the oxidation of biomolecules such as lipids, proteins and DNA by ROS decrease molecular function, which may lead to chronic disease susceptibility (Halliwell & Chirico, 1993; Uprichard & Sutherland, 1999; Kim et al., 2006; Shukla et al., 2004). Typically, oxidative stress is thought to be an end-result and not the primary cause of the disease process. However, oxidative stress is believed to play an important role in the development of human diseases. Oxidative stress can result from the following situations (Halliwell, 2001):

1) Diminished amount of antioxidants due to decrease in antioxidant defense enzymes, increase in toxins that deplete antioxidant defense, or deficiency in dietary antioxidants.

2) Increased production of reactive species, due to increased production of toxins (that are themselves reactive species or are metabolized to generate reactive species) or over-activation of natural biological systems that produce reactive species.

Oxidative stress on cellular components may result in: 1) adaption of the cell or organism through the up-regulation of the defense systems, 2) tissue injury, and 3) cell death by apoptosis or necrosis.

Antioxidants

Antioxidants are reducing agents capable of donating one or more electron(s) to a free radical, thereby terminating the chain reaction. Antioxidants can prevent or delay the oxidation of biological molecules (Tabassum et al., 2007). Cells produce antioxidants such as superoxide dismutase, catalase and glutathione in order to combat free radicals generated via cellular processes such as metabolism or environmental factors. Dietary antioxidants also contribute to free radical scavenging (Briviba & Sies, 1994). The primary role of antioxidants in the body is to prevent or inhibit cellular degradation mediated by free radical reactions (Slater & Cheeseman, 1985; Halliwell, 1995; Morello et al., 2002).

Dietary antioxidants prevent or inhibit radical reactions by hydrogen abstraction or metal-ion assisted electron transfer (Morello et al., 2002). The process of hydrogen abstraction requires an antioxidant to donate a hydrogen atom to the free radical, thereby inhibiting propagation. Classes of dietary antioxidants capable of hydrogen abstraction include phenols, polyphenols, ascorbic acid, vitamin E and carotenoids. Metal-ion assisted electron transfer reactions involve the inhibition of transition metal reactions by polyphenols. Polyphenols act by binding to metals, thereby preventing electron transfer from the metal compounds.

2. Immune function

The immune system is a complex network of cells and organs intended to protect the body from infection and disease. Immune cells are highly active and produce ROS in response to a foreign antigen; therefore, oxidative damage to the cell membrane can be detrimental to immune cell function (Gruner et al., 1986). For optimal effector function, immune cells such as macrophages must take up more antioxidants compared to other cells in order to reduce the effect increased ROS produced during an immune response (Coquette et al., 1986)

Carotenoids & immunity

The immune system is composed of innate and acquired immunity. The acquired immunity branch involves lymphocytes. Lymphocytes generate ROS as a part of their normal function. Carotenoids are important for optimal immune function. These dietary antioxidants have the ability to quench free radicals such as the superoxide anion (O_2^-) and other ROS created from O_2^- by radical chemistry (Chew & Park, 2004). During an innate immune response to a pathogen, macrophages take up molecular oxygen during respiratory burst to kill bacteria (Halliwell, 2006). Although the body produces many endogenous antioxidants which can effectively scavenge ROS, over-production of O_2^- by phagocytes can lead to an oxidant:antioxidant imbalance and ensuing ROS-induced cell damage. Immune cells are particularly susceptible to damage caused by ROS due to the high concentration of polyunsaturated fatty acids (PUFAs) in the cell membrane (Meydani et al., 1995). The immune system depends on cell-to-cell communication via membrane-bound receptors. Consequently, oxidative damage to the cell membrane or receptors can be detrimental to lymphocyte function because it decreases the expression of surface molecules (Gruner et al., 1986). It is critically

important for immune cells to take up antioxidants to neutralize the increased level of ROS produced during an immune response (Coquette et al., 1986).

Cell signaling via antigen presentation must effectively occur in order for a cell-mediated immune response to begin. This happens through antigen presentation by antigen-presenting cells (APCs) such as monocytes, macrophages and dendritic cells. APCs present foreign antigens to immune system cells through membrane-bound molecules major histocompatibility complex II (MHC II) molecules that are unique to APCs (Janeway et al., 2005). The CD4^{+} T cells have T-cell receptors that readily recognize antigen presented by an APC. The expression of MHC II molecules in humans is dependent on a set of three human leukocyte antigen (HLA) genes, HLA-DR, HLA-DP and HLA-DQ. The primary function of the HLAs is to present either foreign or self-antigens to immune cells. Foreign antigens presented by MHC II will elicit a cell-mediated immune response. The MHC II expression on immune cells directly affects the immunogenic response to an antigenic stimulant (Reith & Mach, 2001). Increased expression of MHC II is beneficial to the elicitation of an immune response.

β-Carotene may enhance antigen presentation, a process vital to the immune response required to eradicate pathogenic infections and neoplastic cells. The expression of HLA-DR in human monocytes decreased in response to ROS exposure. However, carotenoids such as β-carotene can upregulate HLA-DR expression (Gruner et al., 1986).

Carotenoids, lymphoblastogenesis and co-stimulatory molecule regulation

Lymphoblastogenesis is used to assess the ability of T and B lymphocytes to proliferate in response to a mitogen or antigen (Chew & Park, 2004). Current research on lymphoblastogenesis involves animal and human *ex vivo* studies, as well as *in vitro* cell cultures.

The MHC II molecules on APCs, such as monocytes, are measured. The combination of an increased T helper (Th) population and the up-regulation of MHC II and adhesion molecules, or increased lymphocyte functional antigen (LFA), are good markers for enhanced immune response.

Prabhala et al. (1989) cultured human peripheral blood mononuclear cells (PBMC) with β-carotene and cantaxanthin to measure lymphoblastogenesis. Physiologically achievable concentrations (10^{-8} M) of β-carotene and cantaxanthin increased of the Th subset. When human subjects were given 30 mg/day β-carotene for 3 months, no effect on total T cells or T cell subsets was observed (Prabhala et al., 1991). Results of these studies indicate a different lymphoblastogenic response to β-carotene and cantaxanthin in *in vitro* versus *in vivo* studies.

In *ex vivo* animal studies, Kim et al. (2000a) demonstrated that domestic cats fed lutein for 12 weeks had higher Th (CD4+) and B (CD21+) cell populations. Increases in these lymphocyte subsets are indicative of enhanced immunity. In a similar study, Kim et al. (2000b) demonstrated that domestic dogs fed lutein/zeaxanthin (20 mg/day) for 8 weeks had higher total T cell population and expression of MHC II molecules on APCs. The authors hypothesized that up-regulation of T cell subsets may be the result of mitogenesis induced by dietary lutein supplementation. The enhanced Th response may also be due to the upregulation of MHC II molecules by APCs from the lutein supplementation.

In human studies, Murata et al. (1994) demonstrated that after four weeks of supplementation with β-carotene (20 mg/day) in healthy males, the uptake of β-carotene by PBMC increased two fold. While no change in the total lymphocyte population was observed, the $CD4^+$ lymphocyte subset and ratio of $CD4^+$:$CD8^+$ increased. This ratio did not change until

after 9 months of supplementation, suggesting that it may take a long time for β-carotene to affect lymphocyte populations.

Hughes et al. (1997a) reported that β-carotene supplementation (15 mg/day) in healthy male non-smokers may enhance immune response by increasing cell-surface expression of MHC II molecules. An increase in MHC II expression is indicative of an enhanced immune response by allowing better cell-to-cell adhesion and up-regulating co-stimulatory molecules. After 26 days of β-carotene supplementation, participants showed a significant increase in the expression of MHC II and HLA-DR molecules on monocytes, which was maintained after a cross-over to a 26 day placebo treatment. Intercellular adhesion molecule-1(ICAM-1) and LFA-3 adhesion molecules significantly increased after supplementation, but returned to baseline levels after placebo treatment. Hughes et al. (1997b) reported that human subjects supplemented with 15 g β-carotene 28 days increased the percentage of monocytes expressing the HLA-DR MHC II molecule. Furthermore, the number of cells expressing the MHC II molecules remained significantly higher than baseline after the crossover placebo period. Additionally, monocytes expressing ICAM-1 and LFA-3 significantly increased. The carotenoid concentrations used are equivalent to the amount found in 150 g of carrots (β-carotene). Similarly, Hughes et al. (2000) supplemented healthy male nonsmokers (mean age=41) with lycopene or lutein (15 mg/day). A significant increase in the monocytes expressing MHC II HLA-DR and LFA-1 with lycopene supplementation; however, no increases in HLA-DP, HLA-DQ, ICAM-1, or LFA-3 were observed. Lutein supplementation had no effect on the relative numbers of MHC II molecules expressed on monocytes. Lutein significantly decreased the expression of HLA-DQ.

Corridan et al. (2001) reported no significant changes in lymphocyte populations,

cytokine production or cell surface markers in humans supplemented with β-carotene (8.2 mg/day) and lycopene (13.3 mg/day) after 12 weeks of supplementation. Participants fed 2, 4 or 8 servings of whole fruits/vegetables containing high concentrations of carotenoids for 4 weeks showed no significant differences in lymphoproliferation (Watzl et al., 2005).

The results of these studies indicate that diets supplemented with carotene and xanthophyll carotenoids may increase immune function. However, results generally have been inconsistent, likely due to differences in the type of study (*in vitro* vs. *in vivo*), type of carotenoid, dose, length of supplementation, and whether challenged with any vaccine.

Polyphenols and immune function

In comparison to carotenoids, research on polyphenols and their effect on immune function are limited. Human studies involving polyphenols have demonstrated mixed results. However, evidence suggests that immune function may be enhanced by polyphenol supplementation. Recent human studies have focused on polyphenol administration via food and beverages.

Álvarez et al. (2006) investigated the immunomodulatory effects of cereal polyphenols on mice. The cereals contained varying amounts of gallic acid, *p*-hydroxybenzoic acid, vanillic acid, sinapic acid, *p*-coumaric acid, ferulic acid quercetin, catechin, rutin and oryzanol as the major polyphenolic constituents. Immune markers such as lymphoproliferation response to mitogens, tumor necrosis factor-α (TNF-α) and IL-2 were measured in mice fed the polyphenol-rich cereals after 5 weeks. Leukocytes obtained from mice on supplemental diets showed a significantly greater proliferative response to concanavalin A or LPS. Significant increases in IL-2 production were observed in all treatment groups except the group with no *p*-hydrobenzoic

acid. A decrease in TNF-α production was observed in all treatment groups except in those that did not receive *p*-coumaric acid. Therefore, *p*-hydrobenzoic and *p*-coumaric acids may be important phenolics in immune-modulation in mice. This study demonstrates that cereals naturally rich in polyphenols can increase immune function through increased IL-2 production and lymphoproliferation. This study also demonstrates that TNF-α, an inflammatory cytokine, can be inhibited by a polyphenol-rich diet.

Chen et al. (2005) investigated the action of purple sweet potato leaves (PSPL) on immune function in humans. The PSPL are typically grown in tropical regions of Asia and are excellent sources of polyphenols and carotenoids. In their study, participants consumed 200 g of PSPL for 2 weeks. The control group diet had low polyphenols and had the carotenoid concentrations adjusted to match the PSPL group; therefore, any immunomodulatory effects is attributed to the polyphenols. After two weeks of PSPL supplementation, PBMC proliferation and concentrations of differentiation cytokines, IL-2 and IL-4, were significantly higher in the PSPL group. Watzl et al. (2004) reported that red wine polyphenols (500 mL/day) had no significant effects on lymphocyte proliferation, secretion of IL-2, IL-4 and TNF-α, NK lytic activity, phagocytic activity and apoptosis.

Comparing these results, polyphenols appear to have a beneficial effect on immune response. Discrepancies among studies may be due to the type and concentration of the polyphenol used and the length of time the antioxidant was administered.

3. Inflammation and chronic disease

Oxidative stress caused by ROS initiates an inflammatory response from the immune system (Closa & Folch-Puy, 2004). Chronic inflammation is categorized by a continuous cycle of tissue infiltration by mononuclear cells, tissue destruction and tissue repair (Kumar et al., 2003). Chronic inflammation is a response to a variety of conditions that produce ROS, such as persistent infection, prolonged exposure to toxic agents, autoimmune diseases and obesity.

Pathogenesis

The immune response plays a major role in the initiation of the inflammation process in response to tissue injury, causing blood monocytes to migrate to the site of injury or infection. Stimulation by endotoxins (gram-positive bacteria) or interferon- γ (IFN-γ) produced by activated T cells facilitates the differentiation of monocytes to macrophages. Macrophages are a major mediator of chronic inflammation due to their ability to produce active pro-inflammatory substances such as ROS and reactive nitrogen species (RNS), chemotactic factors, coagulation factors, arachidonic acid metabolites, nitric oxide (NO) and cytokines (Ding et al., 1988; Kumar et al., 2003).

Upon exposure to an immunological stimulant such as IFN-γ, macrophages secrete the proinflammatory cytokine TNF- α, which stimulates the production of NO by inducible nitric oxide synthase (iNOS) (Taylor et al., 1998). Various studies have demonstrated that TNF-α plays a pivotal role in the production of NO by working synergistically with other cytokines such as IFN-γ or IL-1 (Ding et al., 1988; Moncada et al., 1991). In addition to stimulation of NO production, TNF-α also augments the release of ROS from macrophages; the ROS can react with NO to produce other reactive nitrogen species (Kobuchi et al., 1999). The chronic presence of

these substances can lead to inflammation and tissue injury that is associated with many diseases.

Elevated concentrations of IL-1, TNF-α and ROS can activate nuclear factor-κB (NFκB), a transcription factor responsible for inducing the expression of genes involved in inflammation and cellular stress, injury and infection (Bowie & O'Neill, 2000). The heightened cellular stress response caused by the activation of NFκB is implicated in the development and progression of cancer by regulating the expression of genes involved in cellular proliferation, migration and apoptosis (Dolcet et al., 2005).

In addition to activating NFκB, IL-1 and TNF-α promote the secretion of a secondary cytokine, IL-6. The IL-6 is produced by macrophages, although a number of other blood cell types are capable of secreting IL-6; these include lymphocytes, endothelial cells, smooth muscle cells and fibroblasts (Rattazzi et al., 2005). The body responds to IL-6 during infection or inflammation by producing an acute phase protein by the liver, C-reactive protein (CRP) (Castell et al., 1990). CRP is an indicator of inflammation and is currently used a marker for chronic disease development and progression (Griselli et al., 1999; Smolen et al., 2006)

In recent years, research has confirmed that obesity causes chronic inflammation and oxidative stress, thereby increasing the risk of heart disease, type II diabetes and cancer (Stehno-Bittel, 2008; de Ferranti & Mozaffarian 2008). Such diseases are characterized by systemic inflammation and by elevated concentrations of circulating pro-inflammatory cytokines and CRP. Concentrations of circulating cytokines increase with disease severity (Yndestad et al., 2006). Research demonstrates that obese individuals have lower plasma antioxidant concentrations than non-obese individuals, and interventions such as exercise and a low-fat diet that increases daily antioxidant consumption can reduce inflammation in obese persons (Vincent

et al., 2007). Limiting the production of cytokines and NFκB activation can lower inflammation and is an approach to prevent chronic inflammatory diseases.

Carotenoids and inflammation

Evidence suggests that dietary carotenoid serum concentrations are inversely associated with inflammation markers, and therefore, with cardiovascular disease by positively affecting the oxidative stress pathway (Hozawa et al., 2007). Lutein inhibits the activation of NF-κB gene expression by scavenging superoxide and hydrogen peroxide, thereby inhibiting the inflammation process (Kim et al., 2008). Additionally, lutein decreases skin inflammation associated with UV-exposure (Lee et al., 2004) and choroidal neovascularization associated with age-related macular degeneration (Izumi-Nagai et al., 2007).

Recently, whole-food intervention studies have shown reduced inflammation after supplementation with fruits and vegetables containing high amounts of carotenoids. In the first phase of a study conducted by Watzl et al. (2005), volunteers consumed 2 servings of fruit and vegetables per day (one serving = 100 g or 200 mL juice) for 4 weeks. During the second phase of treatment, volunteers consumed 2, 4, or 8 servings/day of whole fruits and vegetables. Inflammation as measured by blood CRP concentrations were significantly lower in the 8 servings/day intervention group; there was a dose-dependent effect of fruit/vegetable consumption. Additionally, the CRP was inversely correlated with plasma β-carotene concentrations. No other significant correlation was observed for other carotenoids. Though fruits/vegetables (red cabbage, radishes, apples, cherries, strawberries and red currants) consumed in this study are good sources of polyphenols, the authors did not report on their possible effect on plasma CRP concentrations. It is unknown if lutein can reduce plasma CRP.

Polyphenols and inflammation

Dietary compounds that have the ability to inhibit the production of NO by blocking

iNOS synthesis may be beneficial in preventing chronic inflammation (Kobuchi et al., 1999).

The effect of polyphenols on NO production in RAW 264.7 LPS/IFN-γ activated macrophages

demonstrated that anthocyanins such as pelargonidin, peonidin, cyanidin, delphinidin, malvidin

and malvidin glycosides inhibited NO production by at least 50% (Wang & Mazza, 2002a).

Interestingly, glycosylated anthocyanins are less inhibitory on NO production than their

aglycones. In addition to individual polyphenols, anthocyanin extracts from blueberries,

blackberries, black currents and Saskatoon berries were investigated for their anti-inflammatory

properties. Only high concentrations (250-500 µg/mL) of purified blackberry and black current

extracts induced a moderate decrease in NO production. The observed inhibitory effects on NO

production by the purified berry extracts was positively correlated with the total phenolics and

total anthocyanin concentrations (R^2 = 0.76-0.90, P < 0.01) (Wang & Mazza, 2002a). Pergola et

al. (2006) found that blackberry extract containing primarily cyanidin-3-*O*-glucoside inhibited

iNOS protein expression and production dose-dependently in rat lung cells. These results show

different NO inhibitory activity of an aglycone, a glycosylated anthocyanin or an extract

containing many glycosylated anthocyanins.

Cyanidin-3 glucoside (C3G) inhibited iNOS and NO production in a murine model using

zymosan-induced inflammation (Tsuda et al., 2002). The C3G treated group produced greater

NO inhibition compared to the non-C3G treated group. Treatment with C3G also inhibited the

production of IL-1β, TNF-α and IL-6, indicating that C3G inhibits inflammation in mice.

Although anthocyanins and flavonols have been shown to inhibit NO production in RAW

264.7 macrophages, one study has demonstrated a dose-dependent increase in TNF-α production after treatment with anthocyanins (16, 31, 63, 125, 250, 500 µg/mL) (Wang & Mazza, 2002b). Potential mechanisms of polyphenol inhibition of NO production include the scavenging of NO radicals, inhibition of iNOS enzyme activity and inhibition of iNOS gene expression (Kobuchi et al., 1999). The effects of anthocyanins on TNF-α may be due to their ability to decrease NO production post-transcriptionally, resulting in the upregulation of TNF-α mRNA expression, which accounts for the increase in TNF-α secretion (Wang & Mazza, 2002b; Wadsworth & Koop, 1999).

The ability of anthocyanins to inhibit NFκB has been reported in several studies. Hämäläinen et al. (2007) found that pelargonidin inhibited the activation of NFκB by 80% in macrophages, thereby reducing iNOS expression and NO production. Anthocyanin-rich pomegranate juice extract decreased gene expression and production of IL-6 in basophils by blocking MAP kinase and NFκB activation (Rasheed et al., 2009). In a human intervention study, the expression of pro-inflammatory cytokines involved in the activation of NFκB was examined following anthocyanin supplementation. Participants who consumed anthocyanin supplements (300 mg/d, 3 wk) made from bilberries and blackcurrants had a 40-45% decrease in IL-8 and IFN-γ compared to the control group (Karlsen et al., 2007).

Procyanidins are polymer chains of flavonoids that commonly found in grape seeds. The effect of procyanidin supplementation on cytokine expression was investigated in rats fed a low fat, high fat or high fat + procyanidin diet. Rats fed the high fat + procyanidin diet had a significantly lower plasma CRP concentration compared to the high fat diet. Expression of TNF-α and IL-6 was also significantly reduced by procyanidin feeding (Terra et al., 2009).

The combined results of these studies indicate that anthocyanins are potent anti-inflammatory compounds. Anthocyanins exert their anti-inflammatory effects by inhibiting NFκB activation and by regulating pro-inflammatory cytokines and NO production. Therefore, anthocyanin supplementation may reduce the risk of inflammatory chronic diseases such as rheumatoid arthritis and cardiovascular diseases.

4. Coronary heart disease

Inflammation plays a major role in the development and progression of coronary heart disease (CHD) (Kotur-Stevuljevic et al., 2006). CHD caused by atherosclerosis (ATH) is the narrowing and hardening of the coronary arteries by plaque deposits, and is the highest cause of death in the U.S. (American Heart Association, 2007).

Pathogenesis

The initiation of the pathogenesis of ATH is thought to start with endothelial cell dysfunction. Endothelial cells comprise the innermost layer of vascular walls and are responsible for maintaining a level of homeostasis in blood vessel wall and lumen by producing NO. The NO acts as a vasodilator, prevents platelet adhesion and aggregation and inhibits vascular smooth muscle cell proliferation (Vita & Keaney, 2002). Endothelial cell dysfunction decreases NO, resulting in inflammation, thrombosis, vasoconstriction, and formation of atherosclerotic lesion (Levine et al., 1995). This results in increased endothelial cell permeability, followed by increased leukocyte adhesion and altered expression of endothelial cell proteins. These changes are likely to occur from disturbances in blood flow and hypercholesterolemia.

Hypercholesterolemia is a major risk factor for the development of CHD (Kumar et al., 2003). Elevated total lipoprotein concentrations and a high low-density lipoprotein (LDL) to

high-density lipoprotein (HDL) ratio are contributing factors. Increased cholesterol concentrations may affect endothelial cell function by increasing the production of ROS, which decreases the production and bioavailability of NO. As NO production is decreased, endothelial cell permeability increases, resulting in an influx of lipoproteins and leukocytes into the intima layer of the blood vessel. Plasma LDL is oxidized by ROS produced by endothelial cells. Oxidized LDL can transverses the epithelial cell layer into the intima, where it is internalized by macrophages, creating foam cells (Tegos et al., 2001). The accumulation of foam cells, cholesterol, fibrous proteins and calcium deposits in the intima results in an atherosclerotic plaque. Plaque build-up causes protrusions from the intima into the vessel lumen, causing the epithelial layer to weaken and possibly rupture. The rupturing of the epithelial layer can lead to heart attack, stroke, aneurisms and peripheral vascular disease.

The role of cytokines & C-reactive protein in coronary heart diseases

Inflammation associated with heart disease is induced by cytokines secreted from immune cells that infiltrate the intima at an early stage in the development of atherosclerosis (Tedgui & Mallat, 2006). Macrophages are typically the first immune cells to respond (Watson et al., 1997). Th1 cells are the major cytokine-secreting cells responsible for the initiation of inflammation in the development of atherosclerosis, although other cell types such as macrophages, Th2 cells and B cells are also present (Frostegard et al., 1999).

Numerous cytokine pathways exist in the development of atherosclerosis, with the pro-inflammatory Th1 pathway being the most predominant (Frostegard et al., 1999). This pathway is initiated by the secretion of IL-12 from macrophages, which results in the differentiation of Th0 to Th1. In turn, Th1 cells generate IFN-γ and IL-2, which then results in suppressed

secretion of anti-inflammatory cytokines from other immune cells, recruitment of additional T cells (Wuttge et al., 2004) and secretion of IL-1β and TNF-α from macrophages (Young et al., 2002). The Th1 cytokine pathway is favored in the presence of oxidized LDL (Harlan, et al., 2006). The Th2 response has also been implicated in atherogenesis, but this remains controversial (Tedgui & Mallat, 2006).

The Th1 pro-inflammatory pathway is also responsible for the production of CRP that is secreted from the liver in response to IL-6 produced mainly by macrophages (Heinrich et al., 1990). Cytokines such as IL-1 and TNF-α are also responsible for CRP production. Until recently, elevated serum CRP concentrations were simply viewed as a marker of inflammation and not a causative agent (Li & Fang, 2004). However, more recent research has demonstrated an active role of CRP in the progression of atherosclerosis (Paffen & deMaat, 2006), and proposed the use of CRP as an important inflammatory predictor of cardiovascular risk (Haverkate et al, 1997; Ridker et al., 2000). CRP can directly induce pro-inflammatory cytokines (IL-1β & TNF-α) from monocytes and promote endothelial cell apoptosis (Nabata et al., 2007).

Griselli et al. (1999) demonstrated that CPR plays a direct role in complement activation, which in turn induces inflammation and cell destruction. Additionally, the combined presence of CRP and IFN-γ induce NO production, leading to increased inflammation and oxidative stress (Hattori et al., 2003). Other research indicates that CRP may play a direct role in enhancing procoagulant activity involved in thrombosis (Libby & Simon, 2001). Although current research suggests the multi-faceted roles of CRP in the progression of atherosclerosis, much controversy still exists and the exact role is yet to be elucidated (Paffen & deMaat, 2006).

Polyphenols, carotenoids and the prevention of heart disease: epidemiological evidence

Epidemiological evidence suggests an inverse relationship between polyphenol intake and the development of heart disease. However, only a limited number of flavonoid subclasses have been studied. These included flavonols (e.g. quercetin), flavones (e.g. apigenin), flavanones (e.g. hesperetin) or catechins (e.g. epicatechin) (Arts & Hollman, 2005), with little attention paid to phenolic acids or anthocyanins. The results of these studies are varied. Several studies reported no inverse relationship found between flavonol and flavone consumption and CHD risk in middle-aged women (Lin et al., 2007; Sesso et al., 2003; Rimm et al, 1996), while others have found an inverse association between flavonoid intake and CHD (Knekt et al., 1996; Yochum et al., 1999; Hirvonen et al. 2001).

Few studies have investigated the relationship between dietary anthocyanin intakes and heart disease (Hooper et al., 2008). Mink et al. (2007) investigated anthocyanin consumption in post-menopausal women. Results suggest that the highest quintiles of total flavonoid and anthocyanin intake were inversely related with mortality. The highest quintile of anthocyanin intake was found to be inversely related to the development of CHD and CVD, but not incidence of stroke. Anthocyanin food sources that are beneficial against heart disease include strawberries (CHD) and red wine (CHD & CVD).

Epidemiological evidence regarding heart disease and phenolic acids is limited. Two studies have indicated that moderate coffee consumption (<300 mL/day) may help to reduce the risk of the development of acute coronary events (Panagiotakos et al., 2003; Happonen et al., 2004). Chlorogenic acid is the primary phenolic acid in coffee. Heavy coffee consumption (300-600 mL/day) has demonstrated a significant risk increase, although the US Physician's and

Nurse's Health Study reported no relation between the amount of coffee consumed and heart disease (Hammar et al, 2003).

Anthocyanins and inflammatory signaling associated with heart disease

Recent evidence suggests that anthocyanins may reduce CD40 stimulated inflammation through NF-κB (Xia et al., 2007). CD40 is a cell receptor on immune and non-immune cells involved in cell signaling. Additionally, TNF receptor-associated factors (TRAFs) aid in the CD40 cellular signaling cascade and are important mediators of pro-inflammatory signaling (Zirlik et al., 2007). Through these signaling mechanisms, CD40 can recruit inflammatory cytokines IL-6 and IL-8, and the monocyte chemoattractant protein-1, which promote plaque development associated with atherosclerosis.

Xia et al. (2007) demonstrated that upon stimulation, CD40 induces cytokine production from endothelial cells (ECs). Cytokine production was inhibited in the presence of cyanidin-3-glucoside and peonidin-3-glucoside (100 μmol/L). Anthocyanins decreased the cholesterol content of the ECs, which led to decreased TRAF-2 expression in the lipid raft. Anthocyanin inhibition of TRAF-2 translocation may be ultimately responsible for the inhibition of CD40 and therefore inhibition of NF-κB activation. These results demonstrate the important role that anthocyanins play in cellular lipid regulation and inflammatory signaling pathways.

Anthocyanins and ischemia-reperfusion injury

Reperfusion results in tissue injury when blood flow is restored after a period of ischemia, and is partly due to the inflammatory response of the damaged tissue. Many inflammatory factors such as free radicals and interleukins are released by blood cells, and are typically seen in patients with a heart attack or stroke. Toufektsian et al. (2008) found that rats

supplemented with an anthocyanin diet containing pelargonidin and cyanidin glucosides demonstrated resistance to induced ischemia and reperfusion injury. In rats fed the anthocyanin diet, infarct sizes were significantly smaller than in the unsupplemented group, and were the result of increased production of reduced glutathione (GSH). GSH is a major antioxidant in human tissues: it functions in the transfer of electrons in redox reactions involving glutathione peroxidase-catalyzed reduction of hydrogen peroxide and lipid hydroperoxides. The higher GSH concentrations in anthocyanin fed rats suggests a potential benefit in preventing tissue injury associated with heart attack and stroke.

Polyphenols and LDL oxidation

LDL oxidation is a risk factor for the development of atherosclerosis. *Hibiscus sabdariffa* L. is a flower whose leaves are used to make infused beverages such as tea. A study was conducted to determine the anti-oxidative properties of an anthocyanin-rich *Hibiscus sabdariffa* L. extract (85-95% purity), containing 3.7 and 0.9% of delphinidin and cyanidin, respectively (Chang et al., 2006). Pre-treatment with anthocyanin extract (1-2 mg/mL) significantly inhibited LDL oxidation in a $CuSO_4$ induced LDL oxidation model. The anthocyanin extract (1.0-1.5 mg/mL) also reduced thiobarbituric acid-reactive substances (TBARS) production.

The ability of phenolic acids to prevent LDL oxidation was investigated in a study by Laranjinha et al. (1996). *In vitro* oxidation to LDL was initiated by ferrylmyoglobin. The phenolic acids that were most inhibitory against LDL oxidation were *o*-dihydroxy derivatives of cinnamic and benzoic acids, namely chlorogenic, caffeic and procatechuric acids. In a related study, the ability of regular coffee consumption was investigated with regards to its anti-LDL oxidation properties because coffee is a good source of chlorogenic and caffeic acids (Yukawa et

al., 2004). After 7 days of coffee supplementation, blood cholesterol, LDL-oxidation and TBARS were lower compared to baseline values. Chlorogenic acid was not detected in plasma after supplementation, suggesting that it may have been metabolized into its cinnamic and quinic acid derivatives.

Safari & Sheikh (2003) examined the *in vitro* investigated of the prevention of LDL oxidation by flavonoids. Human blood plasma was incubated with apigenin, genistein, morin, naringin, pelargonidin and quercetin (50, 100, and 200 μmol/L), and LDL was subsequently separated from plasma and oxidized with a copper catalyst. Oxidation was measured by the production of TBARS. Quercetin and morin inhibited LDL oxidation at 50 μmol/L, whereas pelargonidin, genistein and naringin inhibited TBARS formation at 100 μmol/L. All flavonoids were inhibitory at 200 μmol/L. The order of inhibition was: quercetin > morin > pelargonidin > genistein > naringin > apigenin. Although pelargonidin was not the most inhibitory flavonoid, it did display powerful antioxidant capabilities.

Carotenoids and the prevention of heart disease: epidemiological evidence

Sufficient epidemiological evidence suggests that an inverse relationship exists between carotenoid consumption and the development of heart disease (Connor et al., 2004; Ito et al., 2006). Ito et al. (2006) found that Japanese men and women with high serum α-carotene, β-carotene and lycopene concentrations had a lower mortality rate for cardiovascular diseases. Connor et al. (2004) found an increased risk of CHD in people with low serum concentrations of lutein, zeaxanthin and β-carotene. Low blood CRP concentrations have been associated with low serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin and lycopene (Kritchevsky et al., 2000; Hozawa et al., 2007). These results from these studies suggest that a

diet high carotenoids of may reduce the risk of atherogenesis by reducing inflammation. In contrast, others (Evans et al., 1998; Yochum et al., 2000; Hak et al., 2003) have failed to show the relationship between serum carotenoid concentrations and the development of heart disease.

Carotenoids and LDL oxidation

In vitro, lutein reduced LDL oxidation and inhibited monocyte chemotaxis into the arterial wall, preventing macrophage and oxidized LDL interactions (Dwyer et al., 2001). In the same study, mice supplemented with dietary lutein showed a 43% reduction in lesion (plaque) size and lower plasma lipid hyroperoxides. This demonstrates that lutein may be cardio-protective.

Carotenoids and heart disease: clinical studies

In response to mounting epidemiological evidence, clinical trials were initiated to study the effects of carotenoid supplementation, mainly β-carotene, on risk factors associated with heart disease. Results of these studies are relatively inconclusive, as some studies have shown a positive relationship between carotenoid supplementation and heart disease, while others have not.

The α-tocopherol β-carotene cancer prevention (ATBC) study was designed to investigate the effects of α-tocopherol (50 mg/day) and/or β-carotene (20 mg/day) supplementation on smokers and other groups at high risk for the development of cancer (Alpha-tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). A subset of participants who had previously experienced myocardial infarction was used to examine ensuing myocardial events (Rapola et al., 1997). Subjects given β-carotene or β-carotene/α-tocopherol supplementation groups had a higher instance of death from myocardial infarction compared to

unsupplemented groups; there was a trend towards increased death rate in the α-tocopherol group (Rapola et al., 1997). This study indicates that β-carotene supplementation may increase the risk of death from myocardial infarction in smokers who have previously experienced a major coronary event.

Additional studies published after the ATBC cancer prevention study have failed to demonstrate cardio-protection from β-carotene (Törnwall et al., 2004). The beta-carotene and retinol efficacy trial (CARET) examined supplementation of β-carotene (30 mg/day) and vitamin A (25,000 IU/day) for 4 years to long-term smokers and people exposed to occupational asbestos (Omenn et al., 1996). The supplemented group showed a 26% higher mortality rate from cardiovascular causes. In Physicians' Health Study I, healthy male physicians in the U.S were given 50 mg of β-carotene every other day for 12 years (Hennekins et al., 1996). β-Carotene supplementation had no beneficial or adverse effect on the incidence or death from cardiovascular events.

Overall, clinical trials involving β-carotene supplementation do not show any beneficial effects regarding the prevention of heart disease. However, only β-carotene was used in these trials. Additional trials need to be conducted to determine if supplementation of xanthophyll carotenoids, such as lutein can provide cardio-protection.

5. Cancer

Cancer is the second leading cause of death in the U.S., preceded only by cardiovascular disease (Centers for Disease Control and Prevention, 2006). Factors contributing to cancer development include genetics, geography, microbial exposure and environmental factors, such as

smoking and diet (Kumar et al., 2003). Tobacco use, poor diet and physical inactivity contributed

to the largest number of deaths from cancer; increasing death rates in this category are attributed

to diet and physical inactivity (Mokdad et al., 2005). Interest on nutrition in preventing cancer

has gained momentum.

Pathogenesis

Cancer is characterized by abnormal, invasive cellular growth that disables and destroys

adjacent cellular function. Regardless of the myriad of environmental or genetic factors that may

contribute to the initiation of cancer growth, the molecular basis of cancer initiation begins with

cellular inability to repair damaged DNA, thereby leading to gene mutation (Kumar et al., 2003).

DNA repair genes regulate normal regulatory genes, which are responsible for suppressing

abnormal cell growth and initiating apoptosis. Once damaged DNA goes unrepaired, oncogenes

are expressed and promote cancer progression; genes responsible for apoptosis and cancer

suppression are inactivated. Oncogenes enable cancerous cells to grow and replicate via a series

of growth signals for angiogenesis, invasion and metastasis.

Carcinogenesis and immune surveillance

As previously described, the onset of carcinogenesis is characterized by gene

transformation. The expression of antigens or oncogene proteins by MHC molecules on cancer

cells is recognized as non-self by the immune system. This immune surveillance is facilitated

primarily by natural killer (NK) cells (Kumar et al., 2003), although many cancers do

metastasize without substantial impact from immune surveillance (Janeway et al., 2005).

The frequent incidence of cancer in the human population implies that cancer cells can

proliferate by escaping detection by the immune system. Cancer cells escape immune detection

through the 1) lack of antigen presentation by MHC molecules, 2) tolerance of cancer antigen, 3) degradation of cancer antigen: antibody complexes by endocytosis, 4) secretion of immune-suppressive cytokines, and 5) generation of a physical barrier (Janeway et al., 2005).

Natural killer cells and cancer

The NK cells are lymphocytes associated with the innate immune response and are the first line of defense against tumor cells. NK cells circulate between peripheral blood passageways and lymph nodes. The main effector function of NK cells is to kill virus-infected and cancerous cells by releasing cytotoxic granules that penetrate the target cell's membrane and induce apoptosis, as well as attracting other innate and adaptive cells to the active site (Janeway et al., 2005).

The expression of MHC I by normal cells prevents NK cell cytotoxic perforin release. However, during transformation, tumor cells lose MHC I expression, thereby enabling NK cell detection (Aptsiauri et al., 2007). Tumors cells affect the ability of NK cells to destroy cancerous cells through cytokine inhibition and regulation of NK surface molecules (Konjević et al., 2007). Research involving diet and immunotherapy has demonstrated that NK cell function increases with carotenoid and polyphenol supplementation, making them more efficient at detecting and killing cancer cells (Santos et al., 1996; Chen et al., 2005).

Polyphenols and cancer prevention: epidemiological evidence

Evidence suggests that fruit and vegetable consumption is associated with a lower risk for the development of cancer. The anti-carcinogenic activity of these foods is attributed to phytochemical antioxidants (Johnson, 2007). Studies have attempted to determine which antioxidants exert beneficial health effects. Bosetti et al. (2006) found no relationship between

anthocyanin consumption and prostate cancer incidence, while Rossi et al. (2006) found that anthocyanin consumption was inversely related to the development of colorectal cancer. As with many types of epidemiological research, results vary as to whether anthocyanins, as well as other flavonoids and polyphenols, help reduce the risk of cancers.

Polyphenols and NK cells

Warner & Dennert (1982) used NK-deficient mice to demonstrate the importance of NK in tumor surveillance. The NK-deficient mice that received a NK injection had a 50-85% reduction in the incidence of melanoma tumors as compared to the NK deficient mice. The latter group also experienced a high rate of metastasis. This experiment shows that NK cells play an active role in the prevention of tumor growth.

Few studies have examined the effects of polyphenols on the effector functions of NK cell cancer surveillance. One study (Dedoussis et al., 2005) investigated whether cancer cells would be more susceptible to killing by NK cells after incubation with phenolic acid. NK cells were harvested from healthy volunteers and incubated with K562 human leukemic cells that were pre-treated with benzoic acid, syringic acid, ferulic acid, *p*-coumaric acid, *o*-coumaric acid, gallic acid, and rutin. Results indicated that flavonoid pre-treatment with the gallic acid induced NK mediated necrosis and rutin induced apoptosis. No significant difference was observed in cells incubated with phenolic acids.

Chen et al. (2005) tested the lytic activity of NK cells from participants supplemented with purple sweet potato leaves (200 g) for 2 weeks. The treatment group demonstrated a greater ability to lyse K562 (leukemia) cells, indicating that a diet rich in anthocyanin polyphenols may be chemopreventive.

Polyphenols and oxidative DNA damage

Oxidative DNA damage is an indication of oxidative stress and is a major risk factor for the onset of cancer (Block & Schwarz, 1994). In a recent study, Schaefer et al. (2006) reported an antioxidant protective effect of a phenolic-acid containing in human colon cell lines, Caco-2 and HT29. Menadione-induced superoxide and *tert*-butylhydroperoxide-induced peroxyl radicals were generated to simulate *in vivo* radical chemistry in pathological conditions of DNA oxidation. The juice extracts were reconstituted to contain different concentrations of phenolic acids and flavonoids. Reconstituted extracts containing phloridzin (26.1%), rutin (1.8%), chlorogenic acid (43.3%), caffeic acid (25.9%) and epicatechin (2.9%) significantly inhibited oxidative DNA damage measured by the comet assay. Another extract containing phloridzin (44.9%), rutin (47.2%), chlorogenic acid (7.8%), caffeic acid (1.6%) and epicatechin (7.2%) inhibited DNA damage. These results indicate that varying combinations of phenolic acids and flavonoids have the potential to reduce oxidative DNA damage.

Møller et al. (2004a) conducted a similar *in vivo* study measuring DNA damage in PBMC from healthy, young-adult females after anthocyanin supplementation. Supplementation consisted of blackcurrant juice (397 g/day) or anthocyanin drink (365 g/day) for 3 weeks. Anthocyanin supplementation did not influence DNA damage. In addition, no difference was observed between baseline and endpoint DNA damage. The lack of effect may be explained by inadequate anthocyanin dose or the absence of oxidative stress in the participants. Other studies involving antioxidant supplementation in smokers have shown protection against DNA damage (Møller et al. 2004b).

Polyphenols and chemoprevention

Polyphenols can reduce DNA damage, induce apoptosis, increase cancer cell death, decrease cell proliferation and decrease angiogenesis (Lambert et al., 2005; Prior & Wu, 2006). There is sufficient *in vivo* evidence to suggest that phenolic acids, including caffeic and chlorogenic acids can prevent or reduce tumor incidence in animal models (Takana et al., 1993). Matsunaga et al. (2002) demonstrated that rats fed chlorogenic acid (250 ppm) before carcinogen induction had a lower incidence of colon tumors compared to the control group. A decreased incidence of tumors also was observed in the rats fed the chlorogenic acid diet post-tumor initiation. Shimizu et al. (1999) also observed tumor prevention in rats fed 250 and 500 ppm chlorogenic acid. These studies demonstrate that a diet enriched with chlorogenic acid may help reduce the risk of tumor development. Additionally, caffeic acid extracts from dietary sources significantly decreased DNA damaged in S30 breast cancer cells (Burdette et al., 2002), and chlorogenic acid may prevent the formation of the lipid peroxide induced DNA adduct, 8-hydroxydeoxyguanosine (8-OHdG) in rats (Kasai et al., 2000). More studies are needed to determine the effects of dietary phenolic acids on cancer development.

Phenolic acids show pro-apoptotic activity in cancer cells. Kampa et al. (2003) found that physiologic concentrations of phenolic acids, especially caffeic acid were anti-proliferative actions and induced apoptosis in T47D, a human breast cancer line. Watabe et al. (2004) was able to show that caffeic acid phenethyl ester induced apoptosis in MCF-7 breast cancer cells through inhibition of NFκB. The latter induces Fas/FasL binding that initiates the caspase pathway, resulting in cell death. These studies have provided the molecular mechanisms by which phenolic acids act as chemopreventive agents.

Studies involving anthocyanins demonstrate many different mechanisms in chemoprevention. Azevedo et al. (2006) investigated the effects of anthocyanin extract from eggplant and a purified form of its primary anthocyanin, delphinidin. The eggplant extract did not prevent cyclophosphamide-induced mutagenesis in mice. On the other hand, purified delphinidin (10 and 20 mg/kg) reduced mutagenesis. It is important to note that purified delphinidin concentrations used in this study are unachievable via the diet. These results indicate that anthocyanins may help reduce the risk of cancer only at high concentrations. Pool-Zobel et al. (1999) found similar results. Various berry extracts did not prevent DNA strand breaks in a human colon cell line (HT29 clone 19A), while purified cyanidin (100 µM) significantly inhibited DNA strand breaks.

Other studies have demonstrated that anthocyanin extracts are capable of cancer inhibition. Reddivari et al. (2007b) recently reported on the anti-cancer effects of anthocyanin, phenolic acid and whole potato extracts from pigmented potatoes. The extracts were incubated with two different prostate cancer cell lines, PC-3 and LNCaP. Results indicated that at concentrations of 2.5-5 µg/mL, the anthocyanin and whole potato extract had the highest anti-proliferative effects. The cause of cell death from the extracts was determined to be from apoptosis, namely from both caspase-dependent and independent pathways. Seeram et al. (2006) reported similar findings from berry extracts. Concentrations ranging from 25-200 µg/mL produced a dose-dependent anti-proliferative effect in colon, prostate, breast and oral cancer cell lines. Only black raspberry and strawberry extracts (200 µg/mL) induced apoptosis, indicating that different extracts containing various combinations of antioxidants may inhibit cancer growth through different mechanisms.

Duthie et al. (2006) conducted an experiment investigating the effects of cranberry juice on the 8-OHdG biomarker in light smokers (3 cigarettes/day).Participants consumed cranberry juice (750 mL/day) for two weeks. No difference in DNA damage was observed with cranberry supplementation.

The investigation of cancer prevention mechanisms by anthocyanins has yielded valuable information. Lazzé et al. (2004) determined that delphinidin but not cyanidin (200 µM) inhibited tumor cell growth via apoptosis. The authors concluded that these results may be due to the higher antioxidant capacity of delphinidin due to the presence of a third hydroxyl group on the B ring. Hou et al. (2003) found that of the six aglycone structures investigated, delphinidin (100 µg/mL) was the most potent inducer of apoptosis. Cyanidin and petunidin also induced apoptosis, but to a lesser extent. Pelargonidin, peonidin and malvidin did not induce apoptosis. Taken together, these results indicate that the number of OH groups on the B ring of the aglycones may be responsible for their apoptotic activities. Delphinidin displayed the most potent effects (three OH groups), followed by cyanidin and petunidin (two OH groups), whereas pelargonidin, peonidin and malvidin (one OH group) displayed no apoptotic activities. Feng at al. (2007) found that cyanidin-3-glucoside (50 and 120 µM) upregulated the production of hydrogen peroxide in leukemia cells by activating p38 MAPK and JNK, which initiates a cascade of proteins involved in the apoptotic process. Hydrogen peroxide did not increase in cyanidin-3-glucoside treated non-cancer cells, suggesting that anthocyanins induce apoptosis in cancer cells by up-regulating the production of ROS.

Angiogenesis is the growth of new blood vessels in order to deliver nutrients and oxygen to tissues (Kumar et al., 2003).The process is required by cancer cells to grow and metastasize.

Therefore, the prevention of angiogenesis may prevent the growth and spread of cancers. Favot et al. (2003) investigated the effects of delphinidin on endothelial cell migration and proliferation, both of which play a pivotal role in angiogenesis. Endothelial cell migration and proliferation were both inhibited in a dose-dependent fashion when incubated with delphinidin (5, 10 and 20 µg/mL). Lamy et al. (2006) reported similar results. Out of six anthocyanin aglycones tested, delphinidin (15 µM) most effectively inhibited endothelial cell migration and proliferation (~76%). Pelargonidin, peonidin, petunidin and malvidin (15 µM) suppressed endothelial cell migration but to a lesser extent (~25%). The mechanism of angiogenesis inhibition in this study was the inhibition of vascular endothelial cell growth factor, which is a regulatory protein involved in endothelial cell migration.

The anti-cancer action of phenolic acids and anthocyanins is mediated through angiogenesis inhibition, ROS up-regulation, induction of apoptosis and DNA oxidative damage reduction. However, conflicting evidence exists, likely due to differences in experimental model, the concentration and the type of polyphenol used.

Carotenoids and cancer: epidemiological evidence

Epidemiological evidence suggests an inverse relationship between dietary carotenoid intake and the development of different cancers (van Poppel & Goldbohm, 1995). Männistö et al. (2004) conducted a pooled analysis of seven cohort studies investigating carotenoid intake and the risk of lung cancer. Results indicated that β-carotene intake did not affect the development of lung cancer, while β-cryptoxanthin was inversely related to lung cancer risk. Other studies have demonstrated an inverse relationship between carotenoid consumption and the risk of breast (Sato et al., 2002), prostate (Mettlin et al., 1989) and colon cancers (Kune et al., 1987). A few

epidemiological studies have shown an increased risk of cancer with elevated carotenoid consumption, but many have shown no relationship (van Poppel & Goldbohm, 1995; Cooper et al., 1999). Overall, the role of dietary carotenoids on the incidence of cancer remains uncertain.

Carotenoids and NK cells

Carotenoids improve immune function and surveillance by increasing the NK cells' ability to effectively detect and kill tumor cells (Hughes, 2001). Studies have demonstrated that carotenoid supplementation can increase the NK population (Prabhala et al., 1989). Often, increases in NK populations also result in an increased effector function. However, an increase in NK effector function can occur without a corresponding increase in the NK population (Santos et al., 1996). Cell cultures supplemented with β-carotene and cantaxanthin (10^{-8} M) for 72 hours significantly increased NK population, with cantaxanthin being more effective than β-carotene (Prabhala et al., 1989). Additionally, carotenoids also augmented the expression of IL-2 receptors on NK cells. IL-2 is an activation cytokine secreted by Th cells, which causes NK activation and increased cytotoxic activity (Male, 2004).

Human, *in vivo* experimentation have yielded similar results. Supplementation with 30 mg/day β-carotene for 3 months increased the NK population and cytotoxicity effector function in adult men with leukoplakia or Barrett's esophagus (Prabhala et al., 1991). By the second month, PBMC from the β-carotene group expressed more IL-2, HLA-DR and transferrin receptors. At the conclusion of the trial, the expression of the activation markers had decreased but remained significantly higher than baseline.

Immune function, including NK activity, is inversely related to age (Mocchegiani & Malavolta, 2004). Supplementation of β-carotene to a healthy elderly population (50

mg/alternate days, 65-86 years) maintained NK cell activity at levels comparable to middle-aged men (51-64 years), and activity was significantly greater than the elderly placebo group (Santos et al., 1996). The middle-aged placebo and β-carotene groups did not show a significant difference in NK activity. Treatment did not affect NK population or IL-2 receptors, in contrast to Prabhala et al. (1989). Santos et al. (1996) suggests that the discrepancy in the expression of IL-2 receptors may be due to differences in resting versus mitogen-stimulated PMBCs.

Research regarding the human consumption of carotenoids from whole, unprocessed fruits and/or vegetables and NK cell activity is limited. However, a recent human study involving healthy men assessed immune function following carotenoid-rich vegetable juice supplementation (Watzl et al., 2003). Participants on a low-calorie diet were given 330 mL/day of tomato juice (37 mg/day lycopene) or carrot juice (27 mg/day β-carotene and 13.1 mg/day α-carotene). Results indicated that NK activity and IL-2 production by PBMC significantly increased a week after the washout period. There was no difference between tomato and carrot juice, suggesting that lycopene and β/α-carotene increased the effector functions of PBMC. In another study (Watzl et al., 2005), subjects supplemented with 2, 4 or 8 servings of whole fruits/vegetables containing high concentrations of carotenoids for 4 weeks showed no significant effect on NK cytotoxicity.

Overall, studies suggest that carotenes and xanthophylls can affect NK activity in humans. The mechanism(s) as to how carotenoids increase NK lytic activity is currently unknown. It is likely controlled by the multi-faceted actions and interactions between immune cells and cytokine production (Santos et al., 1998). Additional studies supplementing carotenoids from fruit/vegetable juices or whole fruits/vegetables are warranted.

Carotenoids and oxidative DNA damage

Bioactive compounds found in watercress have been shown to prevent lymphocyte DNA damage in humans (Gill et al., 2006). In smokers, whole-food supplementation with watercress (85 g/day, 8 wk) decreased oxidative DNA damage. Because watercress is high in lutein, β-carotene and glucosinolates, it is unknown which compound(s) is/are responsible for the observed action.

Another study investigating the effect of carotenoids on lymphocyte DNA damage was conducted on post-menopausal women (Zhao et al., 2006). Diets were supplemented with a combination of lutein, β-carotene and lycopene (4 mg of each carotenoid) or with the individual carotenoids (12 mg/day) for 56 days. Women who received individual or mixed carotenoids had lower oxidative DNA damage compared to the unsupplemented group. Therefore, the action of watercress on the reduction of DNA damage may be due to its carotenoid content.

Carotenoids and cancer: clinical studies

Clinical trials have failed to demonstrate a chemopreventive effect of β-carotene supplementation. Results of these studies are relatively inconclusive.

The α-tocopherol β-carotene cancer prevention (ATBC) study was designed to investigate the effects of α-tocopherol (50 mg/day) and/or β-carotene (20 mg/day) supplementation on smokers and other groups at high risk for the development of cancer (Alpha-tocopherol, Beta Carotene Cancer Prevention Study Group, 1994). Results show higher incidence of lung cancer from β-carotene supplementation compared to the α-tocopherol or control groups. α-Tocopherol supplementation did produce any beneficial effects, and β-carotene supplementation may actually be harmful to long-term smokers.

Other studies similarly have failed to show a chemo-preventive effect of β-carotene. The beta-carotene and retinol efficacy trial (CARET) supplemented β-carotene (30 mg/day) and vitamin A (25,000 IU/day) for 4 years to long-term smokers and people exposed to occupational asbestos (Omenn et al., 1996). The CARET trial was ended early due to alarming results. The supplemented group had a 28% higher incidence of lung cancer and a 26% higher mortality rate from cardiovascular causes. The Physicians' Health Study I was a randomized trial that studied the effects of β-carotene (50 mg/alternate days) on healthy male physicians in the U.S for 12 years (Hennekins et al., 1996). The results demonstrated that β-carotene supplementation had no beneficial or adverse effects on the incidence of malignant neoplasms or death from cancer.

Taken together, these studies indicate that β-carotene supplementation does not appear to decrease the incidence of cancer in healthy individuals. Furthermore, supplementation appears to have adverse health effects in long-term smokers and those at high risk for cancer and cardiovascular diseases. Additional trials involving xanthophyll carotenoid supplementation need to be conducted to determine their role in the prevention of cancer.

6. Pigmented potatoes: origin and antioxidants

History of the Potato

The history of the potato, *Solanum tuberosum* L., can be traced back to 200 A.D. in Peru, where Incans cultivated potatoes in the foothills of the Andes Mountains (Talburt, 1987). Potatoes are native to South America; however, they were distributed throughout the Americas as far north as southern U.S. states around the time of the discovery of the Americas. Spanish explorers brought the potato to Europe, and their popularity soon spread. Potatoes were re-

introduced to North American colonies by English settlers in the early 1600s.

The genetic diversity of potatoes is extensive, with many desired genes introgressed from wild and cultivated species (Lehesranta et al., 2005). Different cultivars have developed through cross-pollination (Thompson, 1987). Thousands of years of potato cultivation in the Andes has led to the development of cultivars with yellow, orange, red, purple and blue skinned and/or flesh (Talburt, 1987). It is unknown why white-flesh potatoes were cultivated in Europe and North America and not pigmented-flesh cultivars. Currently, it is rare to find red or purple cultivars outside of the Andes region of South America (Brown et al., 2005a).

Potato consumption in the U.S.

Potatoes are the leading vegetable crop in the United States both in sales and in total consumption. Reports indicate that Americans consume approximately 130 pounds/person of fresh and processed potatoes annually (USDA, 2007a). The potato is a source of many essential and important nutrients, and therefore can be an important source of vitamins and minerals in the American diet (Chun et al., 2005). The most popular potato in the U.S. is the Russet Burbank (USDA, 2007b), a white-fleshed cultivar. Based on consumption patterns as well as antioxidant capacity, reports placed white-flesh potatoes as the greatest source of antioxidants in the U.S. diet (Chun et al., 2005).

Glycemic index

Research suggests that high carbohydrate diets are associated with the obesity epidemic (Ludwig et al., 1999). Another dietary consideration in food consumption that has received increased awareness is glycemic index, a commonly used indicator of the glucose response in blood to dietary carbohydrates. The glycemic index of a food is determined by comparing

glucose concentrations after ingesting 50 g of a particular food against a reference (glucose or white bread) (Jenkins et al., 1981). Some epidemiological evidence suggests that foods with high glycemic index are associated with the risk of the development of chronic diseases such as type II diabetes (Schultze et al., 2004) and coronary heart disease (Liu et al., 2000). However, conflicting reports exists regarding these associations (Stevens et al., 2002) and they remain controversial (Katz, 2001).

The glycemic index associated with potato consumption is of interest because many popular potato cultivars in the U.S. tend to have high ($\geq$70) glycemic index (Fernandes et al., 2005; Soh & Brand-Miller, 1999). The consumption of potatoes tends to be discouraged based on epidemiological evidence that suggests high glycemic index foods may attribute to the development of chronic diseases.

Differences in glycemic index among potato cultivars may be attributed to a variety of factors, such as cultivar and cooking method. Fernandes et al. (2005) showed a 4-point higher glycemic index with baked Russet compared to Prince Edward Island potato cultivar. Soh and Brand-Miller (1999) showed a 14-point difference between Pontiac cultivars cooked by microwaving versus baking. Additionally, Fernandes et al. (2005) demonstrated the glycemic index of boiled potatoes served hot (GI=89) was significantly higher than chilled boiled potatoes (GI=56). The confounding effects of cultivar and method of cooking may also influence the glycemic index of potatoes. Currently, no studies exist in addressing the glycemic index of colored Andean cultivars of *S. tuberosum*.

Potato antioxidants

More and more foods are now sold with accompanying labels stating their content of bioactive compounds that influence immune function and disease development. The most widely studied dietary antioxidants include vitamins E and C, as well as polyphenols and carotenoids. Consumption of diets rich in antioxidants is associated with a lower incidence of chronic diseases such as cardiovascular disease, cancer and macular degeneration (Galassetti & Pontello, 2007; Gonzalez, 2006; Snodderly, 1995). In addition to containing essential vitamins and minerals, potatoes are rich in polyphenols and carotenoids (Lewis et al., 1998; Brown, 2005b; Andre et al., 2007a). Polyphenols, plant-derived nutrients, are a diverse class of antioxidants that include flavonoids, phenolic acids and phytoestrogens. Thousands of polyphenols have been identified in plant tissues, many of which are used as food sources for mammals (Shahidi & Naczk, 2003).

The yellow, red and purple colors present in the skin and flesh of potato cultivars occur from two major classes of plant pigments, carotenoids and anthocyanins (Lewis et al., 1998). The antioxidant composition of the potato cultivars is dependent on the color of the skin and flesh (Lewis et al., 1998; Brown et al., 2003).

6-1.   Phenolic Acids

Phenolic acids, which constitute one of the two major subclasses of polyphenolics, are typically the most abundant polyphenol antioxidants among white-, yellow-, red- and purple-flesh potato cultivars (Andre et al., 2007a). Chlorogenic acid derivatives are the predominate phenolic acid found in potato flesh, accounting for 60-90% of total phenolics found in potato

skin and flesh (Lewis et al., 1998; Andre et al., 2007b). Caffeic, procatechuic, vanillic, *p*-coumaric, ferulic, sinapic and salicylic acids are also found in potatoes, but they are most concentrated in the skin. Different pigmented-flesh potatoes contain the same types of phenolics in the skin and flesh, but in varying concentrations.

In general, purple-flesh cultivars tend to have the highest total phenolic acid concentration compared to white, yellow or red flesh. The purple Guincho Negra cultivar was found to have 15 to 30 fold higher total phenolics than the yellow Amarilla del Centro cultivar, and the white Jancko Anckanchi cultivar, respectively (Andre et al., 2007b). Lewis et al. (1998) found red and purple cultivars to contain similar concentrations of total phenolics, which were also 2 to 3-fold greater than white potatoes. It is important to note that antioxidant concentrations vary by cultivar, year and growing location (Reddivari et al., 2007a).

6-2. Anthocyanins

Anthocyanins are secondary plant metabolites responsible for red, purple and blue hues in plants. There are negligible amounts of anthocyanins in white- and yellow-flesh cultivars (Andre et al., 2007b), but they are the primary source of antioxidants in red and purple cultivars (Lewis et al., 1998). Acylated glycoside derivatives of pelargonidin and peonidin are the most predominant anthocyanins in the red cultivar Red Flesh; glycosylated delphinidin and malvidin are also found, but at lower concentrations (Lewis et al., 1998). Purple cultivars (Urenika, Stage II Blue and I53) contained mostly acylated petunidin and malvidin glycosides and lesser amounts of glycosylated delphinidin (Lewis et al., 1998). These findings are in agreement with others (Harborne, 1960).

In a recent potato breeding study conducted by Brown et al. (2003), the progeny of

breeding crosses were analyzed for anthocyanin identification and total anthocyanin concentrations. Red-flesh tubers contained primarily cinnamic acid acylated derivatives of pelargonidin-3-rutinoside-5-glucoside, ferulic and *p*-coumaric acids, whereas purple-flesh tubers contained acylated derivatives of petunidin-3-rutinoside-5-glucoside and peonidin-3-rutinoside-5-glucoside, cyanidin and malvidin. As much as 86% of the total anthocyanin content in the red cultivars (PA97B37-3 & PA97B37-7) were acylated pelargonidin glycosides, and as much as 60% of the total anthocyanin content of the purple cultivars (PA97B29-2 & PA97B29-4) was derived from acylated petunidin glycosides, whereas about 20% was derived from acylated peonidin glycosides.

Urenika purple cultivars have higher concentration of anthocyanins in the skin (2-fold) and flesh (11-fold) than in Red Flesh (Lewis et al., 1998). Interestingly, Brown et al. (2003) found that the total anthocyanin content of red cultivars was typically greater than that of purple cultivars. They reported that red-flesh tubers have as much as 5 times higher anthocyanin concentrations than purple-flesh potatoes. These studies demonstrate the wide variation in anthocyanin content between different cultivars, and agree with other studies (Harborne, 1960; Fossen et al., 2003). The growing season, harvest time and genotype may affect anthocyanin content (Reyes, 2004; Lewis et al., 1998).

6-3. Flavonoids

Polyphenol flavonoids other than anthocyanins constitute a small portion of the hydrophilic antioxidants found in the skin and flesh, with catechin, epicatechin, eriodictyol, kaempherol and naringenin as the predominant flavonoids (Lewis et al., 1998). Quercetin and myricetin were found in lower concentrations, while only traces of rutin were detected.

Epicatechin was the only flavonoid not found in tuber flesh. Small differences in flavonoid concentrations exist among cultivars.

6-4. Carotenoids

Carotenoids are naturally occurring antioxidants found in white, yellow and orange (Brown et al., 1993), as well as red and purple-fleshed potatoes (Brown et al., 2005a). Xanthophylls are the major class of carotenoids found in pigmented potatoes; these include lutein, zeaxanthin, violaxanthin and antheraxanthin. The relative concentration of these carotenoids is dependent on the cultivar, although lutein is the predominant carotenoid found in potatoes (Andre et al., 2007b; Brown et al., 1993). These carotenoids belong to the xanthophyll subclass, and therefore do not possess pro-vitamin A activity. Pro–vitamin A carotenoids such as β-carotene, β-cryptoxanthin and neoxanthin are also present, but in lower concentrations (Breithaupt & Bamedi, 2002).

Yellow and orange-flesh potato cultivars have the highest total carotenoid concentrations compared to other cultivar (Andre et al., 2007b). Red and purple flesh cultivars may also contain high concentrations of carotenoids. Andre et al. (2007b) found that the Guincho Negra purple cultivar had between 2 and 5 fold higher concentrations of carotenoids than white cultivars and concentrations were similar to the lowest concentration found in yellow cultivars. In a similar study, Brown et al. (2005a) reported that purple and red flesh cultivars had total carotenoid concentrations similar to white-flesh cultivars, with the exception of two red cultivars (PA99P32-5 and POR00PG3-1) that were significantly higher.

Carotenoid bioavailability

Except for lutein, little is known about the potential health benefits of other xanthophylls (Breithaupt & Bamedi, 2002). In a study conducted by Barua and Olsen (2001), violaxanthin or its metabolites were not detected in human plasma after supplementation, leading to the conclusion that violaxanthin is not absorbed by humans. However, a recent study identified the presence of violaxanthin in both healthy and cancerous ovarian tissue isolated from women (Czeczuga-Semeniuk & Wolczynski, 2005). Antheraxanthin was not found in normal ovarian tissue in their study, but was detected in certain ovarian tumors. This indicates that most xanthophylls are present in plasma after ingestion and are transported to different tissues in the body.

Polyphenol bioavailability

Humans consume primarily two major subclasses of plant polyphenols, phenolic acids and flavonoids, with the flavonoid subclass being the most predominant (Scalbert & Williamson, 2000). A typical American diet contains > 1 g polyphenols/day, with one-third phenolic acids and two-thirds flavonoids. The bioavailability of polyphenols from plants remains controversial due to a lack of *in vivo* studies (Karakaya, 2004). It is currently unknown if polyphenolic compounds remain in the body long enough in active chemical form to produce beneficial effects.

Chlorogenic acid, the predominant phenolic acid present in red and purple-flesh potatoes, is comprised of an esterified caffeic acid molecule combined with quinic acid. Chlorogenic acid remains intact in human small intestine epithelial cells, liver and plasma (Karakaya, 2004). The metabolites caffeic acid and quinic acid were found in fecal extracts, implying that chlorogenic

acid is enzymatically cleaved by large intestine microflora (Plumb et al., 1999). One study in humans demonstrated that 33% of ingested chlorogenic acid enters the blood circulation via small intestinal absorption (Olthof et al., 2001). The presence of the intact molecule in plasma allows it to be transported to target tissues.

Anthocyanins from the flavonoid subclass are the second most predominant group of antioxidants in red and purple potatoes (Lewis et al., 1998). In humans, anthocyanin absorption is low, and is rapidly eliminated from the body (Manach et al., 2005). Manach et al. (2005) currently proposes that anthocyanin bioavailability may appear to be low due to as yet unidentified metabolites, and to rapid degradation under *in vivo* pH conditions. Recently, the stability of anthocyanins in frozen urine samples has come under question, which may necessitate new studies to determine the validity of past reports on urinary anthocyanin concentrations (Felgines et al., 2003). Because HPLC analysis of anthocyanins typically measures the flavylium cation molecular structure under neutral pH, it is possible that the low concentration reported in plasma and urine are due to the conversion of the cation structure to quininoid, hemiketal and chalcone tautomers (McGhie & Walton, 2007).

Pelargonidin glucosides are the most predominant anthocyanins in red potatoes, while petunidin glucosides are predominant in purple potatoes (Lewis et al., 1998; Brown et al., 2003). Only 2% of the pelargonidin-3-glucoside ingested from strawberries was excreted within 24 hours; two-thirds of the metabolite was excreted within 4 hours (Felgines et al., 2003). The concentration of pelargonidin and pelargonidin metabolites was not measured in the study. However, pelargonidin and pelargonidin metabolites have been detected in rat kidney, liver and lung tissues after supplementation (Abd El Mohsen et al., 2006)

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The bioavailability of petunidin and other anthocyanin aglycones present in bilberry extract was investigated in rats (Ichiyanagi et al., 2006). Results demonstrated that the sugar moiety attached to the aglycone affected bioavailability; galactosides were absorbed the most efficiently, and the *O*-methyl analog of petunidin, peonidin and malvidin positively affected absorption. *O*-methyl analog had higher affinity for kidney and liver tissues, indicating that tissues do not take up more hydrophilic anthocyanins such as delphinidin. Total anthocyanin absorption from the bilberry extract was estimated to be 0.93%. Petunidin, because of its B-ring *O*-methylation, was the most highly absorbed. The bioavailability of non-acylated anthocyanins from carrot juice is higher than the acylated form (Charron et al., 2009). Absorption of anthocyanins such as malvidin with more OCH_3 groups was more bioavailable than those with hydroxyl groups (e.g. delphinidin) (Yi et al., 2006). These findings demonstrate the importance of the anthocyanin structure on bioavailability.

Not all ingested anthocyanins are absorbed by the small intestine. Lala et al., (2006) studied chemoprotective activity of anthocyanin-rich extracts against colon cancer. They reported the presence of intact monomeric anthocyanins in the fecal matter, and that anthocyanins may exert their antioxidant effects directly on colon epithelial cells.

Anthocyanin metabolites differ according to which aglycones have been ingested (Felgines et al., 2005). Anthocyanins typically have glycoside groups attached; the latter can be enzymatically cleaved (Scalbert & Williamson, 2000), and products have been identified as the primary metabolites in the urine (Felgines et al., 2003; Manach et al., 2005). Anthocyanins with intact sugar moieties could be directly absorbed. Recent studies indicate anthocyanins are cleaved into phenolic acids. Vitaglione et al. (2007) observed that in participants that consumed

a fruit juice containing 71 mg of cyanidin glucosides as the major anthocyanin, 44% of the anthocyanin was recovered as procatechuic acid in the plasma. This is a significant finding because many *in vitro* cell; culture studies use native polyphenol structures rather than their metabolites.

7. Processing methods and antioxidant retention

Consumption patterns indicate that potatoes are the greatest source of antioxidants in the diet (Chun et al., 2005). Processed potato products constitute 68% of the potatoes sold on the U.S. market (Lucier et al., 2006). Therefore, it is important to retain their nutritional components.

Drum drying, freeze drying and Refractance Window™ dehydration methods

Food drying can be defined as a unit operation where water is removed by heat application from a liquid, solid or semi-solid product to produce a solid product of significantly lower moisture content (Mujumdar, 1997). Drying has been an effective method of food preservation for hundreds of years (Barbosa-Cánovas & Vega-Mercado, 1996). In the advent of current technologies, large-scale drying methods have been developed to retain optimal quality of the preserved food. However, during the drying process, many quality-related properties change, including structural, optical, textural, thermal, sensory, rehydration and nutritional qualities (Krokida & Marloulis, 2000). The retention of nutritional components of dehydrated products, such as vitamins and antioxidants are of increasing interest to the food industry (Nindo & Tang, 2007). Many vitamins and antioxidants present in fruits and vegetables are readily destroyed during food processing due to long periods of elevated temperature and pressure, and exposure to oxygen. Carotenoids phytochemicals are easily oxidized, resulting in the loss of

biological function (Rodriguez-Amaya, 1999). Drying processes that minimize heating time, temperature and oxygen exposure may provide products with the highest nutrient retention.

Drum dryers are types of contact dryer in which fluids, suspensions, pastes, or purees are typically dried by contact with condensing steam in a revolving drum container (Oakley, 1997). Due to their versatility and low operating cost, drum drying is widely used in the food industry. They are particularly beneficial for the drying of heat-sensitive products because contact with high temperature (120-170ºC) is minimal (seconds). Additionally, products can be dried under vacuum pressure; therefore, a lower temperature may be used. The final dried product is typically a powder, flake or chip; therefore, drum drying is commonly used for potato flake production.

Freeze drying is a multi-step drying process that employs freezing, sublimination and desorption (Snowman, 1997). The first step of freeze drying involves freezing the product in order to solidify the water. Next, the atmospheric pressure is lowered and low-heat (40ºC) is applied to cause sublimination. Sublimination occurs when frozen liquid in the product is directly transformed from the solid phase to the gas phase, bypassing the liquid phase. Desorption, or secondary drying, is the final step that involves heating the product under vacuum pressure to remove bound water. Freeze drying is a novel drying method because there is minimal damage to heat-liable nutrients in products; however, a major disadvantage of freeze drying is cost.

Refractance window™ (RW) drying is a relatively new drying technology developed in response to consumer interest in the retention of vitamins and other biologically active compounds (Nindo & Tang, 2007). This drying method is carried out by spreading a food slurry

over a transparent mylar film mounted on a conveyor belt. The slurry is thermally heated as it is moved along the conveyor belt, resulting in rapid drying of the product. RW drying is similar to drum drying, but is conducted at a lower temperature (95-97ºC) and without direct contact with the heat source. The actual product temperature during processing is about 70ºC. RW drying is an ideal drying method for industry use because equipment cost is one-third to one-half less than a freeze drying system and energy costs are less than half of a freeze drying system. Additionally, the RW drying method enables superior quality retention, including vitamin and bioactive phytochemicals in the final product. Factors that contribute to the retention of product components obtained from RW drying include short drying time, low product temperature and minimal product-oxygen interaction.

Dehydration methods and antioxidant retention

Due to the interest in nutritional retention of dehydrated products, including powdered neutraceuticals, many studies have examined the effect of different processing methods on vitamin C (Nindo et al., 2003; Abonyi et al., 2002). Vitamin C retention is the gold standard of nutrient retention due to its high sensitivity to processing conditions (Nindo et al., 2003).

Nindo et al. (2003) investigated nutrient retention in asparagus by measuring vitamin C concentrations and total antioxidant activity (TAA) after RW and freeze drying. The vitamin C retention in asparagus was highest when processed with the RW method (2.2 mg/g, DW), followed by freeze drying (1.9 mg/g DW). The RW processing method resulted in vitamin C concentrations equal to the control (raw) asparagus (2.2 mg/g, DW). The RW drying method resulted in the greatest TAA retention (76.2 μmol/g, trolox equivalents), but was not different from freeze drying (73.2 μmol/g, trolox equivalents). However, both methods retained TAA in

significantly greater concentrations than other methods studied, including tray drying, spouted bed and microwave/spouted bed drying. The authors explained that the high retention of vitamins and antioxidants was due to the short drying time (<5 min) and low product temperature (70-80ºC) during processing. Although freeze drying utilized an even lower temperature (20ºC), the greater loss of vitamin C was attributed to the longer processing time (18-24 hours).

In an additional study comparing dehydration methods, Abonyi et al. (2002) investigated the nutrient retention of strawberry and carrot purees among RW, freeze drying and drum drying. The loss of vitamin C after RW and freeze drying were comparable, and was about 6%. Drum drying was not investigated for vitamin C retention. Comparing total carotene concentrations (α- & β-carotene), freeze drying resulted in a 5% carotene loss, followed by RW with a 10% loss. There was no significant difference between these two methods; however, they both had significantly higher carotene retention compared to the drum drying method, which resulted in a 57% loss of carotenes. Desobry et al. (1997) reported only a 14% loss of β-carotene in encapsulated β-carotene by drum drying. Encapsulation of β-carotene provides a protective covering around the molecule, creating a matrix in which oxygen is less permeable, thereby preventing oxidation. Additional factors that may contribute to the large discrepancy include the use of different drum dryers, different operational specificities and differences in carotene concentrations between the foods (Abonyi et al., 2002).

In contrast to the concept that fruit and vegetable processing lowers the nutritive value of foods because of vitamin and phytochemical loss, some studies have demonstrated that the total antioxidant activity actually increases after thermal processing (Dewanto et al., 2002a; Dewanto et al., 2002b). Increased antioxidant activity was observed in thermally processed tomatoes (88ºC

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for 30 minutes) even though significant decreases in vitamin C content were observed (Dewanto et al., 2002b). The loss of vitamin C from processed foods may not significantly decrease the nutritive value of the food because vitamin C activity has been reported to contribute < 2% of total antioxidant activity in common fruits and vegetables (Eberhardt et al., 2000; Dewanto et al., 2002a).

Overall, the processing of foods has a multi-faceted effect on nutritive qualities. Heat processing appears to decrease vitamin C and β-carotene in fruits and vegetables. However, other bioactive molecules such as lycopene and phenolic acids tend to remain unchanged or increase, leading to a net increase in total antioxidant activity. RW and freeze drying typically result in higher retention of antioxidants. Additionally, drying methods increase in the release of phenolic compounds bound in the cell matrix, creating more bioavailable antioxidants (Nindo et al., 2003).

In summary, dehydration processing such as RW and freeze drying appear to produce the least destruction of the nutrient content of foods. Energy and time efficiency of these methods are important economic considerations for the industry. The final nutritive value of a processed food can be affected by the type and concentration of vitamins and phytochemicals present, specific processing method employed, temperature, time, and oxygen exposure.

8. Sensory evaluation

Even though consumers link diet with development of chronic diseases, consumer acceptance plays a critical role in the overall success of a food, regardless of its health benefit.

Consumers often associate color with healthy and appealing food products (Baker & Günther, 2004). β-Carotene is added to products such as cheese to enhance color; more recently, lutein has been added for its health benefits related to macular degeneration (Jones et al., 2005). Polyphenol antioxidants are presently being added to milk products (Axten et al., 2008) and soups (Llorach et al., 2005) as a functional food ingredient.

Influence of color on acceptability

The color of food plays a major role in perception of flavor, aroma and texture: color tends to give the perception of stronger odor intensity in foods when compared to non-colored counterparts (Christensen, 1983). Color may affect the perceived flavor of a food because flavor is a combination of aroma (volatile compounds), tastes (sweet, sour, salty and bitter), and chemical feeling sensations (cooling or spice heat) (Meilgaard et al.1991)

To date, there is no published research on sensory preferences and pigmented *S. tuberosum* cultivars. However, the affect of color has been investigated in specialty carrots and sweet potatoes. Comparing pale-flesh and high β-carotene orange-flesh sweet potato cultivars, Tomlins et al. (2007) found that adults preferred the orange-flesh sweet potato. Similar results were found in a study comparing white-, yellow-, orange-, red-, and purple-flesh carrot cultivars (Surles et al., 2004). Participants tended to prefer the orange-flesh cultivar for flavor, sweetness and crispiness. Importantly, panelists found all pigmented carrot cultivars acceptable.

Due to the relatively recent introduction of pigmented potatoes into the U.S. market

(Stelljes 2001), there is little information on preference and factors affecting preference. However, research from other studies suggest that consumers find pigmented vegetable cultivars acceptable; therefore, they can potentially provide additional health benefits due to the higher content of phenolic acids, anthocyanins and carotenoids.

9. Summary and hypothesis

Phenolic acids, anthocyanins and carotenoids found in pigmented potatoes are bioactive compounds that reduce LDL oxidation, reduce DNA damage, inhibit cell proliferation, and decrease CRP production, while improving immune cell function. White-flesh potatoes are the most popular vegetable in the U.S. and significantly contribute to dietary antioxidant intake. Pigmented cultivars such as yellow- and purple-flesh have higher antioxidant concentrations compared to their white-flesh counterparts. Therefore, it is important to evaluate the potential health benefit from the consumption of different potato cultivars in humans. Consumer acceptance and antioxidant retention after processing of these potatoes are other important considerations. We hypothesize that (1) pigmented potato consumption will decrease oxidative stress and inflammatory damage, while enhancing immune function, (2) different processing methods will retain antioxidant concentrations differentially, and (3) there will be no difference in consumer acceptance of different pigmented potato cultivars.

REFERENCES

Abd El Mohsen, M., Marks, J., Kuhnle, G., Moore, K., Debnam, E., Srai, S. K., et al. (2006). Absorption, distribution and excretion of pelargonidin and its metabolites following oral administration to rats. *British Journal of Nutrition, 95*, 51-58.

Abonyi, B. I., Feng, H., Tang, J., Edwards, C. G., Chew, B. P., Mattinson, D. S., et al. (2002). Quality retention in strawberry and carrot purees dried with refractance window™ system. *Journal of Food Science, 67*(3), 1051-1056.

Alpha-tocopherol, Beta Carotene Cancer Prevention Study Group. (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine, 330*(15), 1029-1035.

Álvarez, P., Alvarado, C., Mathieu, F., Jiménez, L., De la Fuente, M. (2006). Diet supplementation for 5 weeks with polyphenol-rich cereals improves several functions and the redox state of mouse leukocytes. *European Journal of Nutrition, 45*, 428-38.

American Heart Association. (2007). Heart disease and stroke statistics. Retrieved June 13, 2007 from http://www.americanheart.org/presenter.jhtml?identifier=4478.

Andre, C. M., Ghislain M., Bertin, P., Oufir, M., Herrera, M., Hoffman, L., et al. (2007a). Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *Journal of Agriculture and Food Chemistry, 55*, 366-378.

Andre, C. M., Oufir, M., Guignard, C., Hoffman, L., Hausman, J., Evers, D., et al. (2007b). Antioxidant profiling of native Andean potato tubers (*Solanum tuberosum* L.) reveals cultivars with high levels of β-carotene, α-tocopherol, chlorogenic acid, and petanin. . *Journal of Agriculture and Food Chemistry, 55*, 10839-10849.

Aptsiauri N., Cabrera T., Garcia-Lora A., Lopez-Nevot M. A., Ruiz-Cabello F., Garrido,
F. (2007). MHC class I antigens and immune surveillance in transformed cells.
International Review of Cytology, 256, 139-189.

Arts, I., Hollman, P. (2005). Polyphenols and disease risk in epidemiological studies.
American Journal of Clinical Nutrition, 81, 317S-325S.

Axten, L. G., Wohlers, M. W. and Wegrzyn, T. (2008). Using phytochemicals to enhance
health benefits of milk: Impact of polyphenols on flavor profile. *Journal of Food Science*,
73, H122-126.

Azevedo, L., Alves de Lima, P. L., Gomes, J. C., Stringheta, P. C., Ribeiro, D. A.,
Salvadori, D. (2007). Differential response related to genotoxicity between eggplant
(*Solanum melanogena*) skin aqueous extract and its main purified anthocyanin
(delphinidin) *in vivo*. *Food and Chemical Toxicity, 45*, 852-858.

Baker, R. and Günther, C. (2004). The role of carotenoids in consumer choice and the likely
benefits from their inclusion into products for human consumption. *Trends in Food
Science and Technology*, *15*, 484-488.

Barbosa-Cánovas, G. V., Vega-Mercado, H. (1996). Introduction to dehydration of
food. In Barbosa-Cánovas & Vega-Mercado (Eds), *Dehydration of foods* (pp.1-7). New
York, NY: International Thomson Publishing.

Barua, A. B., Olson, J. A. (2002). Xanthophyll epoxides, unlike β-carotene
monoepoxides, are not detectibly absorbed by humans. *Journal of Nutrition, 50*(24),
7175-7181.

Block, G., Schwarz, R. (1994). Ascorbic acid and cancer: animal and cell culture data.

In B. Frei (Ed), *Natural antioxidants in human health and disease* (pp. xix-xxiii). San

Diego, CA: Academic Press, Inc.

Bosetti, C., Bravi, F., Talamini, R., Parpinel, M., Gnagnarella, P., Negri, E. (2006).

Flavonoids and prostate cancer risk: a study in Italy. *Nutrition and Cancer, 56*(2), 123-

127.

Bowie, A., O'Neill, L. (2000). Oxidative stress and nuclear factor-κB activation.

Biochemical Pharmacology, *59*, 13-23.

Breithaupt, D. E., Bamedi, A. (2002). Carotenoids and carotenoid esters in potatoes

(*Solanum tuberosum* L.): new insights into an ancient vegetable. *Journal of Agricultural

and Food Chemistry, 50*, 7175-7181.

Briviba, K., Sies, H. (1994). Nonenymatic antioxidant defense systems. In B. Frei (Ed),

Natural antioxidants in human health and disease (pp. 25-55). San Diego, CA: Academic

Press, Inc.

Brown, C. R., Edwards, C. G., Yang, C. P., Dean, B. B. (1993). Orange flesh trait in

potato: inheritance and carotenoid content. *Journal of the American Society for

Horticultural Science, 118*(1), 145-50.

Brown, C. R., Wrolstad, R., Durst, R., Yang, C. P., Clevidence, B. (2003). Breeding

studies in potatoes containing high concentrations of anthocyanins. *American Journal of

Potato Research, 80*, 241-250.

Brown, C. R., Culley, D., Yang, C. P., Durst, R., Wrolstad R. (2005a). Variation of

anthocyanin and carotenoid contents and associated antioxidant values in potato breeding

lines. *Journal of the American Society for Horticultural Science, 130*(2), 174-180.

Brown, C. R. (2005b). Antioxidants in Potato. *American Journal of Potato Research*, *82*, 163-172.

Burdette, J. E., Chen, S. N., Lu, Z. Z., Xu, H., White, B., Fabricant, D. S., et al. (2002). Black Cohosh (*Cimicifuga racemosa* L.) protects against menadione-induced DNA damage through scavenging of reactive oxygen species: bioassay-directed isolation and characterization of active principles. *Journal of Agriculture and Food Chemistry, 50*, 7022-7028.

Castell, J. V., Gomez-Lechon, M. J., David, M., Fabra, R., Trullenque R., Heinrich P. C. (1990). Acute-phase response of human hepatocytes: regulation of acute phase protein synthesis by interleukin-6. *Hepatology, 12*, 1179-86.

Centers for Disease Control and Prevention: National Center for Health Statistics (2006). Retrieved August 1, 2007 from http://www.cdc.gov/nchs/fastats/lcod.htm.

Chang, Y. C., Huang, K. X., Huang, A. C., Ho, C. H., Wang, C. J. (2006). *Hibiscus* anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. *Food and Chemical Toxicology, 44*, 1015-1023.

Charron, C. S., Kurilich, A. C., Clevidence, B. A., Simon, P. W., Harrison, D. J., Britz, S., et al. (2009). Bioavailability of anthocyanins from purple carrot juice: effects of acylation and plant matrix. *Journal of Agricultural and Food Chemistry*, *57*(4), 1226-1230.

Chen, C. M., Li, S. C., Lin, Y. L., Hsu, C. Y., Shieh, M. J., Liu, J. F. (2005). Consumption of purple sweet potato leaves modulates human immune response: T-lymphocyte functions, lytic activity of natural killer cell and antibody production. *World Journal of Gastroenterology, 11*(37), 5777-5781.

Chew, B. P., Park, J. S. (2004). Carotenoid action on the immune response. *Journal of Nutrition, 134*(1), 257S-261S.

Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., Han, J. T., Lee, C. Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture, 85*, 1715-1724.

Closa, D., Folch-Puy, E. (2004). Oxygen free radicals and the systemic inflammatory response. *IUBMB Life, 56*, 185-191.

Connor, S. L., Ojeda, L. S., Sexton, G., Weidner, G., Connor, W. E. (2004). Diets lower in folic acid and carotenoids are associated with the coronary disease epidemic in central and eastern Europe. *Journal of the American Dietetic Association, 104*(12), 1793-98.

Cooper, D. A., Eldridge, A. L., Peters, J. C. (1999). Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: a review of recent research. *Nutrition Reviews, 57*(7), 201-214.

Coquette, A., Vray, B., Vanderpas J. (1986). Role of vitamin E in the protection of the resident macrophage membrane against oxidative damage. *Archives Internationales de Physiologie et de Biochimie, 94*(5), S29-S34.

Corridan, B. M., O'Donoghue, M., Hughes, D. A., Morrissey, P. A. (2001). Low-dose supplementation with lycopene or β-carotene does not enhance cell-mediated immunity in healthy free-living elderly humans. *European Journal of Clinical Nutrition, 55*, 627-

635.

Christensen, C. M. (1983). Effects of color on aroma, flavor and texture judgments of foods. *Journal of Food Science*, *48*, 787-90.

Czeczuga-Semeniuk, E., Wolczynski, S. (2005). Identification of carotenoids in ovarian tissue in women. *Oncology Reports, 14*, 1385-1392.

Dedoussis, G., Kaliora, A. C., Andrikopoulos, N. K. (2005). Effect of phenols on natural killer (NK) cell-mediated death in the K562 human leukemic cell line. *Cell Biology International, 29*, 884-889.

de Ferranti, S., Mozaffarian, D. (2008). The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clinical Chemistry*, *54*, 945-955.

Desobry, S. A., Netto, F. M., Labuza, T. P. (1997). Comparison of spray-drying, drum-drying, and freeze drying for β-carotene encapsulation and preservation. Journal *of Food Science, 62*(6), 1158-62.

Dewanto, V., Wu, X., Liu, R. H. (2002a). Processed sweet corn has higher antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*, 4959-4964.

Dewanto, V., Wu, X., Liu, R. H. (2002b). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*, 3010-3014.

Ding, A. H., Nathan, C. F., Stuehr, D. J. (1988). Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. *The Journal of Immunology, 141*(7), 2407-2412.

Dolcet, X., Llobet, D., Pallares, J., Matias-Guiu. (2005). NF-κB in development and

progression of human cancer. *Virchows Archiv, 446,* 475-482.

Duthie, S. J., Jenkinson , A. M., Crozier, A., Mullen, W., Pirie, L., Kyle, J., et al. (2006).

The effects of cranberry juice consumption on antioxidant status and biomarkers relating

to heart disease and cancer in health individuals. *Eurpoean Journal of Nutrition, 45*, 113-

122.

Dwyer, J. H., Navab, M., Dwyer, K. M., Hassan, K., Sun, P., Shircore, A., et al. (2001).

Oxygenated carotenoid lutein and progression of early atherosclerosis. *Circulation*, *103*,

2922-2927.

Eberhardt, M. V., Lee, C. Y., Lui, R. H. (2000). Antioxidant activity of fresh apples.

Nature, 405, 903-904.

Evans, R.W., Shaten, B. J., Day, B. W., Keller, L. H. (1998). Prospective association between

lipid soluble antioxidants and coronary heart disease in men. The Multiple Risk Factor

Intervention Trial. *American Journal of Epidemiology, 147*(2), 180-186.

Favot, L., Martin, S., Keravis, T., Andriantsitohaina, R., Lugnier, C. (2003). Involvement

of cyclin-dependent pathway in the inhibitory effect of delphinidin on angiogenesis.

Cardiovascular Research, 59, 479-487.

Felgines, C., Talavera, S., Gonthier, M. P., Texier, O., Scalbert, A., Lamaison, J. L., et al.

(2003). Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates

in humans. *Journal of Nutrition, 133*(5), 1296-1301.

Felgines, C., Talavera, S., Texier, O., Gil-Izquierdo, A., Lamaison, J. L., Remesy, C.

(2005). Blackberry anthocyanins are mainly recovered from urine as methylated and

glucuronidated conjugates in humans. *Journal of Agriculture and Food Chemistry,*

53(20), 7721-7727.

Feng, R., Ni, H. M., Wang, S. Y., Tourkova, I. L., Shurin, M. R., Harada, H. (2007).

    Cyanidin-3-rutinoside, a natural polyphenol antioxidant, selectively kills leukemic cells

    by induction of oxidative stress. *Journal of Biological Chemistry, 282*(18), 13468-13476.

Fernandes, G., Velangi, A., Wolever, T. M. (2005). Glycemic index of potatoes

    commonly consumed in North America. *Journal of the American Dietetic Association,*

    105(4), 557-562.

Fossen, T., Øvstedal, D. O., Andersen, Ø. M. (2003). Anthocyanins from a Norwegian

    potato cultivar. *Food Chemistry, 81*, 433-437.

Frei, B. (1994). Forward. In B. Frei (Ed), *Natural antioxidants in human health and*

    disease (pp. xix-xxiii). San Diego, CA: Academic Press, Inc.

Frostegard, J., Ulfgren, A., Nyberg, P., Hedin, U., Swedenborg, J., Andersson, et al.

    (1999). Cytokine expression in advanced human atherosclerotic plaques: dominance of

    pro-inflammatory (Th1) and macrophage-stimulating cytokines. *Atherosclerosis, 145*, 33-

    43.

Galassetti, P., Pontello, A. (2006). Dietary effects on oxidation of low-density

    lipoprotein and atherogenesis. *Current Atherosclerosis Report, 8*(6), 523-29.

Gill, C., Haldar, S., Boyd, L. A., Bennett, R., Whiteford, J., Butler, M., et al.

    (2007).Watercress supplementation in diet reduces lymphocyte DNA damage and alters

    blood antioxidant status in healthy adults. *American Journal of Clinical Nutrition*, *85*,

    504-510.

Gonzalez, C. A. (2006) Nutrition and cancer: the current epidemiological evidence.

British Journal of Nutrition, 96(1), 42S-45S.

Griselli, M., Herbert, J., Hutchinson, W. L., Taylor, K. M., Sohail, M., Krausz, T., et al. (1999). C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *Journal of Experimental Medicine, 190*(12), 1733-1739.

Gruner, S., Volk, H. D., Falck, P., Baehr, R. V. (1986). The influence of phagocytic stimuli on the expression of HLA-DR antigens; role of reactive oxygen intermediates. *European Journal of Immunology, 16*, 212-215.

Hak, A. E., Stampfer, M. J.., Campos, H., Sesso, H. D., Gaziano, J. M., Willett, W., et al. (2003). Plasma carotenoids and tocopherols and risk of myocardial infarction in a low-risk population of US male physicians. *Circulation, 108*(7), 802-807.

Halliwell, B., Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement, and significance. *American Journal of Clinical Nutrition, 57*(5), 715S-724S.

Halliwell, B. (1995). How to characterize an antioxidant: an update. In C. Rice-Evans, B. Halliwell & G. Lunt (Eds.), *Free radicals and oxidative stress: environment, drugs and food additives* (pp. 73-93). London: Portland Press.

Halliwell, B. (2001). Free radical reactions in human disease. In J. Fuchs & L. Packer (Eds.), *Environmental stressors in health and disease* (pp. 1-16). New York, NY: Marcel Dekker, Inc.

Halliwell, B. (2006). Phagocyte-derived reactive species: salvation or suicide? *TRENDS in Biochemical Sciences, 31*(9), 509-514.

Hämäläinen, M., Nieminen, R., Vuorela, P., Heinonen, M., Moilanen, E. (2007). Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit

STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and
pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on
iNOS expression and NO production in activated macrophages. *Mediators of
Inflammation, 2007,* 45673.

Hammar, N., Anderson, T., Alfredsson, L., Reuterwall, C., Nilsson, T., Hallqvist, J., et al.
(2003). Association of boiled and filtered coffee with incidence of first nonfatal
myocardial infarction: the SHEEP and the VHEEP study. *Journal of Internal Medicine,
253*(6), 653-659.

Happonen, P., Voutilainen, S., Salonen, J. T. (2004). Coffee drinking is dose-dependently
related to the risk of acute coronary events in middle-aged men. *Journal of Nutrition,
134*(9), 2381-2386.

Harborne, J. B. (1960). Anthocyanin production in the cultivated potato. *Biochemical
Journal, 74*, 262-269.

Harlan, L. M., Chan, M. A, Benedict, S. H. (2007). Two
different modes of costimulation predispose human T lymphocytes to differential
responses in the presence of HDL or oxidized LDL. *Atherosclerosis, 193*(2), 309-320.

Hattori, Y., Matsumura, M., Kasai, K. (2003). Vascular
smooth muscle cell activation by C-reactive protein. *Cardiovascular Research,
58*, 186-195.

Haverkate, F., Thompson, S. G., Pyke, S. D., Gallimore, J. R., Pepys, M. B. (1997).
Production of C-reactive protein and risk of coronary events in stable and unstable
angina. *Lancet, 349,* 462-66.

Heinrich, P. C., Castell, J. V., Andus, T. (1990). Interleukin-6 and the acute phase

    response. *Biochemical Journal*, *265*, 621-636.

Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R.,

    et al. (1996). Lack of effect of long-term supplementation with beta carotene on the

    incidence of malignant neoplasms and cardiovascular disease. *The New England Journal*

    of Medicine, 334(18), 1145-1149.

Hirvonen, T., Pietinen, P., Virtanen, M., Ovaskainen, M. L., Hakkinen, S., Albanes, D.,

    et al. (2001). Intake of flavonols and flavones and risk of coronary heart disease in male

    smokers. *Epidemiology, 12*(1), 62-67.

Hooper, L., Kroon, P. A., Rimm, E. B., Cohn, J. S., Harvey, I., Le Comu, K. A., et al.

    (2008). Flavonoids, flavonoi-rich foods, and cardiovascular risk: a meta-analysis of

    randomized controlled trials. *American Journal of Clinical Nutrition*, *88* (1), 38-50.

Hou, D. X., Ose, T., Lin, S., Harazoro, K., Imamura, I., Kubo, M., et al. (2003).

    Anthocyanins induce apoptosis in human promyelocytic leukemia cells: structure-activity

    relationship and mechanisms involved. *International Journal of Oncology, 23*, 705-712.

Hozawa, A., Jacobs, D. R., Steffes, M. W., Gross, M. D., Steffen, L. M., Lee, D. H.

    (2007). Relationships of circulating carotenoid concentrations with several markers of

    inflammation, oxidative stress, and endothelial dysfunction: the coronary artery risk

    development in young adults (CARDIA)/young adult longitudinal trends in antioxidants

    (YALTA) study. *Clinical Chemistry, 53*(3), 447-455.

Hughes, D. A., Wright, A. J., Finglas, P. M., Peerless, A. C., Bailey, A. L., Astley, S. B., et al.

    (1997a). The effect of β-carotene supplementation on the immune function of blood

monocytes from healthy male nonsmokers. *Journal of Laboratory and Clinical Medicine, 129*(3), 309-316.

Hughes, D. A., Wright, A. J., Finglas, P. M., Peerless, A. C., Bailey, A. L., Astley, S. B., et al. (1997b). Comparison of effects of β-carotene and lycopene supplementation on the expression of functionally associated molecules on human monocytes. *Biochemical Society Transactions, 25*(2), 206S.

Hughes, D. A., Wright, A. J., Finglas, P. M., Polley, A. C., Bailey, A. L., Astley, S. B., et al. (2000). Effects of lycopene and lutein supplementation on the expression of functionally associated surface molecules on blood monocytes from healthy male nonsmokers. *The Journal of Infectious Diseases, 182*(Suppl 1), S11-S15.

Hughes, D. A. (2001). Dietary carotenoids and human immune function. *Nutrition, 17*, 823-827.

Ichiyanagi, T., Shida, Y., Rahman, M. M., Hatano, Y., Konishi, T. (2006). Bioavailability and tissue distribution of anthocyanins in bilberry (*Vaccinium myrtillus* L.) extract in rats. *Journal of Agriculture and Food Chemistry, 54*, 6578-6587.

Ito, Y., Kurata, M., Suzuki, K., Hamajima, N., Hishida, H., Aoki, K. (2006). Cardiovascular disease mortality and serum carotenoid levels: a Japanese population-based follow-up study. *Journal of Epidemiology, 16*(4), 154-160.

Izumi-Nagai, K., Nagai, N., Ohgami, K., Satofuka, S., Ozawa, Y., Tsubota, K., et al. (2007). Macular pigment lutein is anti-inflammatory in preventing choroidal neovascularization.. *Atherosclerosis, Thrombosis and Vascular Biology*, *27*, 2555-2562.

Janeway, C. A., Travers, P., Walport, M., Shlomchik, M. J. (2005). Immunobiology:

the immune system in health and disease, 6th Ed. New York, NY: Garland Science.

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M. et al. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *The American Journal of Clinical Nutrition*, *34*, 362-366.

Johnson, I. T. (2007). Phytochemicals and cancer. *Proceedings of the Nutrition Society*, *66*(2), 207-215.

Jones, S. T., Aryana, K. J. and Losso, J. N. (2005). Storage stability of lutein during ripening of cheddar cheese. *Journal of Dairy Science*, *88*, 1661-70.

Kampa, M., Alexaki, V. I., Notas, G., Nifli, A. P., Nistikaki, A., Hatzoglou, A., et al. (2003). Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. *Breast Cancer Research, 6*, R63-R74.

Karakaya, S. (2004). Bioavailability of phenolic compounds. *Critical Reviews in Food Science and Nutrition, 44*, 453-464.

Karlsen, A., Retterstøl, L., Laake, P., Paur, I., Kjølsrud-Bøhn, S., Sandvik, L. (2007). Anthocyanins inhibit nuclear factor-κB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. *Journal of Nutrition*, *137*, 1951-1954.

Kasai, H., Fukada, S., Yamaizumi, Z., Sugie, S., Mori, H. (2000). Action of chlorogenic acid in vegetables and fruits as an inhibitor of 8-hydroxydeoxyguanosine formation *in vitro* and a rat carcinogenesis model. *Food and Chemical Toxicity, 38*, 467-71.

Katz, D. L. (2001). Glycemic load and the risk of coronary heart disease. *American Journal of Clinical Nutrition, 73*(1), 131-132.

Kehrer J. P., Smith, C. V. (1994). Free radicals in biology: sources, reactivities, and roles in the etiology of human diseases. In B. Frei (Ed), *Natural antioxidants in human health and disease* (pp. 25-55). San Diego, CA: Academic Press, Inc.

Kim, H. W., Chew, B. P., Wong, T. S., Park, J. S., Weng, B. B., Byrne, K. M., et al. (2000a). Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. *Veterinary Immunology and Immunopathology, 73*, 331-341.

Kim, H. W., Chew, B. P., Wong, T. S., Park, J. S., Weng, B. B., Byrne, K. M., et al. (2000b). Dietary lutein stimulates immune response in the canine. *Veterinary Immunology and Immunopathology, 74*, 315-327.

Kim, J., Na, H., Kim, C., Kim, J., Ha, K., Lee, H., et al. (2008). The non-provitamin A carotenoid, lutein, inhibits NF-κB-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-κB-inducing pathways: Role of H_2O_2 in NF-κB activation. *Free Radical biology and Medicine*, *45*, 885-896.

Kim, N. H., Jeong M. S., Choi S. Y., Kang J. H. (2006). Oxidative modification of cytochrome c by hydrogen peroxide. *Molecules and Cells, 22*(2), 220-227.

Knekt, P., Jarvinen, R., Reunanen, A., Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. *British Medical Journal, 312*, 478-481.

Kobuchi, H., Virgili, F., Packer, L. (1999). Assay of inducible form of nitric oxide synthase activity: effects of flavonoids and plant extracts. *Methods in Enzymology, 301*,

504-513.

Konjević G., Martinović K. M., Vuletić A., Jović V., Jurisić V., Babović N., et al.

(2007). Low expression of CD161 and NKG2D activating NK receptor is

associated with impaired NK cell cytotoxicity in metastatic melanoma patients.

Clinical Experimental Metastasis, 24, 1-11.

Kotur-Stevuljevic, J., Memon, L., Stefanovic, A., Spasic, S., Spasojevic-Kalimanovska,

V., Bogavac-Stanojevic, N., et al. (2007). Correlation of oxidative stress

parameters and inflammatory markers in coronary artery disease patients. *Clinical*

Biochemistry, *40*, 181-187.

Kritchevsky, S. B., Bush, A. J., Pahor, M., Gross, M. D. (2000). Serum carotenoids
and markers of inflammation in nonsmokers. *American Journal of Epidemiology,*

152(11), 1065-71.

Krokida, M., Maroulis, Z. (2000). Quality changes during drying of food materials. In

A. S. Mujumdar (Ed), *Drying Technology in Agriculture and Food Sciences* (pp. 61-97).

Enfield, NH: Science Publishers, Inc.

Kumar, V., Cotran, R. S., Robbins, S. L. (2003). *Robbins Basic Pathology* (7th ed.).

Philidelphia, PA: Saunders.

Kune, S., Kune, G. A., Watson, L. F. (1987). Case-control study of dietary etiological

factors: the Melbourne Colorectal Cancer Study. *Nutrition and Cancer, 9*(1), 21-42.

Lambert, J. D., Hong, J., Yang, G., Liao, J., Yang, C. S. (2005). Inhibition of

carcinogenesis by polyphenols: evidence from laboratory investigations. *American*

Journal of Clinical Nutrition, 81, 284S-291S.

Lala, G., Malik, M., Zhao, C., He, J., Kwon, Y., Giusti, M. M., et al. (2006).

  Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. *Nutrition
  and Cancer, 54*(1), 84-93.

Lamy, S., Blanchette, M., Michaud-Levesque, J., Lafleur, R., Durocher, Y., Moghrabi.,

  A., et al. (2006). Delphinidin, a dietary anthocyanin, inhibits vascular endothelial growth
  factor receptor-2 phosphorylation. *Carcinogenesis, 27*(5), 989-996.

Laranjinha, J., Vieira, O., Almeida, L., Madeira, V. (1996). Inhibition of

  metmyoglobin/H_2O_2-dependent low density lipoprotein lipid peroxidation by naturally
  occurring phenolic acids. *Biochemical Pharmacology, 51*, 395-402.

Lazzé, M. C., Savio, M., Pizzala, R., Cazzalini, O., Perucca, P., Scovassi, A. I., et al.

  (2004). Anthocyanins induce cell cycle perturbations and apoptosis in different human
  cell lines. *Carcinogenesis, 25*(8), 1427-33.

Lee, E. H., Faulhaber, D., Hanson, K. M., Ding, W., Peters, S., Kodali, et al. (2004).

  Journal of Investigative Dermatology, *122*, 510-517.

Lehesranta, S. J., Davies, H. V., Shepard, L. V., Nunan, N., McNicol, J. W., Auriola,

  S., et al. (2005). Compairison of tuber proteomes of potato varieties, landraces, and
  genetically modified lines. *Plant Physiology, 138*, 1690-1699.

Levine, G. N., Keaney, J. F.,Vita, J. A. (1995). Cholesterol reduction in

  cardiovascular disease: Clinical benefits and possible mechanisms. *New England
  Journal of Medicine, 332*(8), 512-521.

Lewis, C. E., Walker, J. R., Lancaster, J. E., Sutton, K. H. (1998). Determination of

  anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum*

tuberosum L. *Journal of the Science of Food and Agriculture, 77*, 45-57.

Li, J. J., Fang, C. H. (2004). C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases. *Medical Hypotheses, 62*, 499-506.

Libby, P., Simon, D. I. (2001). Inflammation and thrombosis, the clot thickens. *Circulation, 103*, 1718-1720.

Liu, S., Willett, W. C., Stampfer, M. J., Hu, F. B., Franz, M., Sampson, M., et al. (2000). A prospective study of dietary glycemic index load, carbohydrate intake, and risk of coronary heart disease in US women. *American Journal of Clinical Nutrtion, 71*(6), 1455-1461.

Lin, J., Rexrode, K. M., Hu, F., Albert, C. M., Chae, C. U., Rimm, E. B., et al. (2007). Dietary intakes of flavonols and flavones and coronary heart disease in the US women. *American Journal of Epidemiology, 165*(11), 1305-1313.

Llorach, R., Tomás-Barberán, F. A. and Ferreres, F. (2005). Functionalisation of commercial chicken soup with enriched polyphenol extract from vegetable by-products. *European Food Research and Technology, 220*, 31-36.

Lucier, G., Pollack, S., Ali, M., Perez, A. (2006) Fruit and vegetable backgrounder. *USDA Outlook Report*,VGS-31301, 1-56

Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics. 103*, E26.

Male, D. (2004). Immunology: an illustrated outline, 4th Ed. London, UK: Mosby.

Manach, C., Williamson, G., Morand, C., Scalbert, A., Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability

studies. *American Journal of Clinical Nutrition, 81*, 230S-242S.

Männistö, S., Smith-Warner, S. A., Spiegelman, D., Albanes, D., Anderson, K., van den
   Brandt, P. A., et al. (2004). Dietary carotenoids and risk of lung cancer in a pooled
   analysis of seven cohort studies. *Cancer Epidemiology, Biomarkers & Prevention, 13*(1),
   40-48.

Matsunaga, K., Katayama, M., Sakata, K., Kuno, T., Yoshida, K., Yamada, Y., et al.
   (2002). Inhibitory effects of chlorogenic acid on azoxymethane-induced colon
   carcinogenesis in male F344 rats. *Asian Pacific Journal of Cancer Prevention, 3*(2), 163-
   166.

McGhie, T. K., Walton, M. C. (2007). The bioavailability and absorption of
   anthocyanins: towards a better understanding. *Molecular Nutrition and Food Research,
   51*,702-713.

Meilgaard, M., Civille, G. V. and Carr, B. T. (1991). Sensory attributes and the way we
   perceive them. In *Sensory Evaluation Techniques, 2nd Ed.* , pp. 7-21, CRC Press, Inc.,
   Boca Raton, FL.

Mettlin, C., Selenskas, S., Natarajan, N., Huben, R. (1989). Beta-carotene and animal
   fats and their relationship to prostate cancer risk. A case-control study. *Cancer, 64*(3),
   605-612.

Meydani, S. N., Wu, D., Santos, M. S., Hayek, M. G. (1995). Antioxidants and immune
   response in aged persons: overview of present evidence. *American Journal of Clinical
   Nutrtion, 62*, 1462S-76S.

Mink, P. J., Scrafford, C. G., Barraj, L. M., Harnack, L., Hong, C. P., Nettleton, J. A. et

al. (2007). Flavonoid intake and cardiovascular disease and mortality: a prospective study in postmenopausal women. *American Journal of Clinical Nutrition, 85*, 895-909.

Mocchegiani, E., Malavolta, M. (2004). NK and NKT cell functions in immunosenescence. *Aging Cell, 3*(4):177-84.

Mokdad, A. H., Marks, J. S., Stroup, D. F., Gerberding, J. L. (2005). Correction: actual causes of death in the United States, 2000. *Journal of the American Medical Association, 293*(3), 293-294.

Møller, P., Loft, S., Alfthan, G., Freese, R. (2004a). Oxidative damage in circulating mononuclear blood cells after ingestion of blackcurrent juice or anthocyanin-rich drink. *Mutation Research, 551*, 119-126.

Møller, P., Loft, S. (2004b). Interventions with antioxidants and nutrients in relation to oxidative DNA damage and repair. *Mutation Research, 551*(1-2), 79-89.

Moncada, S., Palmer, R. M., Higgs, E. A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological Reviews, 43*(2), 109-134.

Morello, M. J., Shahidi, F., Ho, C. T. (2002). Free radicals in foods: Chemistry, nutrition and health effects. In M. Morello, F. Shahidi, & C. Ho (Eds.), *Free radicals in foods: Chemistry, nutrition and health effects* (pp. 1-8). Washington, DC: American Chemical Society.

Mujumdar, A. S. (1997). Drying fundamentals. In C. J. G. Baker (Ed.), *Industrial drying of foods* (pp. 7-29). London, UK: Blackie Academic and Professional.

Murata, T., Tamai, H., Morinobu, T., Manago, M., Takenaka, H., Hayashi, K., et al. (1994). Effect of long-term administration of β-carotene on lymphocyte subsets in

humans. *American Journal of Clinical Nutrition, 60*, 597-602.

Nabata, A., Kuroki, M, Ueba, H., Hashimoto, S., Umemoto, T., Wada, H. (2008). C-reactive protein induces endothelial cell apoptosis and matrix metalloproteinase-9 production in human mononuclear cells: implications for the destabilization of atherosclerotic plaque. *Atherosclerosis, 196*(1), 129-135.

Nindo, C. I., Tang, J. (2007). Refractance window™ dehydration technology: a novel contact drying method. *Drying Technology, 25*, 37-48.

Nindo, C. I., Sun, T., Wang, S. W., Tang, J., Powers, J. R. (2003). Evaluation of drying technologies for the retention of physical quality and antioxidants in asparagus (*Asparagus officinalis*, L.). *Lebensmittel-Wissenschaft+Technologie, 36*, 507-516.

Oakley, D. (1997).Contact dryers. In C. J. G. Baker (Ed.), *Industrial drying of foods* (pp. 115-132). London, UK: Blackie Academic and Professional.

Olthof, M. R., Hollman, P., Katan, M. B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *Journal of Nutrition, 131*, 66-71.

Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J. Cullen, M. R., Glass, A. et al. (1996). Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *The New England Journal of Medicine, 334*(18), 1150-1154.

Paffen, E., deMaat, M. (2006). C-reactive protein in atherosclerosis: a causal factor? *Cardiovascular Research, 71*, 30-39.

Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., Kokkinos, P., Toutouzas, P., Stefanadis, C. (2003). The J-shaped effect of coffee consumption on the risk of developing acute coronary syndromes: the CARDIO2000 case-control study. *Journal of*

Nutrition, 133(10), 3228-3232.

Pergola, C., Rossi, A., Dugo, P., Cuzzocrea, S., Sautebin, L. (2006). Inhibition of nitric
oxide biosynthesis by anthocyanin fraction of blackberry extract. *Nitric Oxide, 15*, 30-39.

Plumb, G. W., Garcia-Conesa, M. T., Kroon, P. A., Rhodes, M., Ridley, S., Williamson,
G. (1999). Metabolism of chlorogenic acid by human plasma, liver, intestine and gut
microflora. *Journal of the Science of Food and Agriculture, 79*, 390-392.

Pool-Zobel, B. L., Bub, A., Schröder, N., Rechkemmer, G. (1999). Anthocyanins are
potent antioxidants in model systems but do not reduce endogenous oxidative DNA
damage in human colon cells. *European Journal of Nutrition, 38*, 227-234.

Prabhala, R. H., Maxey, V., Hicks, M. J. & Watson, R. R. (1989). Enhancement of
activation markers on human peripheral blood mononuclear cells by *in vitro* culture with
retinoids and carotenoids. *Journal of Leukocyte Biology, 45*, 249-254.

Prabhala, R. H., Garewal, H. S., Hicks, M. J., Sampliner, R. E., Watson, R. R. (1991).
The effects of 13-cis-retinoic acid and beta-carotene on cellular immunity in humans.
Cancer, 67(6), 1556-60.

Prior, R. L., Wu, X. (2006). Anthocyanins: structural characteristics that result in
unique metabolic patterns and biological activities. *Free Radical Research, 40*(10), 1014-
1028.

Rasheed, Z., Akhtar, N., Anbazhagan, A. N., Ramamurthy, S., Haqqi, T. M. (2009).
Polyphenol-rich pomegranate fruit extract (POMx) suppresses PMACI-induced
expression of pro-inflammatory cytokines by inhibiting the activation of MAP Kinases
and NF-kappaB in human KU812 cells. *Journal of Inflammation, 8*, 6:1.

Rapola, J. M., Virtamo, J., Ripatti, S., Huttunen, J. K., Albanes, D., Taylor, P. R., et al.
(1997). Randomized trial of α-tocopherol and β-carotene supplements on incidence of
major coronary events in men with myocardial infarction. *The Lancet, 349*, 1715-1719.

Rattazzi, M., Faggin, E., Bertipaglia, B., Pauletto, P. (2005). Innate immunity and
atherogenesis. *Lipids, 14*, 747-751.

Reddivari, L., Hale, A. L., Miller, J. C. (2007a). Genotype, location, and year influence
antioxidant activity, carotenoid content, phenolic content, and composition in specialty
potatoes. *Journal of Agricultural and Food Chemistry*, *55* (20), 8073-8090.

Reddivari, L., Vanamala, J., Chintharlapalli, S., Safe, S. H., Miller, J. C. Jr. (2007b).
Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through
activation of caspase-dependent and caspase-independent pathways. *Carcinogenesis, 10*,
2227-2235.

Reith, W., Mach, B. (2001). The bare lymphocyte syndrome and the regulation of
MHC expression. *Annual Review of Immunology, 19*, 331-373.

Reyes, L. F., Miller, J. C., Cisneros-Zevallos, L. (2004). Environmental conditions
influence the content and yield of anthocyanins and total phenolics in purple and red-
flesh potatoes during tuber development. *American Journal of Potato Research, 81*, (3),
187-193.

Ridker, P. M., Hennekens, C. H., Burning, J. E., Rifai, N. (2000). C-reactive protein
and other markers of inflammation in the prediction of cardiovascular disease in women.
The New England Journal of Medicine, 342, 836-843.

Rimm, E. B., Katan, M. B., Ascherio, A., Stampfer, M. J., Willett, W. C. (1996).

Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Annals of Internal Medicine, 125*(5), 384-389.

Roberfroid, M., Buc Calderon, P. (1995). Free radicals and oxidative phenomena in biological systems (pp. 99-128). New York, NY: Marcel Dekker, Inc.

Rodriguez-Amaya, D. B. (1999). Changes in carotenoids during processing and storage of foods. *Archivos Latinoamericanos de Nutricion*, 49(3), 38S-46S.

Rossi, M., Negri, E., Talamini, R., Bosetti, C., Parpinel, M., Gnagnarella, P., et al. (2006). Flavonoids and colorectal cancer in Italy. *Cancer Epidemiology, Biomarkers & Prevention, 15*(8), 1555-1558.

Safari, M. R., Sheikh, N. (2003). Effects of flavonoids on the susceptibility of low-density lipoprotein to oxidative modification. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 69*, 73-77.

Santos, M. S., Meydani, S. N., Leka, L., Wu, D., Fotouhi, N. & Meydani, M., et al. (1996). Natural killer cell activity in elderly men is enhanced by β-carotene supplementation. *American Journal of Clinical Nutrition, 64*, 772-777.

Santos, M. S., Gaziano, J. M., Leka, L., Beharka, A. A., Hennekens, C. H., Meydani, S. N. (1998). β-carotene-induced enhancement of natural killer cell activity in elderly men: an investigation of the role of cytokines. *American Journal of Clinical Nutrition, 68*, 164-170.

Sato, R., Helzlsouer, K. J., Alberg, A. J., Hoffman, S. C., Norkus, E. P., Comstock, G. W. (2002). Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. *Cancer Epidemiology, Biomarkers & Prevention, 11*, 451-457.

Scalbert, A., Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition, 130*, 2073S-2085S.

Schaefer, S., Baum, M., Eisenbrand, G., Janzowski, C.(2006). Modulation of oxidative cell damage by reconstituted mixtures of phenolic apple juice extracts in human colon cell lines. *Molecular Nutrition & Food Research, 50*(4-5), 413-7.

Seeram, N. P., Adams, L. S., Zhang, Lee, R., Sand, D., Scheuller, H. S., et al. (2006). Blackberry, black raspberry, blueberry, cranberry, red raspberry, strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. *Journal of Agriculture and Food Chemistry, 54*, 9329-9339.

Sesso, H. D., Gaziano, J. M., Liu, S., Buring, J. E. (2003). Flavonoid intake and the risk of cardiovascular disease in women. *American Journal of Clinical Nutrition, 77*(6), 1400-1408.

Shahidi, F., Naczk, M. (2003). *Phenolics in Food and Nutraceuticals*. Boca Raton, FL: CRC Press.

Shimizu, M., Yoshimi, N., Yamada, Y., Matsunaga, K., Kawabata, K., Hara, A., et al. (1999). Suppressive effects of chlorogenic acid on N-methyl-N-nitrosourea-induced glandular stomach carcinogenesis in male F344 rats. *Journal of Toxicological Sciences, 24*(5), 433-439.

Schulze, M. B., Lui, S., Rimm, E. B., Manson, J. F., Willett, W. C., Hu, F. B. (2004). Glycemic index, glycemic load, and dietary fiber intake and incidence of type II diabetes in younger and middle-aged women. *American Journal of Clinical Nutrition, 80*(2), 348-56.

Shukla, L. I., Adhikary, A., Pazdro, R., Becker, D., Sevilla, M. D. (2004). Formation of

    8-oxo-7,8-dihydroguanine-radicals in gamma-irradiated DNA by multiple one-electron

    oxidations. *Nucleic Acids Research, 32*(22), 6565-6574.

Sies, H. (1985). Oxidative stress: Introductory remarks. In H. Sies (Ed.), *Oxidative stress*

    (pp. 1-8). London: Academic Press.

Slater, T. F., Cheeseman, K. H. (1985). Free radical mechanisms of tissue injury and

    mechanisms of protection. In A. Quintanilha (Ed.), *Reactive oxygen species in chemistry,*

    biology, and medicine (pp.1-12). New York, NY: Plenum Press.

Smolen, J. S., Van Der Heijde, D. M., St Clair, E. W., Emery, P., Bathon, J. M.,

    Keystone, E., et al. (2006). Predictors of joint damage in patients with early

    rheumatoid arthritis treated with high-dose methotrexate with or without

    concomitant infliximab: results from the ASPIRE trial. *Arthritis and Rheumatism,*

    54(3), 702-710.

Snodderly, D. M. (1995). Evidence for protection against age-related macular

    degeneration by carotenoids and antioxidant vitamins. *American Journal of*

    Clinical Nutrition, 62, 1448S-1461S.

Snowman, J. W. (1997). Freeze dryers. In C. J. G. Baker (Ed.), *Industrial drying*

    of foods (pp. 134-155). London, UK: Blackie Academic and Professional.

Soh, N. L., Brand-Miller. (1999). The glycaemic index of potatoes: the effect of

    variety, cooking method and maturity. *European Journal of Clinical Nutrition, 53*, 249-

    254.

Stehno-Bittel, L. (2008). Intricacies of fat. *Physical Therapy, 88*, 1265-1278.

Stelljes, K. B. (2001). Colorful potatoes offer nutrition, variety. *Agricultural Research*, *49*, 6.

Stevens, J., Ahn, K., Juhaeri, Houston, D., Steffan, L., Couper, D. (2002). Dietary fiber intake and glycemic index and incidence of diabetes in African-American and white adults: the ARIC study. *Diabetes Care, 10*, 1715-1721.

Surles, R. L., Weng, N., Simon, P. W., Tanumihardjo, S. A. (2004). Carotenoid profiles and consumer sensory evaluation of specialty carrots (*Daucus carota* L.) of various colors. *Journal of Agricultural and Food Chemistry*, *52*, 3417-21.

Tabassum, H., Parvez, S., Rehman, H., Banerjee, B. D., Raisuddin, S. (2007). Catechin as an antioxidant in liver mitochondrial toxicity: Inhibition of tamoxifen-Induced protein oxidation and lipid peroxidation. *Journal of Biochemical and Molecular Toxicology, 21*(3), 110-117.

Talburt, W. F. (1987). History of Potato Processing. In W. Talburt & O. Smith (Eds.), *Potato Processing, 4th* Ed. (pp. 1-9). New York, NY: Van Nostrand Reinhold Company Inc.

Tanaka, T., Kojima, T., Kawamori, T., Wang, A., Suzui, M., Okamoto, K., et al. (1993). Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. *Carcinogenesis, 14*(7), 1321-5.

Taylor, B. S., Alarcon, L. H., Billiar, T. R. (1998). Inducible nitric oxide synthase in the liver: regulation and function. *Biochemistry*, *63*, 766-781.

Tedgui, A., Mallat, Z. (2006). Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiological Reviews, 86*, 515-581.

Tegos, T. J., Kalodiki, E., Sabetai, M. M. (2001). The genesis of atherosclerosis and risk factors: a review. *Angiology*, *52*(2), 89-94.

Terra, X., Montagut, G., Bustos, M., Llopiz, N., Ardévol, A., Bladé, C., et al . (2009). Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. *Journal of Nutritional Biochemistry*, *20*, 210-218.

Thompson, N. R. (1987). Potato Cultivars. In W. Talburt & O. Smith (Eds.), *Potato Processing, 4th* Ed. (pp. 48-68). New York, NY: Van Nostrand Reinhold   Company Inc.

Thomas, M. J. (2000). The role of free radicals and antioxidants. *Nutrition, 16* (7/8), 716- 18.

Tomlins, K., Ndunguru, G., Stambul, K., Neema, J., Ngendello, T., Rwiza, E., et al. (2007). Sensory evaluation and consumer acceptability of pale-flesh and orange-flesh sweetpotato by school children and mothers with preschool children. *Journal of the Science of Food and Agriculture*, *87*, 2436-2446.

Törnwall, M. E., Virtamo, J., Korhonen, P. A., Virtanen, M. J., Taylor, P. R., Albanes, D., et al. (2004). Effect of α-tocopherol and β-carotene supplementation on coronary heart disease during the 6-year post-trial follow-up in the ATBC study. *European Heart Journal, 25*, 1171-1178.

Toufektsian, M. C., de Lorgeril, M., Nagy, N., Salen, P., Donati, M. B., Giordano, L. et al. (2008). Chronic dietary intake of plant-derived anthocyanins protects the rat heart against ischemia-reperfusion injury. Journal of Nutrition, 138(4), 747-742.

Tsuda, T., Horio F., Osawa T. (2002). Cyanidin-3-*O*-β-D-glucoside suppresses nitric oxide production during a zymosan treatment in rats. *Journal of Nutritional*

Science and Vitaminology, 48(4), 305-310.

Upritchard J. E., Sutherland W. H. (1999). Oxidation of heparin-treated low density

lipoprotein by peroxidases. *Atherosclerosis, 146*(2), 211-9.

USDA. (2007b). Potatoes: background. Retrieved June 18, 2007 from

http://www.ers.usda.gov/Briefing/Potatoes/background.htm

van Poppel, G., Goldbohm, R. A. (1995). Epidemiological evidence for β-carotene and

cancer prevention. *American Journal of Clinical Nutrition, 62*, 1393S-402S.

Vincent, H. K., Innes, K. E.,Vincent, K. R. (2007). Oxidative stress and potential

interventions to reduce oxidative stress in overweight and obesity. *Diabetes, Obesity and

Metabolism, 9*, 813-839.

Vita, J. A., Keaney, J. F. (2002). Endothelial function: A barometer for cardiovascular

risk. *Circulation, 106*(6), 640-642.

Vitaglione, P., Donnarumma, G., Napolitano, A., Galvano, F., Gallo, A., Scalfi, L., et al.

(2007). Procatechuic acid is the major human metabolite of cyanidin-glucosides. *Journal

of Nutrition, 137*(9), 2043-2048.

Wadsworth, T. L., Koop, D. R. (1999). Effects of the wine polyphenolics quercetin and

resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages.

Biochemical Pharmacology, 57, 941-949.

Wang, J., Mazza, G. (2002a). Inhibitory effects of anthocyanins and other phenolic

compounds on nitric oxide production in LPS/IFN-g-activated RAW 264.7 macrophages.

Journal of Agricultural and Food Chemistry, 50, 850-857.

Wang, J., Mazza, G. (2002b). Effects of anthocyanins and other phenolic compounds

on the production of tumor necrosis factor-α in LPS/IFN- -activated RAW 264.7

    macrophages. *Journal of Agricultural and Food Chemistry, 50*, 4183-4189.

Warner, J. F., Dennert, G. (1982). Effects of a cloned cell line with NK activity on bone

    marrow transplants, tumor development and metastasis *in vivo*. *Nature, 300*(4),

    31-34.

Watabe, M., Hishikawa, K., Takayanagi, A., Shimizu, N., Nakaki, T. (2004). Caffeic

    acid phenethyl ester induces apoptosis by inhibition of NFκB and activation of

    Fas in human breast cancer MCF-7 cells. *Journal of Biological Chemistry, 279*(7),

    6017-6026.

Watson, A. D., Leitinger, N., Navab, M., Faull, K. F., Hörkkö, S., Witztum, J. L., et al.

    (1997). Structural identification by mass spectrometry of oxidized phospholipids

    in minimally oxidized low density lipoprotein that induce monocyte/endothelial

    interactions and evidence for their presence in vivo. *Journal of Biology and*

    Chemistry, 272(21), 13597-13607.

Watzl, B., Bub, A., Briviba, K., Rechkemmer, G. (2003). Supplementation of a low-

    carotenoid diet with tomato or carrot juice modulates immune functions in healthy

    men. *Annals of Nutrition & Metabolism, 47*, 255-261.

Watzl, B., Bub, A., Pretzer, G., Roser, S., Barth, S. W., Rechkemmer, G. (2004). Daily

    moderate amounts of red wine or alcohol have no effect on the immune system of

    healthy men. *European Journal of Clinical Nutrition, 58*, 40-45.

Watzl, B., Kulling, S. E., Möseneder, J., Barth, S. W., Bub, A. (2005). A 4-wk

    intervention with high intake of carotenoid-rich vegetables and fruit reduces

plasma C-reactive protein in healthy, nonsmoking men. *American Journal of Clinical Nutrition, 82*, 1052-1058.

Wuttge, D. M., Zhou, X., Sheikine Y., Wagsater, D., Stemme, V., Hedin, U., et al. (2004). CXCL16/SR-PSOX is an interferon-γ-regulated chemokine and scavenger receptor expressed in atherosclerotic lesions. *Arteriosclerosis, Thrombosis, and Vascular Biology, 24*(4), 750-55.

Xia, M., Ling, W., Zhu, H., Wang, Q., Ma, J., Hou, M., et al. (2007). Anthocyanin prevents CD40-activated pro-inflammatory signaling in endothelial cells by regulating cholesterol distribution. *Arteriosclerosis, Thrombosis, and Vascular Biology, 27*, 519-524.

Yi, W., Akoh, C. C., Fischer, J., Krewer, G. (2006). Absorption of anthocyanins from blueberry extracts by Caco-2 human intestinal cell monolayers. *Journal of Agricultural and Food Chemistry, 54*, 5651-5658.

Yukawa, G. S., Mune, M., Otani, H., Tone, Y., Liang, X. M., Iwahashi, H., et al. (2004). Effects of coffee consumption on oxidative susceptibility of low-density lipoproteins and serum lipid levels in humans. *Biochemistry (Moscow), 69*(1), 70-74.

Yndestad, A., Damas, J. K., Oie, E., Ueland, T., Gullestad, L., Aukrust, P. (2006). Systemic inflammation in heart failure--the whys and wherefores. *Heart Failure Reviews 11*(1), 83-92.

Yochum, L., Kushi, L. H., Meyer, K., Folsom, A. R. (1999). Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *American Journal of Epidemiology, 149*(10), 943-949.

Yochum, L. A., Folsom, A. R., Kushi, L. H.. (2000). Intake of antioxidant vitamins and

risk of death from stroke in postmenopausal women. *American Journal of*

Clinical Nutrition, 72(2), 476-83.

Young, J. L., Libby, P., Schönbeck, U. (2002). Cytokines in the pathogenesis of

atherosclerosis. *The Journal of Thrombosis and Haemostasis, 88*, 554-67.

Zirlik, A., Bavendiek, U., Libby, P., MacFarlane, L., Gerdes, N., Jagielska, J., et al.

(2007). TRAF-1, -2, -3, -5, and -6 are induced in atherosclerotic plaques and

differentially mediate pro-inflammatory functions of CD40L in endothelial cells.

Arteriosclerosis, Thrombosis, and Vascular Biology, 27(5), 1101-1107.

Zhao, X., Aldini, G., Johnson, E. J., Rasmussen, H., Kraemer, K., Woolf, H., et al.

(2006). Modification of lymphocyte DNA damage by carotenoid supplementation in

postmenopausal women. *American Journal of Clinical Nutrition, 83*, 163-169.

CHAPTER 2

POTATO CONSUMPTION ON OXIDATIVE STRESS, INFLAMMATORY DAMAGE

AND IMMUNE RESPONSE IN HUMANS

Background: Pigmented potatoes contain high concentrations of antioxidants including phenolic acids, anthocyanins and carotenoids, which are implicated in the inhibition or prevention of cellular oxidative damage and chronic disease susceptibility.

Objective: The purpose of this study was to assess the effects of pigmented potato consumption on oxidative stress biomarkers, inflammation and immune response in healthy adult males.

Design: Free living healthy male participants (18-40 yr; n=12/group) were given 150 g of cooked white- (WP), yellow- (YP) or purple-flesh potatoes (PP) once a day for 6 wk in a double-blinded study. Blood was collected at baseline and wk 6 to analyze total antioxidant capacity, DNA damage (8-OHdG), protein oxidation, lipid peroxidation, C-reactive protein (CRP), inflammatory cytokines, lymphoproliferation, NK cytotoxicity and phenotypes. Potatoes were analyzed for total antioxidant capacity, phenolic acids, anthocyanins and carotenoids.

Results: Participants fed YP and PP had lower ($P < 0.08$) plasma IL-6 compared to those fed WP. A concurrent decrease ($P < 0.08$) in CRP concentration was observed in the PP group. Lower concentrations of 8-OHdG were observed in subjects fed either YP ($P < 0.03$) or PP ($P < 0.08$) compared to those fed WP. Percent total Tc cells were lower while B cells were higher in PP compared to the WP group ($P < 0.05$). Compared to WP, YP had high concentrations of phenolic acids ($P < 0.002$) and carotenoids ($P < 0.001$), while purple potatoes had high

concentrations of phenolic acids ($P < 0.002$) and anthocyanins ($P < 0.001$).

Conclusions: Pigmented potato consumption reduced inflammation and DNA damage, and modulates immune cell phenotype in healthy adult males.

INTRODUCTION

Oxidative stress is a disturbance in the prooxidant-antioxidant balance in favor of the former (1), potentially leading to cellular damage. Reactive oxygen species (ROS) are believed to be a major contributor to the onset of oxidative stress in biological systems (2). Increasing evidence has demonstrated that the oxidation of biomolecules such as lipids, proteins and DNA by ROS decreases cellular function, leading to chronic disease susceptibility (3, 4, 5). The primary role of exogenous and endogenous antioxidants in the body is to prevent or inhibit cellular damage mediated by free radical reactions (6).

The immune system is a complex network of cells and organs that function together to protect the body from disease. Immune cells depend on cell-to-cell communication via membrane-bound receptors; consequently, oxidative damage to the cell membrane can be very detrimental to immune cell function by decreasing the expression of surface molecules (7). It is therefore possible that immune cells must take up more antioxidants compared to other cells in order to neutralize the increased amount of ROS produced during an immune response (8). Research has demonstrated a beneficial effect of antioxidant supplementation on immune cell function such as increased natural killer cell (NK) cytotoxicity against cancer cells (9), increased lymphoproliferation (10) and shifts in immune cell populations (11). However, the immune system can also contribute to the development and progression of chronic diseases through the

induction of chronic inflammation by lymphocytes (12). Chronic inflammation may occur in response to persistent infection, prolonged exposure to toxic agents and autoimmune diseases (13). The secretion of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α by lymphocytes is responsible for initiating inflammation in the pathogenesis of chronic diseases such as atherosclerosis and rheumatoid arthritis (14,15). The quantification of cytokines and C-reactive protein (CRP), produced by the liver in response to IL-6, is used to assess disease progression (16).

Diets rich in antioxidants are associated with a lower incidence of chronic diseases such as cardiovascular disease and cancer (17, 18). Research has demonstrated that antioxidants such as phenolic acids, anthocyanins and carotenoids have been shown to reduce LDL oxidation (19), reduce DNA damage (20), inhibit cell proliferation (21) and decrease CRP production (22), while improving immune cell function (23). The potato is the most commonly consumed vegetable in the U.S. (24). In addition to high concentrations of vitamin C and iron (25), some potato cultivars are rich in phenolic acids, anthocyanins and carotenoids (26, 27, 28). Phenolic acids, such as chlorogenic acid, are found in white, yellow and purple potato cultivars (26, 28). Purple-flesh cultivars have 186% more antioxidants compared to white-flesh potatoes, most notably due to the presence of anthocyanins (29), and have 3-4 fold more phenolic acids (26). Yellow-flesh cultivars are rich in lutein and zeaxanthin (30) and can provide up to 10-fold more carotenoids than its white-flesh counterpart (29).

The purpose of this study is to assess the effects of consuming pigmented potatoes on oxidative stress markers, inflammation and immune response in healthy adult males. We hypothesized that carotenoids and anthocyanins from pigmented potatoes would decrease

oxidative stress, inflammation and improve immune status.

SUBJECTS AND METHODS

Subjects and study design

Healthy male participants between 18 and 40 y old were recruited from Washington State University and the surrounding communities. Exclusion criteria included chronic diseases, infection, and the use of tobacco, anti-inflammatory drugs and antioxidant supplements. Antioxidant supplements were avoided, and pigmented potatoes were not consumed outside of the study. The Institutional Review Board of Washington State University approved all study procedures.

Free-living participants (n=12/group) were assigned in a randomized double blind, placebo-controlled experimental design to be fed the following: 1) white-fleshed Russet (WP), 2) yellow-fleshed (YP), or 3) purple-fleshed (PP) potatoes. Randomization was based on their baseline (wk 0) BMI. Participants consumed a total of 150 g of cooked potato daily for 6 wk. In order to maximize compliance, participants consumed their potatoes at our research site between 16:00 and 18:00 h. During the intervention period, participants kept a 3 d dietary log. Height and weight measurements were taken at baseline (wk 0) and the end of the intervention period (wk 6).

The white- (Ranger Russet), yellow- (PORO3PG6-3), and purple-flesh (PORO4PG82-1) potatoes were grown locally in Washington State in 2007 (USDA-ARS, Pomeroy, WA). In order to maximize retention of bioactive compounds, whole potatoes were boiled in a steam kettle for approximately 25 min, immediately cut into quarters, frozen in sealed small plastic bags, and

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stored at -35 °C until use. Appropriate amounts of potatoes were thawed and cooked every day. To minimize destruction of the bioactive compounds, potato recipes utilized quick-cook methods such as soups, mash and stir-fry. Butter, milk or vegetable oil was used in potato preparation, and condiments such as ketchup or hot sauce were made available to the participants.

Fasting blood was collected from all participants at baseline (wk 0) and at the end of the intervention period (wk 6). Samples were immediately centrifuged (400 x g) for 30 min at 20 °C. Plasma was aliquoted, topped with nitrogen gas and stored at -80 °C until assay. The buffy coat leukocyte interface was removed and mononuclear cells obtained by density gradient centrifugation (Histopaque 1077, Sigma-Aldrich, St. Louis, MO). Contaminating RBC were lysed by hypotonic shock, leukocytes washed and cell number were determined using a particle counter (Beckman Coulter Counter, Maimi, FL 33196). One aliquot of the cell suspension was used for phenotyping using flow cytometry (FACSCalibur equipped with Cell Quest software; BD Biosciences, San Diego, CA). The remaining cells were re-suspended in 1 mL of RPMI complete medium containing 10% heat deactivated newborn calf serum, penicillin (100 mg/L) and streptomycin (100 mg/L) (Sigma-Aldrich, St Louis, MO) and used to assess mitogen-induced lymphocyte proliferation and natural killer (NK) cell cytotoxicity.

Analytic procedures

Potato composition

Whole potatoes (8 kg) were randomly selected, washed, abrasively peeled (1.5 min), cut into 6 mm slices and steam-blanched for 8 min at 133 °C by pressurized (207 kPa) steam. The potatoes were rapidly cooled in ice water for 8 min and mixed to a uniform consistency in a Hobart mixer (The Hobart Mfg. Co., Troy, OH) by adding 1.45 kg of recycled potato water/1 kg

blanched potato. The potato slurries were aliquoted, overlayed with nitrogen gas and stored at -35 °C until analysis. Dry matter content was determined by oven drying (48 h at 100 °C).

Total phenolic content. Total phenolic acids were quantified as previously described (31) in triplicate with modifications. Briefly, 2.45 g of potato slurry (1 g blanched potato) was homogenized (OMNI International, Waterbury, CT) in 10 mL distilled water: methanol (1:1, v/v) and centrifuged at 16000 x *g* for 20 min at 4 °C to extract phenolic acids. The supernatant was collected and 0.5 mL was diluted in 8 mL water. Next, 0.5 mL of 0.25 N Folin-Ciocalteu reagent was added and samples were vortexed and allowed to react for 3 min. After incubation, 1 mL of 1 N sodium carbonate was added and samples incubated for 2 h at RT. Absorbance (Beckman DU 640B, Seattle, WA) was measured at 725 nm and total phenolic acid content expressed as gallic acid equivalents (GAE).

Total Anthocyanins. Total anthocyanin content was analyzed in triplicate by the pH differential method (32, 33) with modifications. Briefly, 2.45 g of potato slurry was homogenized in 20 mL 95% ethanol:1.5 N HCl (85:15, v/v). Following a 90 min incubation at 4 °C, samples were centrifuged at 14000 x *g* for 15 min at 4 °C, 5 mL supernatant added to 45 mL potassium chloride buffer (0.025 M, pH 1.0) and 45 mL sodium acetate buffer (0.4 M, pH 4.5). Samples were equilibrated for 15 min and absorbance read at 535 and 700 nm. The final anthocyanin concentration was expressed as malvidin-3-*p*-coumarylglycoside equivalents.

Total Carotenoids. Carotenoids were extracted from 3 g of potato slurry (1.45 kg water/kg potato) with 15 mL acetone. Samples were vortexed on a multi-tube vortexer (Scientific Manufacturing Industry model 2601, Emeryville, CA), and incubated for 30 min in the dark before centrifuging at 1000 x *g* for 15 min at 4 °C. This extraction was repeated twice. The

acetone layer was dried under nitrogen gas at 40 °C. The residues were dissolved in acetone and absorbance was read at 444 nm. Total carotenoids were analyzed in triplicate and expressed as lutein (34).

Total Antioxidant Activity. Total hydrophilic antioxidant activity (TAA) was analyzed in triplicate as previously described (35) with modifications. Briefly, antioxidants were extracted by homogenizing 2.45 g of slurry (1.45 kg water/kg potato) in 10 mL methanol: H_20 (1:1, v/v) in an OMNI homogenizer. The mixture was centrifuged at 16000 x *g* for 20 min at 4 °C. The supernatant was collected, 25 μL added to 975 μL $6x10^{-5}$M DPPH in methanol and incubated 120 min. Absorbance was read at 515 nm and total antioxidant activity expressed as Trolox equivalents.

Plasma total antioxidant capacity

Total antioxidant capacity (TAC) in plasma was assayed by ELISA (Antioxidant Assay kit, Cayman Chemical, Ann Arbor, MI). This assay measures the ability of hydrophilic and lipophilic antioxidants to inhibit the oxidation of 2,2'-Azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS$^{®}$) to ABTS$^{®·+}$ by metmyoglobin. Plasma was assayed at a1:20 dilution. Assay sensitivity was 0.044 mM.

Peripheral blood lymphocyte subsets

Lymphocyte sub-populations were measured by two-color flow cytometry analysis (FACSCalibur equipped with Cell Quest software; BD Biosciences, San Diego, CA) as previously described (36). Briefly, leukocytes were isolated by gradient centrifugation with Histopaque 1.077 (Sigma-Aldrich, St. Louis, MO). Washed cells were labeled with specific monoclonal antibodies conjugated either to fluorescein isothiocyanate (anti-CD3) or

phycoerythrin (anti-CD4, anti-CD8 or anti-CD19) (Invitrogen, Carlsbad, CA). The following

lymphocyte subsets were quantified: total T cells (CD3$^+$CD19$^-$), cytotoxic T cells (Tc;

CD3$^+$CD8$^+$), T helper cells (Th; CD3$^+$CD4$^+$), B cells (CD3$^-$CD19$^+$) and natural killer cells (NK;

CD3$^-$/CD16$^+$56$^+$) (Invitrogen, Carlsbad, CA). Stained cells were fixed in 1% paraformaldehyde

in buffered saline, stored at 4 °C and analyzed within 24 h.

Lymphoblastogenesis

Isolated peripheral blood lymphocytes (100 μL of 2x10^6 cells/mL) were stimulated with

100 μL of the following mitogens (Sigma-Aldrich, St. Louis, MO): phytohemagglutinin (PHA):

20 or 100 mg/L, concanavalin A (ConA): 20 or 100 mg/L, and pokeweed mitogen (PWM): 10 or

50 mg/L (final concentration). Lymphocytes were incubated with mitogens for 72 h (37 °C, 5%

CO$_2$), and tritiated thymidine (1 mCi/L; Specific activity: 60-90 Ci/mmol; MP Biomedicals,

Solon, OH) added. After an additional 4 h incubation, cells were harvested (Perkin-Elmer,

Shelton, CT) and thymidine uptake determined by liquid scintillation (TopCount, Perkin-Elmer,

Shelton, CT). Results were reported as adjusted (stimulated – unstimulated) counts per minute

(cpm).

Natural killer cell cytotoxicity

Natural killer cell cytotoxicity was assayed using K562 as the target cell (ATCC,

Manassas, VA) in Iscove's Modified Dulbeccos (Sigma, St. Louis, MO) medium. Effector

lymphocytes were resuspended at 2.5x10^6 and 5x10^5 cells/mL and incubated overnight (37 °C,

5% CO$_2$) with target cells (5x10^5 cells/mL) to provide final target:effector cell ratios of 1:5 and

1:1. Chromagen (MTT; 5 g/L) (Sigma, St. Louis, MO) was added and incubated for 4 h at 37 °C.

Plates were centrifuged for 5 min (400 x *g*, 4 °C), supernatant removed, and absolute ethanol

were added to solubilize the cells. Optical density was read at 550 nm in a microplate reader (Bio-Tek Instruments, Inc., Winooski, VT). Natural killer cell activity was calculated as percent cytotoxicity using the formula: $100 \times [1-((OD_{effector+target}-OD_{effector})/OD_{target})]$.

C-reactive protein

Plasma CRP concentrations were analyzed using a commercially available ELISA kit (Human C-Reactive Protein ELISA kit, Alpha Diagnostic International, San Antonio, TX). In this assay, CRP simultaneously binds to a capture antibody and a detection antibody conjugated to horseradish peroxidase. Plasma was assayed at a 1:100 dilution. Absorbance was read at 450 nm. The lower limit of detection for CRP was 0.35 ng/mL.

Cytokines

Plasma IL1-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ and TNF-α were analyzed using a commercially available multiplex format ELISA (Quansys Q-Plex Human Cytokine Array, Logan, UT) which allows for the simultaneous detection of multiple cytokines within one sample. The lower limit of detection was 4.10 pg/mL, 1.10 pg/mL, 1.36 pg/mL, ≤ 1.0 pg/mL, 1.36 pg/mL, ≤ 1.0 pg/mL, ≤ 1.0 pg/mL and 2.11 pg/mL for IL1-α, IL-1β, IL-2, IL-4, IL-8, IL-10, IFN-γ and TNF-α respectively. Acquired data was analyzed using Quansys Q-View 2.5.2 software.

Protein Carbonyl

Plasma protein carbonyl concentrations were measured with a commercially available colorimetric assay (Protein Carbonyl Assay kit, Cayman Chemical, Ann Arbor, MI). Concentration of the carbonyls was determined using corrected absorbance at 370 nm. This assay optimally detects protein carbonyls in samples with protein content between 1-10 mg/mL.

Lipid peroxidation

Lipid peroxidation in plasma was analyzed by measuring thiobarbituric acid reactive substances (TBARS), as previously described (37) with modifications. Plasma samples (500 μL) were reacted with 3 mL 1% phosphoric acid, 1 mL 0.6% thiobarbituric acid solution and 50 μL KCl. The mixture was heated in a 90°C water bath for 45 min, cooled on ice and 4 mL n-butanol added. The butanol phase was separated by centrifugation (10 min at 400 x g, 20 °C), and optical density was read at 532 nm and 520 nm (Beckman DU 640B, Seattle, WA). Tetramethoxypropane was used as the standard.

DNA damage

DNA damage was assessed by measuring 8-hydroxydeoxyguanosine (8-OHdG) concentrations in plasma using a competitive ELISA kit (Bioxytech® 8-OHdG-EIA Kit, Oxis Health Products, Inc., Portland, OR). Absorbance was read at 450 nm. The lower limit of detection was 0.5 ng/mL.

Statistical analysis

Potato composition was analyzed by ANOVA using the General Linear Models procedure of SAS statistical software (version 8; SAS Institute, Cary, NC). Blood biomarkers and immune response variables were analyzed by ANCOVA using wk 0 values as a covariate. The initial statistical model included age, BMI and ethnicity. Differences in treatment means were compared using protected LSD.

RESULTS

Subjects

Demographics of the participants are presented in **Table 1**. All participants ($n = 36$) completed the study. No significant treatment differences were observed in age or BMI. Total caloric intake based on a 3 d dietary recall was lower ($P < 0.02$) in the PP treatment compared to YP or WP. There was no significant treatment x wk interaction for BMI. Participant compliance was excellent and no adverse side effects were reported.

TABLE 2-1

Demographic characteristics of participants.

	WP	YP	PP
Age (y)	21.4 ± 1.0	23.1 ± 1.4	22.4 ± 1.4
BMI (kg/m^2)			
Week 0	25.0 ± 1.1	25.8 ± 1.1	25.4 ± 1.0
Week 6	25.1 ± 1.1	25.9 ± 1.1	25.2 ± 1.1
Ethnicity (*n*)			
Caucasian	11	11	10
Asian	1	1	2
Caloric intake, kcal/d	2579 ± 141[a]	2620 ± 141[a]	2100 ± 141[b]

[a, b] Different letters represent significant treatment differences ($P < 0.05$) as analyzed by ANOVA ($n =12$). Values are mean ± SE.

Potato composition

The antioxidant composition of the potato cultivars are shown in **Table 2**. Total phenolics were higher ($P < 0.002$) in yellow and purple potatoes compared to white. Total carotenoid content was highest ($P < 0.001$) in yellow potatoes (58.1 μg/g DW). Total anthocyanins were highest ($P < 0.001$) in purple potatoes (6.2 mg/g DW) and almost non-detectable in white and yellow potatoes. TAA was highest ($P < 0.001$) in purple potatoes compared to yellow or white; yellow potatoes had a higher ($P < 0.001$) TAA than white potatoes.

Estimated amounts of antioxidants consumed daily by study participants are shown in **Table 3**. Total phenolics in YP and PP were about 1.5-fold greater than WP. The YP group consumed between 30 and 38-fold more carotenoids than WP or PP. The WP and YP groups consumed few anthocyanins while the PP consumed at least 24-fold more. Total antioxidants, assayed by TAA, were almost 2-fold higher in PP compared to YP, and YP was 3-fold higher than WP.

C-reactive protein

Plasma CRP concentrations in PP (1.3 ng/L) were lower ($P < 0.08$) than in WP (3.4 ng/L) at wk 6 (**Figure 1**). Concentrations also tended to be lower in YP (1.8 ng/L) than WP.

DNA damage

Plasma 8-OHdG concentrations in YP (27.3 ng/mL, $P < 0.03$) and PP (29.4 ng/mL, $P < 0.08$) were significantly lower than in WP (37.6 ng/mL) at wk 6 (**Figure 2**).

Total antioxidant capacity

There was no significant treatment difference in plasma TAC after 6 wk of potato consumption however, TAC in YP and PP treatments (2.2 mM) tended ($P > 0.05$) to be higher

than in WP (1.9 mM) (**Table 4**).

TABLE 2-2

Potato composition.

Cultivar	Total phenolics (mg/g DW)	Total carotenoids (µg/g DW)	Total anthocyanins (mg/g DW)	Total antioxidant activity (mg/g DW)	Dry matter
White	1.5 ± 0.1 [a]	1.3 ± 0.0 [a]	0.0 ± 0.0 [a]	1.2 ± 0.0 [a]	22.1%
Yellow	3.2 ± 0.1 [b]	58.1 ± 12.3 [b]	0.3 ± 01 [a]	5.5 ± 0.0 [b]	15.2%
Purple	3.1 ± 0.1 [b]	1.3 ± 0.2 [a]	6.2 ± 0.1 [b]	8.4 ± 0.0 [c]	17.9%

[a, b] Different letters denote significant difference ($P < 0.001$) as analyzed by ANOVA. Values are triplicate mean ± SE.

TABLE 2-3

Estimated average daily intake of bioactive compounds based on 150 g potato consumed.

Treatment	Total phenolics[1]	Total carotenoids[2]	Total anthocyanins[1]	Total antioxidant activity[1]
White	49.6	43.0	0.0	39.7
Yellow	72.9	1323.8	6.8	125.3
Purple	83.2	34.9	166.3	225.4

[1] mg/150 g cooked potato

[2] μg/150 g cooked potato

Figure 2-1. Plasma CRP concentrations in subjects fed white (WP), yellow (YP) or purple (PP) potatoes for 6 wk. Data were analyzed by ANCOVA using wk 0 as a covariate. Different symbols above each bar denote significant difference compared to WP ($^{\tau}P < 0.08$).

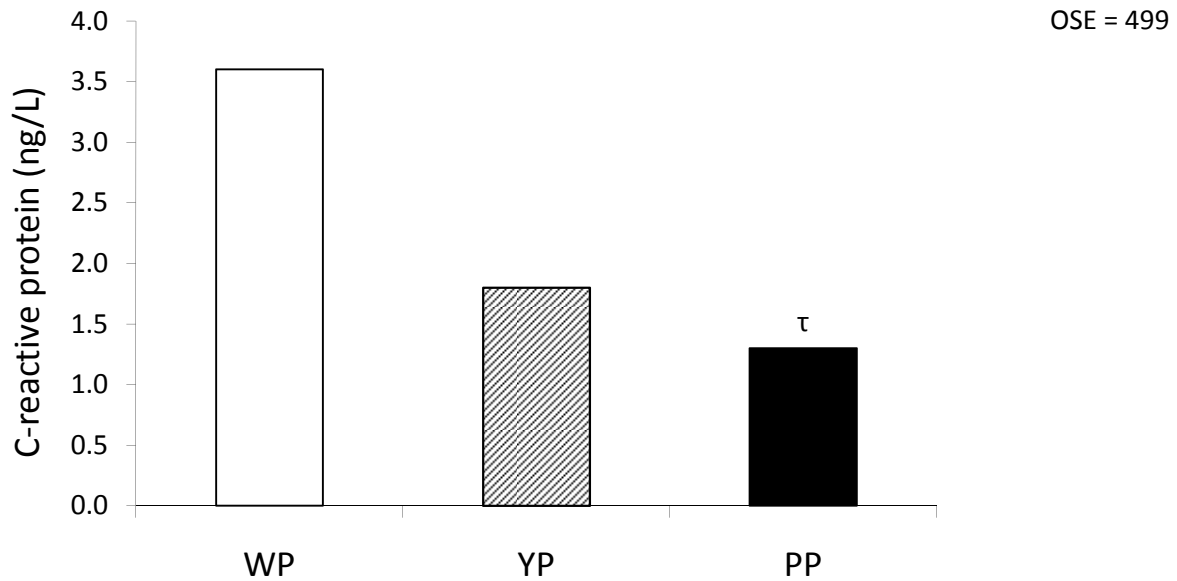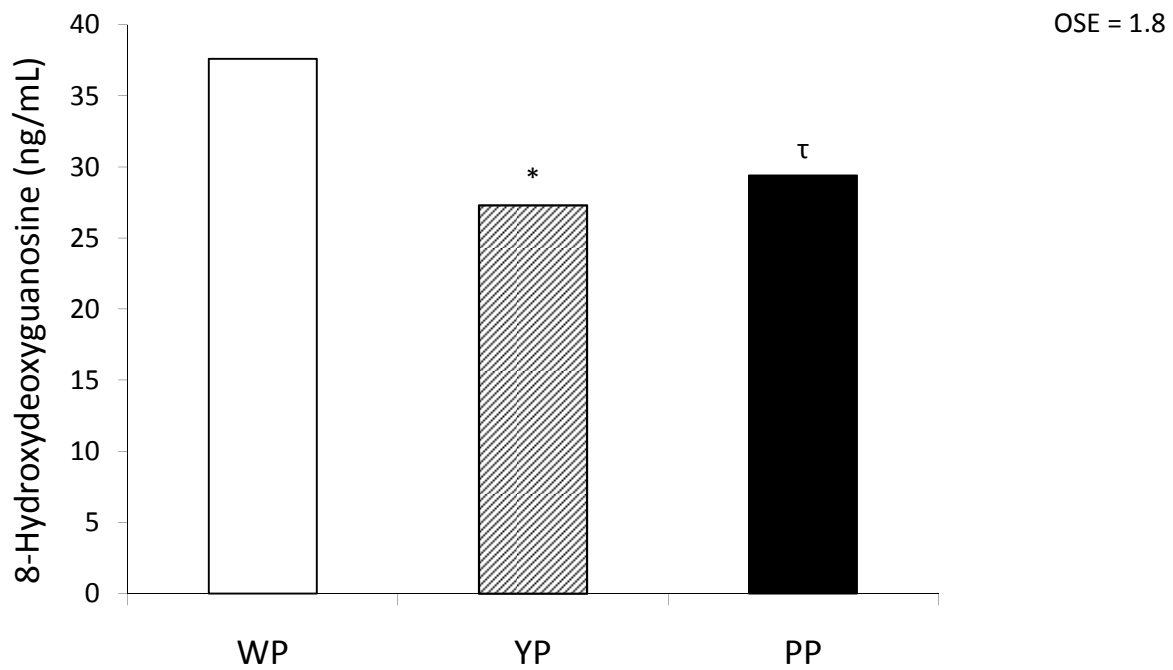**Figure 2-2.** Plasma concentrations of 8-hydroxydeoxyguanosine in subjects fed white (WP), yellow (YP) or purple (PP) potatoes for 6 wk. Data were analyzed by ANCOVA using wk 0 as a covariate. Different symbols above each bar denote significant difference compared to WP ($^{*}P <$ 0.03, $^{\tau}P < 0.08$).

TABLE 2-4

Oxidative stress biomarkers in participants fed white (WP), yellow (YP), or purple (PP) potatoes for 6 wk.[1,2]

Treatment	TAC (mM)	Protein carbonyl (nmol/mL)	TBARS (µM)
WP	1.9 [a]	5.6 [a]	1.1 [a]
YP	2.2 [a]	6.3 [a]	1.1 [a]
PP	2.2 [a]	6.5 [a]	1.1 [a]
Overall SE	0.1	0.2	0.1

[1]Data were analyzed by ANCOVA ($n = 12$) using wk 0 as a covariate.

[2]There were no significant treatment differences.

Protein carbonyl

Plasma protein carbonyl content showed no significant difference between treatments at wk 6. Plasma protein carbonyl averaged 6.1 nmol/mL across all treatments (**Table 4**).

Lipid peroxidation

Concentrations of plasma TBARS were not significantly different among treatments at wk 6 (**Table 4**).

Cytokine production

Plasma cytokine concentrations are shown in **Table 5**. Concentrations of plasma IL-6 in YP ($P < 0.08$) and PP ($P < 0.08$) treatments were lower compared to WP (**Figure 3**). In contrast, no significant differences were observed in plasma IL1-α, IL-1β, IL-2, IL-4, IL-8, IL-10, IFN-γ or TNF-α concentrations among treatments.

Lymphocyte subsets

The distribution of different lymphocyte subpopulations are shown in **Table 6**. The B cell population at wk 6 was higher ($P < 0.03$) in PP (9.3%) than in WP (6.7%) (**Figure 4**). Among the different T cell subsets, subjects in YP generally had higher percentages of total T cells and Th cells than in WP. The Tc cell population in PP (25.0%) but not in YP (28.5%) was lower ($P < 0.05$) than in WP (28.5%) (**Figure 4**). The ratio of Th:Tc was similar among treatments, averaging 1.3. Subjects in PP tended to have the highest percentage of NK cells (26.3%). These results suggest that subjects in YP had numerically lower B and NK cell subpopulations and higher T cell populations than WP. Those in PP tended to have higher B and NK cell populations but lower T cell populations.

TABLE 2-5

Plasma cytokine concentrations (pg/mL) in participants fed white (WP), yellow (YP), or purple (PP) potatoes for 6 wk.[1]

Treatment	IL-1α	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IFN-γ	TNF-α
WP	10.6	38.8	23.8	24.4	30.2[a]	5.2	14.6	3.6	15.4
YP	11.6	40.8	23.8	23.2	16.6[b]	3.8	13.0	3.6	15.0
PP	10.8	39.0	24.4	25.2	16.8[b]	4.4	12.8	4.0	15.0
Overall SE	0.3	0.7	0.4	0.5	3.0	0.4	0.6	0.6	0.4

[1]Data were analyzed by ANCOVA ($n =12$) using wk 0 as a covariate.

[a, b] Different letters denote significant difference ($P < 0.08$).
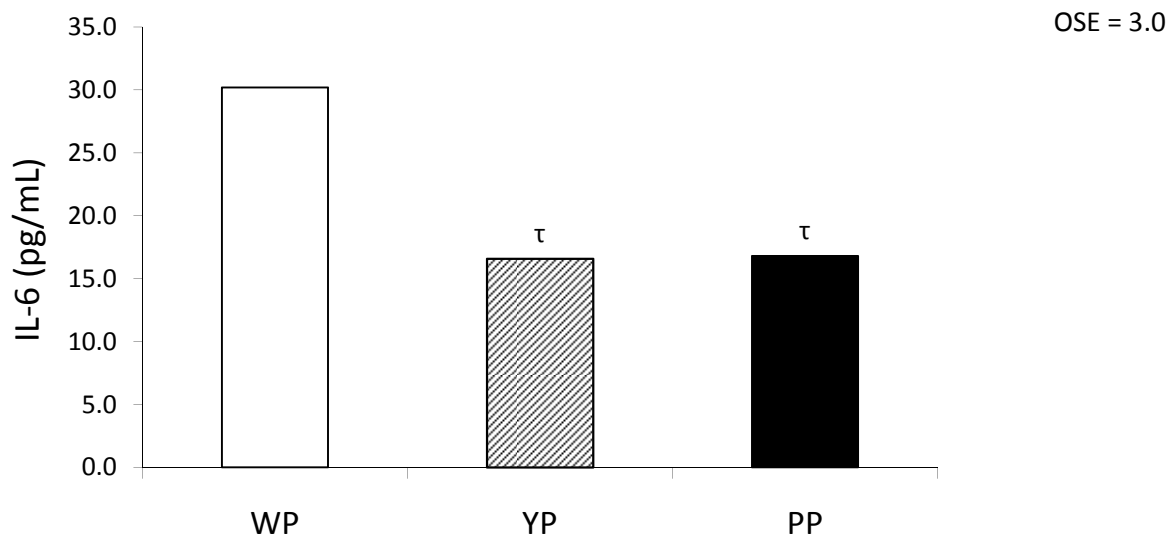
Figure 2-3. Plasma concentrations of IL-6 in subjects fed white (WP), yellow (YP) or purple (PP) potatoes for 6 wk. Data were analyzed by ANCOVA using wk 0 as a covariate. Different symbols above each bar denote significant difference compared to WP ($^{\tau}P < 0.08$).

TABLE 2-6

The percent of lymphocyte subsets in participants fed white (WP), yellow (YP), or purple (PP) potatoes for 6 wk.[1]

Treatment	B	Total T	Th	Tc	Th:Tc	NK
WP	6.7 [a]	64.3	30.6	28.5 [a]	1.2	24.3
YP	6.5 [a]	68.3	35.8	28.5 [a]	1.3	20.6
PP	9.3 [b]	60.4	30.0	25.0 [b]	1.3	26.3
Overall SE	0.5	1.5	1.3	0.6	0.1	1.3

[1] Data were values were analyzed by ANCOVA ($n = 12$) using wk 0 as a covariate.

[a, b] Different letters denote significant difference ($P < 0.04$).
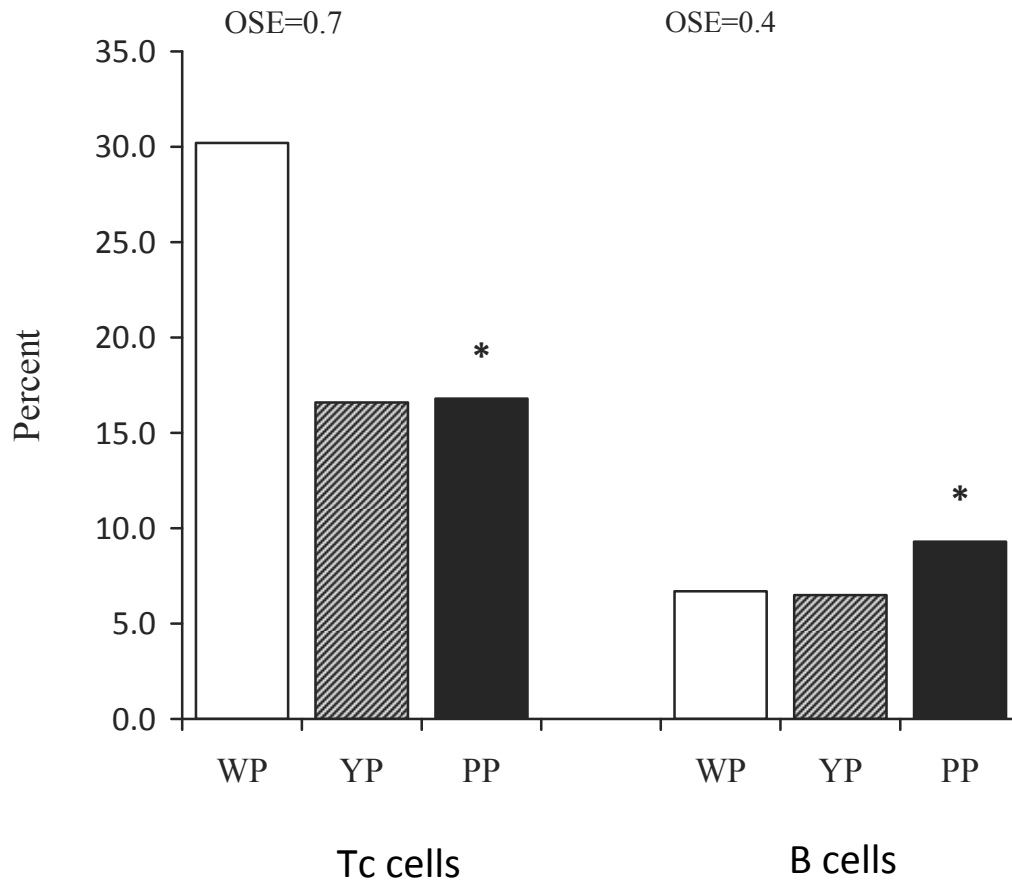
Figure 2-4. Percent lymphocyte Tc and B cells in subjects fed white (WP), yellow (YP) or purple (PP) potatoes for 6 wk. Data were analyzed by ANCOVA using wk 0 as a covariate. Different symbols above each bar denote significant difference compared to WP ($^{*}P < 0.05$).

Lymphoblastogenesis

Lymphoproliferation induced by B and T cell mitogens. No significant treatment differences were observed in lymphocyte proliferation (**Table 7**).

NK cell cytotoxicity

NK cell cytotoxicity showed no significant differences in NK cell killing efficiency among treatments (**Table 8**).

TABLE 2-7

Mitogen-induced lymphoproliferation (cpm) in participants fed white (WP), yellow (YP), or purple (PP) potatoes for 6 wk.[1,2]

Treatment	PHA, 100 mg/L	PHA, 20 mg/L	ConA, 100 mg/L	ConA, 20 mg/L	PWM, 50 mg/L	PWM, 10 mg/L
WP	17456	3574	14846	9483	4080	3661
YP	16199	3981	15201	10190	5094	5079
PP	15672	4740	12930	8015	3688	3413
Overall SE	1360	757	1009	838	453	475

[1] Data were analyzed by ANCOVA ($n = 12$) using wk 0 as a covariate.

[2] No significant treatment difference ($P < 0.05$) in lymphoproliferation was observed.

TABLE 2-8

NK cell cytotoxicity (% killing) in participants fed white (WP), yellow (YP), or purple (PP) potatoes for 6 wk.[1,2]

Treatment	Target : Effector cell ratio	
	(1:1)	(1:5)
WP	97.7	78.2
YP	96.7	86.3
PP	91.2	82.8
Overall SE	4.2	2.6

[1]Data were analyzed by ANCOVA ($n =12$) using wk 0 as a covariate.

[2]No significant treatment difference ($P < 0.05$) in NK cytotoxicity was observed.

DISCUSSION

This is the first study to address the effects of potato consumption on antioxidant status, oxidative stress, inflammation and immune status in humans. Consumption of pigmented potatoes (YP and PP) decreased 8-OHdG concentrations and IL-6, while consumption of PP also decreased CRP concentrations, decreased Tc cells and increased B cells when compared to WP.

Although fasting total antioxidant capacity did not increase, it appears that YP and PP helped prevent oxidative stress associated with DNA damage. Participants in YP and PP had significantly lower concentrations of 8-OHdG, a biomarker of DNA damage. Pool-Zobel et al. (38) reported a significant decrease in DNA damage in healthy males after supplementation with fruit and vegetable juices high in α-carotene, β-carotene, lutein and lycopene for 3-8 wk. Similarly, Gill et al. (39) supplemented smokers and non-smokers with 85 g of watercress containing high concentrations of lutein, β-carotene, phenolics and glucosinolates for 8 wk. They reported no significant difference in fasting plasma antioxidant potential as measured by FRAP; however, there was a significant reduction in lymphocyte DNA damage. One study (40) reported that smokers had a greater beneficial response after 4 wk of daily supplementation with 84 g almonds (rich in vitamin E, phenolics and flavonoids) compared to healthy individuals. In the present study, healthy males supplemented for 6 wk with 150 g of potatoes high in carotenoids or anthocyanins had reduced DNA oxidation, therefore, it is plausible that consumption of yellow and purple potatoes may reduce DNA damage in smokers or individuals with chronic diseases. The prevention of oxidative DNA damage by antioxidants is likely mediated by quenching ROS (41). However, not all studies investigating the relationship between supplemental antioxidants and oxidative stress have demonstrated a beneficial effect. Riso et al. (42) reported no significant

differences in DNA damage (by comet assay) or lipid peroxidation (by urinary 8-isoprostaglandins) in healthy men and women supplemented with carotenoid-enriched tomato juices for 26 d. Additionally, Møller et al. (43) did not observe any significant differences in DNA damage in healthy males and females supplemented for 3 wk with anthocyanin-rich drinks.

As a biomarker of inflammation, elevated CRP concentrations (44) have recently been implicated in chronic disease development (45) and progression (46). CRP is produced by the liver in response to the inflammatory cytokine IL-6; reducing circulating CRP can prevent chronic disease development or disease progression. In this study, purple potatoes, a good source of anthocyanins, significantly reduced CRP and IL-6 concentrations in healthy males; plasma IL-6 concentration was significantly lower in YP than in WP. Plasma CRP was about 2-fold lower in YP than in WP, albeit not significantly. The WP demonstrated a non-significant increase in CRP concentrations at wk 6 compared to baseline. These results imply that PP and YP consumption could potentially alleviate inflammatory symptoms associated with chronic diseases such as cardiovascular disease, rheumatoid arthritis, and inflammatory bowel diseases. Antioxidants likely decrease inflammation by down-regulating the pro-inflammatory NFκB gene (47), which is responsible for cytokine production in immune cells (48). Reduced plasma IL-6 concentrations will therefore inhibit IL-6-stimulated CRP production by the liver (49).

The anti-inflammatory properties of antioxidant supplementation from whole-foods have been demonstrated by Watzl et al. (22) in a multi-phase study design. In the first treatment phase, healthy male volunteers consumed two servings of fruit and vegetables/d for 4 wk. During the second phase of the treatment, volunteers consumed 2, 4 or 8 servings/d of whole fruits and vegetables. Blood CRP concentrations were significantly lower in the eight servings/d

intervention group compared to those given 2 servings/d. The amount of fruits/vegetables consumed, as well as plasma β-carotene concentrations, was inversely related to CRP concentrations.

The antioxidant status of our study participants was only marginally affected by potato supplementation. This result was surprising because a preliminary study in our laboratory indicated that plasma antioxidant status increased by 160% measured 6 h after consumption of 300 g of purple potatoes. Anthocyanins are absorbed into plasma within 15-60 min after consumption; urinary excretion is complete within 6-8 h and typically less that 1% of ingested anthocyanins are absorbed (50). In this study, fasting blood samples were taken at least 14 hr after potato consumption; therefore, the discrepancy in plasma antioxidant status between the two studies is likely due to the low absorption and rapid clearance of anthocyanins from the blood stream. Compliance was not considered an issue in this study, therefore decreases in oxidative damage and inflammation observed in this study may be attributed to phenolic acid, carotenoid or anthocyanin concentrations from the potatoes.

The 3-day dietary assessment revealed that participants in the PP group had a lower ($P <$ 0.02) total kcal intake compared to WP or YP. These results may explain the observed reduction in CRP concentrations; calorie-restricted diets (1,112-1,958 kcal/day) have been shown to decrease CRP, as well as other atherosclerosis risk factors compared to normal calorie diets (1,976-3,537 kcal/day) (51). By definition, only 4 participants in the PP group had calorie restricted diets for the 3-day assessment. However, the YP group had lower CRP concentrations at 6 wk and normal average calorie intakes.

The total phenolic concentration of white potatoes in this study is consistent with

previously published values (25); however, total phenolics in yellow-flesh potatoes in this study were 2-fold greater while that in purple-flesh potatoes were 4-fold lower than reported (25). Total carotenoid concentrations in white and purple potatoes in this study were 2- to 7-fold lower, while concentrations in yellow potatoes were 1.5 fold higher than reported by others (25). Total anthocyanins in white and yellow potatoes were low to non-detectable in this study, similar to other reports (28), whereas purple potatoes in this study had a 3-fold lower anthocyanin concentration compared to others (28). Antioxidant concentrations in potatoes have been reported to vary greatly among cultivars based on genotype, location and year grown (52), which may explain differences in concentrations of bioactive compounds measured in this study. Additionally, differences in extraction methods or efficiencies may exist may attribute to variation.

The possibility exists that bioactive compounds other than antioxidants may be responsible for the effects observed in this study. Potatoes contain angiotensin-converting enzyme (ACE) inhibitors (53) which have been shown to decrease CRP (54) and IL-6 (55) in patients with cardiovascular diseases. To date, no studies have investigated ACE inhibitor concentrations in pigmented potato cultivars.

The beneficial effects of carotenoid antioxidants on immune function have been well documented (23). Studies have shown that carotenoid and polyphenol depletion followed by supplementation results in increased lymphoproliferation and NK cytotoxicity (56, 57), indicating that carotenoids and polyphenols are important for lymphocyte function. In this study, no significant differences in NK cytotoxicity or lymphoproliferation were observed after a 6 wk supplementation with YP or PP. Subjects in our present study did not undergo a

carotenoid/polyphenol depletion period, did not avoid high-antioxidant foods, and were allowed to maintain their normal diets. Others (22) have reported a similar lack of antioxidant supplementation effects on immune status. Based on these studies, a healthy diet without antioxidant depletion, or supplementation from juice or whole food sources, does not appear to modulate lymphocyte function.

Lymphocyte phenotyping is used to diagnose disease and monitor disease progression (58). Changes in lymphocyte subsets may be beneficial or detrimental depending on the physiological state of the individual. For example, an increase in the Th:Tc ratio may be beneficial for an HIV patient, while a decrease in Th:Tc ratio may result in disease progression. The Tc population in the PP was significantly lower compared to WP, whereas the B cell population was significantly higher. No other significant changes in lymphocyte subsets were observed among the potato treatments, and all lymphocyte percentages were within normal range (B cells, 6-19%; Total T, 55-83%; Th, 28-57%; Tc, 10-39%; CD4/8 ratio, 1-3.6; NK, 7-31%) (59). Subjects in YP treatment tended to have lower B and NK cell populations and higher total T cell populations than subjects in WP; conversely, those in PP had higher B and NK cell populations and lower total T cell populations. These results imply that anthocyanins from purple potatoes may decrease Tc cell but increase B cell subpopulations.

In conclusion, consumption of yellow and purple potatoes lowered DNA oxidative damage and inflammation associated with IL-6 production. In addition, consumption of purple potatoes lowered concentrations of the acute phase protein, CRP. The potential physiological benefits of consuming pigmented potatoes should be explored in persons with chronic disease.

REFERENCES

(1) Sies H. Oxidative stress: Introductory remarks. In Sies H, ed. Oxidative stress. London: Academic Press, 1985:1-8.

(2) Halliwell B. Free radical reactions in human disease. In Fuchs J, Packer L, eds. Environmental stressors in health and disease. New York, NY: Marcel Dekker Inc., 2001:1-16.

(3) Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 1993;*57*:715S-24S.

(4) Kim NH, Jeong MS, Choi SY, Kang JH. Oxidative modification of cytochrome c by hydrogen peroxide. Mol Cells 2006;22:220-7.

(5) Shukla LI, Adhikary A, Pazdro R, Becker D, Sevilla MD. Formation of 8-oxo-7,8-dihydroguanine-radicals in gamma-irradiated DNA by multiple one-electron oxidations. Nucleic Acids Res 2004;32:6565-74.

(6) Halliwell B. How to characterize an antioxidant: an update. In Rice-Evans C, Halliwell B, Lunt G, eds. Free radicals and oxidative stress: environment, drugs and food additives. London: Portland Press, 1995:73-93.

(7) Gruner S, Volk HD, Falck P, Baehr RV. The influence of phagocytic stimuli on the expression of HLA-DR antigens; role of reactive oxygen intermediates. Eur J Immunol 1986;16:212-15.

(8) Coquette A, Vray B, Vanderpas J. Role of vitamin E in the protection of the resident macrophage membrane against oxidative damage. Arch Int Phys Bioch 1986;94:S29-S34.

(9) Santos MS, Meydani SN, Leka L, et al. Natural killer cell activity in elderly men is enhanced by β-carotene supplementation. Am J Clin Nutr 1996; 64:772-77.

(10) van Poppel G, Spanhaak S, Ockhuizen, T. Effect of β-carotene on immunological indexes in healthy male smokers. Am J Clin Nutr 1993;57:402-07.

(11) Prabhala RH, Garewal HS, Hicks MJ, Sampliner RE, Watson RR. The effects of 13-cis-retinoic acid and beta-carotene on cellular immunity in humans. Cancer 1991;67:1556-60.

(12) Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358:903-11.

(13) Mitchel, NR, Cotran, RS. 7th ed. Acute and chronic inflammation. In Kumar V, Cotran RS, Robbins SL, eds. Robbins Basic Pathology. Philidelphia, PA: Saunders, 2003:33-59.

(14) Frostegard J, Ulfgren A, Nyberg P, et al. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis 1999;145: 33-43.

(15) Horwood N. Lymphocyte-derived cytokines in inflammatory arthritis. Autoimmunity 2008;41:230-38.

(16) Emery P, McInnes IB, van Vollenhoven R, Kraan MC. Clinical identification and treatment of a rapidly progressing disease state in patients with rheumatoid arthritis. Rheumatology 2008;47:392-98.

(17) Galassetti P, Pontello A. Dietary effects on oxidation of low-density lipoprotein and atherogenesis. Curr Atheroscler Rep 2006;8:523-29.

(18) Gonzalez CA. Nutrition and cancer: the current epidemiological evidence. Brit Journal

of Nutr 2006;96:42S-45S.

(19) Chang YC, Huang K X, Huang AC, Ho CH, Wang CJ. Hibiscus anthocyanin-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. Food Chem Toxicol 2006;44:1015-23.

(20) Schaefer S, Baum M, Eisenbrand G, Janzowski C. Modulation of oxidative cell damage by reconstituted mixtures of phenolic apple juice extracts in human colon cell lines. Mol Nutr Food Res 2006;50: 413-7.

(21) Reddivari L, Vanamala J, Chintharlapalli S, Safe SH, Miller JC Jr. Anthocyanin fraction from potato extracts is cytotoxic to prostate cancer cells through activation of caspase-dependent and caspase-independent pathways. Carcinogenesis 2007;10:2227-35.

(22) Watzl B, Kulling SE, Möseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. Am J Clin Nutr 2005;82:1052-58.

(23) Hughes DA. Dietary carotenoids and human immune function. Nutrition 2001;17:823-27.

(24) Chun OK, Kim DO, Smith N, Schroeder D, Han JT, Lee CY. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J Sci Food and Agr 2005;85:1715-24.

(25) Andre CM, Ghislain M, Bertin P, et al. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. J Agr Food Chem 2007;55:366-78.

(26) Lewis CE, Walker JR, Lancaster JE, Sutton KH. Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of Solanum tuberosum L. J Sci Food and Agr 1998;77:45-57.

(27) Brown CR, Wrolstad R, Durst R, Yang CP, Clevidence B. Breeding studies in potatoes containing high concentrations of anthocyanins. Am J Potato Res 2003;80:241-50.

(28) Andre CM, Oufir M, Guignard C, et al. Antioxidant profiling of native Andean potato tubers (*Solanum Tubersosum* L.) reveals cultivars with high levels of -carotene, -tocopherol, chlorogenic acid, and petanin. J Agr Food Chem 2007;55:10839-49.

(29) Brown CR, Culley D, Yang CP, Durst R, Wrolstad R. Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. J Am Soc for Hortic Sci 2005;130:174-80.

(30) Brown CR, Edwards CG, Yang CP, Dean BB. Orange flesh trait in potato: inheritance and carotenoid content. J Am Soc for Hortic Sci 1993;118:145-50.

(31) Swain T, Hillis WE. The phenolic constituents of *Prunus Domestica I.*—the quantitative analysis of phenolic constituents. J Sci Food Agric 1959;10:63-68.

(32) Fuleki T, Francis FJ. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. J Food Sci 1968;33: 72-77.

(33) Lee J, Durst RW, Wrolstad RE. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J AOAC Int 2005;88:1269-78.

(34) Britton G. General carotenoid methods. Method Enzymol, 1985;111:113-49.

(35) Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate

antioxidant activity. Lebensm Wiss Technol 1995;28:25-30.

(36) Ryan-Borchers TA, Park JS, Chew BP, McGuire MK, Fournier LR, Beerman KA. Soy isoflavones modulate immune function in healthy postmenopausal women. Am J Clin Nutr 2006;83:1118-25.

(37) Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271-78.

(38) Pool-Zobel BL, Bub A, Müller H, Wollowski I, Rechkemmer G. Consumption of vegetables reduces genetic damage in humans: first results of a human intervention trail with carotenoid-rich foods. Carcinogenesis 1997;18:1847-50.

(39) Gill C, Haldar S, Boyd LA, et al. Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. Am J Clin Nutr 2007;85:504-10.

(40) Li N, Jia X, Chen CY, et al. Almond consumption reduced oxidative DNA damage and lipid peroxidation in male smokers. J Nutr 200;137:2717-22.

(41) Burdette JE, Chen S, Lu Z, Xu H, White B, Fabricant DS, Liu J, Fong H, Farnsworth, NR, Constantinou AI, van Breeman RB, Pezzuto JM, Bolton, JL. Black cohosh (*Cimicifuga racemosa* L.) protencts against menadione-induced DNA damage through scavenging of reactive oxygen species: bioassay-directed isolation and characterization of active principles. J Agr Food Chem 2002;50:7022-28.

(42) Riso P, Visiolli F, Grande S, Guarnieri S, Gardana C, Porrini M. Effect of a tomato-based drink on markers of inflammation, immunomodulation and oxidative stress. J Agr Food Chem 2006;54:2563-66.

(43) Møller P, Loft S, Alfthan G, Freese R. Oxidative damage in circulating mononuclear blood cells after ingestion of black current juice or anthocyanin-rich drink. Mutat Res 2004a;551:119-26.

(44) Li JJ, Fang CH. C-reactive protein is not only an inflammatory marker but also a direct cause of cardiovascular diseases. Med Hypotheses 2004;62:499-506.

(45) Paffen E, deMaat M. C-reactive protein in atherosclerosis: a causal factor? Cardiovasc Res 2006;71:30-9.

(46) Smolen JS, Van Der Heijde DM, St Clair EW, et al. Predictors of joint damage in patients with early rheumatoid arthritis treated with high-done methotrexare or without concomitant infliximab. Arthritis and Rheum 2006;54:702-10.

(47) Kim J, Na H, Kim C, Kim J, Ha K., Lee H. The non-provitamin A carotenoid, lutein, inhibits NF-κB-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-κB-inducing pathways: Role of H_2O_2 in NF-κB activation. Free Radic Biol Med 2008;45:885-96.

(48) Rasheed Z, Akhtar N, Anbazhagan AN, Ramamurthy S, Haqqi TM. Polyphenol-rich pomegranate fruit extract (POMx) suppresses PMACI-induced expression of pro-inflammatory cytokines by inhibiting the activation of MAP Kinases and NF-kappaB in human KU812 cells. J Inflamm 2009; 8:6:1.

(49) Castell JV, Gomez-Lechon MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute phase protein synthesis by interleukin-6. Hepatology1990; 12:1179-86.

(50) McGhie TK, Walton MC. The bioavailability and absorption of anthocyanins: towards a

better understanding. Mol Nutr Food Res 2007;51:702-13.

(51) Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proc Natl Acad Sci USA 2004;101:6659-63.

(52) Reddivari L, Hale A, Creighton Miller Jr. J. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. J Agr Food Chem 2007;55:8073-79.

(53) Pihlanto A, Akkanen S, Korhonen AJ. ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). Food Chem 2008;109:104-12.

(54) Di Napoli M, Papa F. Angiotensin-converting enzyme inhibitor use is associated with reduced plasma concentration of C-reactive proteins in patients with first-ever ischemic stroke. Stroke 2003;34:2922-29.

(55) Brull DJ, Sanders J, Rumley A, Lowe GD, Humphries SE, Montgomery HE. Impact of angiotensin converting enzyme inhibition on post-coronary artery bypass interleukin 6 release. Heart 2002;87:252-55.

(56) Watzl B, Bub A, Briviba K, Rechkemmer G. Supplementation of a low-carotenoid diet with tomato or carrot juice modulates immune functions in healthy men. Ann Nutr Metab 2003;47:255-61.

(57) Chen CM, Li SC, Lin YL, Hsu CY, Shieh MJ, Liu JF. Consumption of purple sweet potato leaves modulates human immune response: T-lymphocyte functions, lytic activity of natural killer cell and antibody production. World J Gastroentero 2005;11:5777-81.

(58) Reilly JT, Barnett D. UK NEQAS for leukocyte immunophenotyping: the first 10 years. J Clin Pathol 2001;54:508-11.

(59) Comans-Bitter WM, de Groot R, van den Beemd R, Neijens, HJ, Hop WC, Groeneveld K, Hooijkaas H, van Dongen, JJ. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130:388-93.

CHAPTER 3

PROCESSING OF PIGMENTED FLESH POTATOES (*SOLANUM TUBEROSUM* L.) ON NUTRIENT RETENTION

ABSTRACT

Antioxidant retention in white-, yellow-, red-, and purple-flesh potato cultivars after drum drying (DD), freeze drying (FD) and Refractance Window™ drying (RW) was compared. Dried potatoes were analyzed for total antioxidant activity, phenolics, anthocyanins and carotenoids. Total phenolics were high in all potato cultivars. Processing generally did not influence phenolics, except that DD increased (P <0.001) total phenolics in WP. The FD and RW decreased (P <0.001) phenolics in YP. The RP and PP cultivars had the highest anthocyanin concentration. Total anthocyanins in RP and PP decreased *(P <0.001)* with all drying methods. Total carotenoids were high in YP only, but decreased (P <0.01) after drying processes. Total antioxidant activity decreased (P <0.001) in YP, RP and PP after drying processes, but increased (P <0.001) in WP. Results indicate that drying processes generally decrease antioxidants in the finished product, with FD and RW retaining the highest concentration of antioxidants.

INTRODUCTION

Potatoes are the leading vegetable crop in the United States, both in sales and in consumption, with 68% of potatoes sold used in processed products (*1*). The genetic diversity of potatoes is extensive (*2*). Thousands of years of cultivation in the Andes has led to the development of potatoes with yellow, orange, red, purple and blue skin and flesh, in addition to

the common white potato cultivars (*3*). These different cultivars are rich in phenolic acids, anthocyanins and carotenoids (*4, 5*). Diets rich in these types of antioxidants are associated with a lower incidence of chronic diseases such as cardiovascular disease and cancer (*6, 7*).

Based on consumption patterns and antioxidant capacity, potatoes represent the greatest source of antioxidants in the U.S. diet (*8*). Therefore, it is important to retain their nutritional components (*9*), including carotenoids and polyphenols that promote health. Many bioactive compounds in fruits and vegetables are readily destroyed during food processing due to long periods of elevated temperature and exposure to oxygen (*10, 11*). Most antioxidants are easily oxidized, resulting in the loss of biological function. Drying processes that minimize heating time, temperature and oxygen exposure may provide food with the highest content of bioactive compounds.

Drying has been an effective method of food preservation for hundreds of years (*12*). With current food processing technologies, large-scale drying methods have been developed that strive to retain the nutritional value of the preserved food. However, during the drying process, many quality-related properties tend to change; these include the structural, optical, textural, thermal, sensory, rehydration and nutritional aspects of the food (*13*).

Drum (DD) and freeze drying (FD) are commonly used in the food industry to produce dehydrated products. Drum drying is a type of contact drying process in which fluids, suspensions, pastes, or purees are typically dried by contact with condensing steam in a revolving drum container (*14*). Due to their versatility and lower cost, drum drying is widely used in the food industry. They are particularly beneficial for the drying of heat-sensitive foodstuffs because contact with high temperatures (120-170 °C) is short (seconds). The final

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dried product is typically a powder, flake or chip. Therefore, drum drying is commonly used for potato flake production.

Freeze drying is a multi-step process that employs freezing, sublimination and desorption (*15*). Food is frozen to solidify water, then atmospheric pressure is lowered and low-heat (40 ºC) applied to cause sublimination. Sublimination occurs when frozen liquid in the foodstuff is directly transformed from the solid to the gas phase, bypassing the liquid phase. Desorption, or secondary drying, is the final step that involves heating the foodstuff under vacuum to remove bound water. Freeze drying is a novel drying method because there is minimal damage to heat-labile nutrients in the foods; the however, high cost associated with FD is a major disadvantage.

Refractance Window™ drying (RW) is a relatively new drying technology developed in response to consumer interest in retaining biologically active compounds (*9*). This method is carried out by spreading a thin film of food slurry on a conveyor belt made of a transparent polyester material. The slurry is thermally heated as it moves along the conveyor belt, resulting in rapid drying of the product. The RW drying is similar to drum drying, but at lower temperatures (95-97 ºC) and without direct contact with the heat source. Actual product temperatures average 70 ºC during processing. Equipment cost is one-third to one-half lower and energy cost is less than half compared to FD. The RW method also retains most of the vitamins and antioxidants in the final product compared to other methods that use higher temperatures, such as drum drying (*16*). Factors that enable the retention of food components obtained from RW include short drying times, low product temperatures and minimal product-oxygen interaction.

Studies of nutritive retention in processed foods have demonstrated that FD is the best

drying method to retain antioxidants (*16, 17, 18*); however, some antioxidants may be lost due to longer drying times (18-24 h) (*17*). The RW drying of fruits and vegetables has been shown to retain vitamin C and carotene concentrations similar to FD, while DD decreased more of these antioxidants compared to the other drying methods (*16*). On the other hand, thermal processing at temperatures of 115 °C has been shown to increase the amount of antioxidants in a dried product compared to raw, due to the release of bound phenolic acids (*19*). High temperatures used in blanching and DD may increase total phenolics and thereby increase total antioxidant activities in fruits and vegetables. The RW drying method, with product temperatures no higher than 70 °C, has been shown to an increase the release of phenolic compounds bound in the cell matrix (*17*). Additionally, the loss of vitamin C from processed foods may not be detrimental to the nutritive value of the food because vitamin C activity has been reported to contribute < 2% of the total antioxidant activity in common fruits and vegetables (*19, 20*). The final nutritive value of a processed food can be affected by the type and concentration of vitamins and phytochemicals present in the food, as well as the temperature, time, and oxygen exposure.

The objective of this study is to compare DD, FD and RW drying of white, yellow, red and purple potatoes on the retention of bioactive compounds including total antioxidant activity, phenolics, anthocyanins and carotenoids. To our knowledge, this is the first study to address the effects of different dehydration processes on bioactive molecules from colored potato cultivars.

MATERIALS AND METHODS

Sample Preparation. Potato cultivars used in this study included white- (WP) (Ranger Russet), yellow- (YP) (PORO3PG6-3), red- (RP) (PORO4PG14-2) and purple-flesh potatoes

(PP) (PORO4PG82-1) (*Solanum tuberosum* L.) obtained from USDA-ARS (Pomeroy, WA). The potatoes were processed by using the DD, FD and RW methods.

Whole potatoes (8 kg) were randomly selected, washed, abrasively peeled (1.5 min), cut into 6 mm thick slices, and steam blanched for 8 min at 133 °C by pressurized (207 kPa) steam. The potatoes were rapidly cooled in an ice-water bath for 8 min, then mixed in a Hobart mixer (The Hobart Mfg. Co., Troy, OH) to a uniform consistency by adding 0.166 kg recycled potato water/kg blanched potato for the DD and FD methods, and 1.45 kg water/kg for the RW method. The potato slurries were immediately frozen at -35 °C until processed. On the day of processing, potato slurries were thawed and mixed well before aliquots were used for each processing method.

Drum drying using pressurized steam (147 °C, 345 kPa) was performed with a drum gap setting of 0.3 mm and a drum revolving speed of 1.13 rpm. Potato slurries were freeze dried at 20 °C and 60 mTorr for 41.25 h. The RW drying was conducted at MCD technologies (Tacoma, WA). Frozen slurries were transported to the plant, thawed and homogenized (Silverson L4RT-A, 4500 rpm). Additional water was added to the white, red and purple slurries to attain the proper consistency for drying. The final water:potato ratios (wt/wet wt) for RW were 2.08 kg water/kg white potato, 1.45 kg water/kg yellow potato, 2.15 kg water/kg red potato and 1.80 kg water/kg purple potato. The RW drying was performed with a circulating water temperature of 95 °C and an average belt time of 5 min.

Potato slurries and dried products were analyzed in triplicate for antioxidant activity, phenolic acid, anthocyanin and carotenoid content. Gallic acid, trolox, 2-2'-diphenyl-1picryl-hydrazyl (DPPH), KCl, and Folin-Ciocalteu phenol reagent were obtained from Sigma-Aldrich

Co. (St. Louis, MO). Sodium acetate ($CH_3CO_2Na\cdot3H_2O$) and sodium carbonate (Na_2CO_3) were obtained from J. T. Baker (Phillipsburg, NJ).

Dry Matter Content. Potato slurries were used to determine dry matter content by drying in a 100 °C oven (Precision Scientific, Winchester, VA).

Total Phenolics. Phenolic acids were quantified as previously described (*21*) with modifications. Briefly, phenolic acids were extracted by homogenizing (OMNI International, Waterbury, CT) 2.45 g potato slurry or 1 g dried potato flakes in 10 mL of a 1:1 (v/v) mixture of water and methanol, and centrifuging at 16000 $\times g$ for 20 min at 4 °C. To 0.5 mL of the supernatant, 8 mL water and 0.5 mL Folin-Ciocalteu reagent (0.25 N) were added and vortexed. The mixture was allowed to react for 3 min, then 1 mL Na_2CO_3 (1 N) was added and the mixture incubated for 2 h at RT. Absorbance was measured at 725 nm (Beckman DU 640B, Seattle, WA) and total phenolic concentration was expressed as gallic acid equivalent (GAE).

Total Anthocyanins. Total anthocyanin content was determined by the pH differential method as previously described (*22*, *23*) with modifications. Briefly, 2.45 g potato slurry or 1 g dried potato flakes were homogenized in 20 mL of an 85:15 (v/v) mixture of 95% ethanol and 1.5 N HCl. The tubes were incubated for 90 min at 4 °C and then centrifuged at 14000 $\times g$ for 15 min at 4 °C. Five mL of the supernatant was added to 45 mL 0.025 M KCl (pH 1.0) or 0.4 M $CH_3CO_2Na\cdot3H_2O$ (pH 4.5), and equilibrated for 15 min. Absorbance was read at 535 and 700 nm, and total anthocyanin concentration expressed as malvidin-3-*p*-coumarylglycoside equivalents.

Total Carotenoids. Dried potato flakes (0.2 g) were reconstituted in 1 mL deionized distilled water, topped with nitrogen gas and stored overnight at 4 °C in the dark. Carotenoids

were extracted by adding 5 mL of acetone to the mixture, vortexing for 30 min on a multi-tube vortexer (Scientific Manufacturing Industry model 2601, Emeryville, CA), and centrifuging at 1000 $\times g$ for 15 min at 4 °C. This extraction was repeated twice. The acetone was pooled and dried under nitrogen gas at 40 °C. Potato slurries (3 g) were extracted with 15 mL acetone in a similar manner. The dried residues were dissolved in 1 mL (WP, RP, and PP) or 3 mL (YP) acetone. Absorbance was read at 444 nm. Total carotenoids were calculated using the extinction coefficient for lutein, and expressed as µg total carotenoid/g dry matter (*24*).

Total Antioxidant Activity. Total hydrophilic antioxidant activity (TAA) was analyzed as previously described with modifications (*25*). Antioxidants were extracted by blending 2.45 g of potato slurry or 1 g of dried potato flakes in 10 mL of a 1:1 (v/v) mixture of methanol and H_2O until uniform. The mixture was centrifuged at 16000 $\times g$ for 20 min at 4 °C. The supernatant was collected and 25 µL added to 975 µL DPPH in methanol (6×10^{-5} mol/L), incubated for 120 min, and absorbance read at 515 nm. The TAA was expressed as trolox equivalents.

Statistical Analysis. Total phenolics, anthocyanins, carotenoids and antioxidant activity in slurries and with DD, FD and RW drying methods were analyzed by ANOVA using the General Linear Models procedure of SAS (version 8; SAS Institute, Cary, NC). Statistical significance was set at $P < 0.05$.

RESULTS

The purpose of this study is to compare the effect of different drying methods on the retention of total antioxidant activity, phenolic acids, anthocyanins and carotenoids in white, yellow, red, and purple potato cultivars. Results using these drying methods were compared to

the potato slurries. Antioxidant concentrations are expressed on a dry weight basis; the dry matter content of the different potato cultivars is presented in **Table 1**.

Total phenolics. The effects of processing methods (FD, DD and RW) on total phenolic concentrations are shown in **Figure 1**. Except with YP, processing did not significantly influence the total phenolic acid content. Both FD and RW decreased (P <0.01) phenolic acids in YP. Interestingly, DD increased (P <0.001) total phenolics in WP.

Total anthocyanins. Total anthocyanins were low to undetectable in WP and YP, and were highest in PP (**Figure 2**). All drying methods (FD, DD and RW) significantly decreased *(P* <0.001) anthocyanin concentrations in RP and PP (**Figure 2**). In these potato cultivars, FD retained the highest *(P* <0.001) amount of anthocyanins, and was generally similar to RW. The DD method produced the greatest loss of total anthocyanins, with a 67 and 58 % loss in RP and PP, respectively (**Table 2**).

Total carotenoids. Carotenoids were low to undetectable in WP, RP and PP, and were high in YP (**Figure 3**). In YP, a decrease (P <0.01) in total carotenoids was observed after all drying processes (FD, DD, and RW). Concentrations in YP were 28 to 45-fold higher than in all other potato cultivars. Drying generally decreased (P <0.01) total carotenoid content by 52, 31, and 33% with DD, FD and RW, respectively (**Table 2**).

Total antioxidant activity. The TAA in YP, RP and PP decreased (P <0.001) with all drying methods (**Figure 4**). Losses ranged between 50-71%, with no significant differences observed among the three drying methods. In WP, DD, FD, and RW increased (P <0.001) TAA by 108, 75, and 58% (**Table 2**).

Table 3-1. Dry matter content of potato cultivars.

Potato cultivar	Dry matter, %
White	22.1
Yellow	15.2
Red	17.2
Purple	15.9

Figure 3-1. Total phenolics (g/kg).
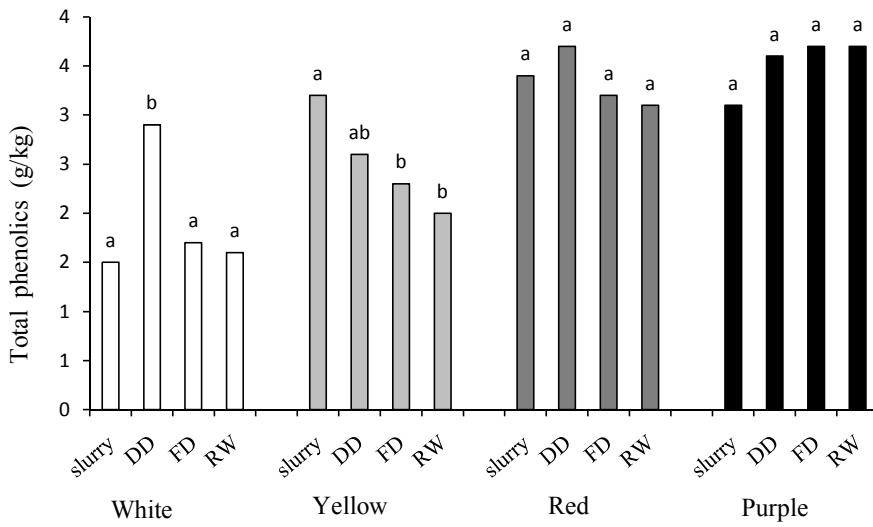
a, b Different symbols above each bar denote significant difference in antioxidant retention among each drying method within potato cultivars (*P* < 0.01).

Figure 3-2. Total anthocyanins (g/kg).
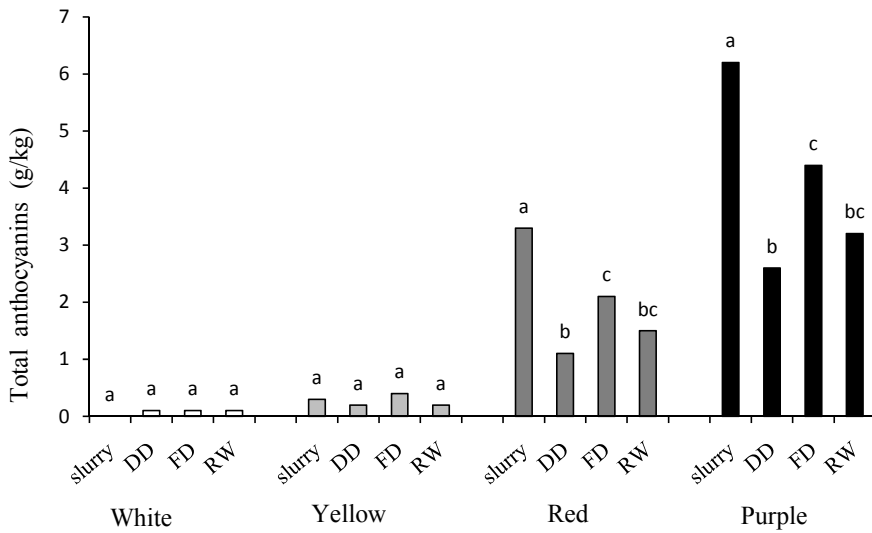
a, b Different symbols above each bar denote significant difference in antioxidant retention among each drying method within potato cultivars ($P < 0.001$).

Table 3-2. Antioxidant profiles (mean ± SE) in white (W), yellow (Y), red (R), and purple (P) potato cultivars when subjected to drum drying (DD), freeze drying (FD), and Refractance Window™ drying (RW)

| Potato cultivar	Processing method	Total phenolics[2]		Total anthocyanins[2]		Total carotenoids[3]		Total antioxidant activity[2]	
W	Slurry	1.5 ± 0.1 ^{a}	(100%)	0.0 ± 0.0 ^{a}	(100%)	1.3 ± 0.0 ^{a}	(100%)	1.2 ± 0.0 ^{a}	(100%)
W	DD	2.9 ± 0.3 ^{b}	(+93%)[1]	0.1 ± 0.0 ^{a}	(------)	1.4 ± 0.3 ^{a}	(+8%)	2.5 ± 0.1 ^{b}	(+108%)
W	FD	1.7 ± 0.2 ^{a}	(+13%)	0.1 ± 0.0 ^{a}	(------)	1.6 ± 0.6 ^{a}	(+23%)	2.1 ± 0.2 ^{c}	(+75%)
W	RW	1.6 ± 0.2 ^{a}	(+7%)	0.1 ± 0.1 ^{a}	(------)	2.3 ± 0.1 ^{a}	(+77%)	1.9 ± 0.1 ^{d}	(+58%)
Y	Slurry	3.2 ± 0.1 ^{a}	(100%)	0.3 ± 0.1 ^{a}	(100%)	58.1 ± 12.3 ^{a}	(100%)	5.5 ± 0.0 ^{a}	(100%)
Y	DD	2.6 ± 0.3 ^{ab}	(-19%)	0.2 ± 0.0 ^{a}	(-33%)	27.8 ± 5.9 ^{b}	(-52%)	2.4 ± 0.1 ^{b}	(-56%)
Y	FD	2.3 ± 0.4 ^{b}	(-28%)	0.4 ± 0.1 ^{a}	(+33%)	39.8 ± 0.2 ^{b}	(-31%)	2.3 ± 0.1 ^{b}	(-58%)
Y	RW	2.0 ± 0.4 ^{b}	(-38%)	0.2 ± 0.0 ^{a}	(-33%)	39.1 ± 0.1 ^{b}	(-33%)	2.3 ± 0.1 ^{b}	(-58%)
R	Slurry	3.4 ± 0.1 ^{a}	(100%)	3.3 ± 0.3 ^{a}	(100%)	2.1 ± 0.3 ^{a}	(100%)	4.8 ± 0.0 ^{a}	(100%)
R	DD	3.7 ± 0.2 ^{a}	(+9%)	1.1 ± 0.1 ^{b}	(-67%)	2.2 ± 0.1 ^{a}	(+5%)	2.4 ± 0.1 ^{b}	(-50%)
R	FD	3.2 ± 0.2 ^{a}	(-6%)	2.1 ± 0.2 ^{c}	(-36%)	2.8 ± 0.2 ^{a}	(+33%)	2.4 ± 0.1 ^{b}	(-50%)
R	RW	3.1 ± 0.1 ^{a}	(-9%)	1.5 ± 0.1 ^{bc}	(-55%)	2.6 ± 0.2 ^{a}	(+24%)	2.3 ± 0.1 ^{b}	(-52%)
P	Slurry	3.1 ± 0.1 ^{a}	(100%)	6.2 ± 0.1 ^{a}	(100%)	1.3 ± 0.2 ^{a}	(100%)	8.4 ± 0.0 ^{a}	(100%)
P	DD	3.6 ± 0.3 ^{a}	(+16%)	2.6 ± 0.3 ^{b}	(-58%)	1.9 ± 0.1 ^{a}	(+46%)	2.5 ± 0.0 ^{b}	(-70%)
P	FD	3.7 ± 0.6 ^{a}	(+19%)	4.4 ± 0.2 ^{c}	(-29%)	2.0 ± 0.0 ^{a}	(+54%)	2.4 ± 0.0 ^{b}	(-71%)
P	RW	3.7 ± 0.7 ^{a}	(+19%)	3.2 ± 0.9 ^{bc}	(-48%)	2.2 ± 0.1 ^{a}	(+69%)	2.4 ± 0.0 ^{b}	(-71%)

[1] Percent increase (+) or decrease (-) when compared to the corresponding potato.
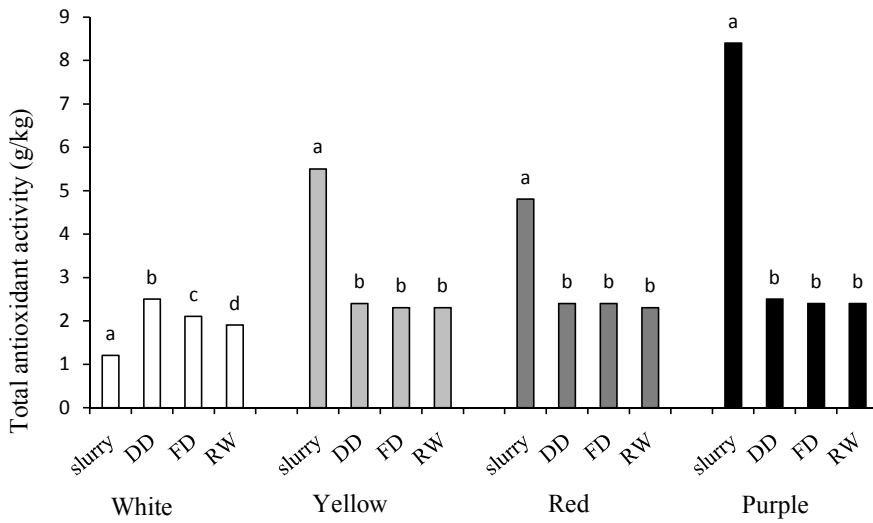
[2] g/kg DW.

[3] mg/kg DW.

[a, b] Different symbols denote significant differences between drying methods within a potato cultivar ($P < 0.01$).

Figure 3-3. Total carotenoids (mg/kg).
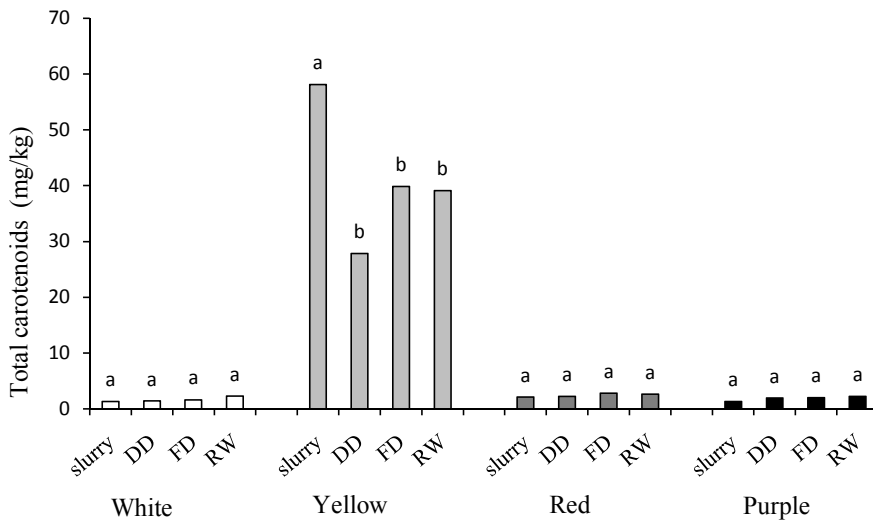
[a, b] Different symbols above each bar denote significant difference in antioxidant retention among each drying method within potato cultivars ($P < 0.01$).

Figure 3-4. Total antioxidant activity (g/kg).

[a, b] Different symbols above each bar denote significant difference in antioxidant retention among each drying method within potato cultivars ($P < 0.001$).

DISCUSSION

Retaining the nutritional quality of processed foods is important in promoting health. Because processed potatoes constitute a major portion of total potato sales, using processing methods that maximize the retention of important bioactive molecules that provide health benefits becomes a major consideration. Indeed, bioactive compounds such as phenolic acids, anthocyanins and carotenoids have been reviewed for their health benefits (*26, 27, 28*). Unfortunately, antioxidants in whole-foods are easily destroyed during processing due to long periods of elevated temperature and exposure to oxygen (*10, 11*). We compared the effects of DD, FD and RW drying methods on antioxidant retention in white, yellow, red and purple potato cultivars. Overall, nutrient retention due to processing is largely dependent on the class of antioxidants present.

Concentrations of total phenolics in the 4 potato cultivars generally agreed with other published values (*5*). Andre et al. (*5*) reported total phenolics in white, yellow, red and purple cultivars to range between 1.1 and 12.4 mg/g DW. The purple-flesh cultivar (Guincho Negra) they reported had a 3-fold greater total phenolic concentration compared to the PP in this study. This difference may be due to the difference in cultivars; year and growing location also can influence total phenolic content (*29*).

Except with YP, processing generally did not influence the total phenolic content of potatoes. In fact, DD increased the total phenolic content of WP. This is likely due to the release of cell matrix-bound phenolic acids (*17, 19*) or the production of Maillard reaction antioxidants (30, 31), both of which are associated with higher processing temperature. Differences in

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extraction efficiency of phenolics may contribute to this difference; however, this is unlikely because no similar effect was observed with YP, RP or PP.

Regardless of the method, processing generally decreased the total anthocyanin content of potatoes, with DD being the most destructive. The RW drying seems to retain as much anthocyanins as FD. Therefore, retention of anthocyanins is inversely related to the processing temperature.

Total anthocyanins averaged 3-6 g/kg DW in RP and PP, and were very low to non-detectable in WP and YP. These are similar to values reported by others (*32*, *33*). Yurdugül (*34*) reported a 5% loss in anthocyanins with freeze drying strawberries. Loss of anthocyanins in RP (36%) and PP (29%) was generally greater than reported with strawberries.

Carotenoids are also heat-labile antioxidants (*35*). Significant amounts of total carotenoids were found only in YP, where concentrations averaged 41 mg/kg DW. These values are similar to others (*32*). Andre et al. (*32*) reported a yellow-flesh cultivar (Amarilla del Centro) to have 54.8 µg/g DW, which is slightly higher than our report of 39.8 µg/g DW (FD). The total carotenoid in a purple-flesh potato used in a study (*32*) was 10-fold greater than that in PP in this study. Total carotenoids in YP decreased by 52, 31 and 33% in DD, FW and RW drying, respectively. In a similar study, total carotene in carrots was decreased by 56% after DD, but only by 4% with FD and 9% with RW (*16*). Both studies showed that high-heat methods such as DD decreased carotenoid concentrations more dramatically than FD or RW, indicating that FD or RW are more suitable drying methods to retain maximal carotenoid content.

The TAA assay used in this study measured total hydrophilic antioxidants. The TAA values in this study are similar to others (*36*). Lachman et al. (*36*) found purple-flesh cultivars to

have TAA ranging from 2.2 to 2.7 mg/g DW, whereas concentrations in PP in this study averaged of 2.4 mg/g DW. The TAA concentration in YP in this study was 2-fold greater than the yellow-flesh cultivars reported by others (*36*).

The TAA content was higher in pigmented potatoes than in WP, with PP having the highest TAA content. However, all three processing methods decreased TAA in YP, RP and PP by 50-71%. Interestingly, TAA was higher in WP, with all 4 processing methods, with DD showing a 108% increase over the slurry. This may be explained by the increased total phenolic content in WP with DD. However, this relationship was not observed with pigmented potatoes, suggesting a contribution to TAA by other hydrophilic antioxidants such as vitamin C. The vitamin C concentration in white, yellow and purple potato cultivars have been reported to be similar (*5*). Therefore, it is possible that the higher TAA in processed WP is due to bioactive molecules such as Maillard reaction products (*30, 31*), which were not analyzed in this study. The high TAA in RP and PP is likely due to the high total phenolics and anthocyanin content in these potato cultivars; TAA is positively correlated with total phenolics and anthocyanin content in potatoes (*37*).

Overall, processing decreased bioactive compounds in potatoes. The degree of destruction is largely dependent on the type of bioactive compound present. Freeze drying was least destructive but is generally not cost effective. The RW method appears to be the most cost-effective option regarding maximal retention of bioactive compounds.

REFERENCES

(1) Lucier, G.; Pollack, S.; Ali, M.; Perez, A. Fruit and vegetable backgrounder. USDA **2006**, 1-56.

(2) Lehesranta, S. J.; Davies, H. V.; Shepard, L. V.; Nunan, N.; McNicol, J. W.; Auriola, S.; Koistinen, K.M.; Suomalainen, S.; Kokko, H. I.; Kärenlampi, S. O. Compairison of tuber proteomes of potato varieties, landraces, and genetically modified lines. *Plant Physiol*. **2005**, *138*, 1690-99.

(3) Talbert, W. F. History of Potato Processing. In *Potato Processing*, 4th; Talbert, W., Smith, O., Eds.; Van Nostrand Reinhold Company Inc.: New York, NY, 1987; pp 1-9.

(4) Brown, C. R.; Culley, D.; Bonierbale, M.; Amorós, W. Anthocyanin, carotenoid content, and antioxidant values in native South American potato cultivars. *HortScience* **2007**, *42*, 1733-1736.

(5) Andre, C. M.; Ghislain M.; Bertin, P.; Oufir, M.; Herrera, M.; Hoffman, L.; Hausman, J.; Larondelle, Y.; Evers, D. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *J. Agric. Food Chem.* **2005**, *55*, 366-378.

(6) Galassetti, P.; Pontello, A. Dietary effects on oxidation of low-densitlipoprotein and atherogenesis. *Curr. Atheroscler. Rep.* **2006** *8*, 523-29.

(7) Gonzalez, C. A. Nutrition and cancer: the current epidemiological evidence. *Br. J. Nutr*. **2006**, *96*, 42S-45S.

(8) Chun, O. K.; Kim, D. O.; Smith, N.; Schroeder, D.; Han, J. T.; Lee, C. Y. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *J. Sci. Food Agric*. **2005**, *85*, 1715-1724.

(9) Nindo, C. I.; Tang, J. Refractance Window™ dehydration technology: a novel contact drying method. *Drying Technology* **2007**, *25*, 37-48.

(10) Rodriguez-Amaya, D. B. Changes in carotenoids during processing and storage of foods. *Arch. Latinoam. Nutr*. **1999**, *49*, 38-S-46-S.

(11) Sadilova, E.; Carle, R.; Stintzing, F. C. Thermal degradation of anthocyanins and its impact on color and *in vivo* antioxidant capacity. *Mol. Nutr. Food Res*. **2007**, *51*, 1461-1471.

(12) Barbosa-Cánovas, G. V.; Vega-Mercado, H. Introduction to dehydration of food. In *Dehydration of foods*; Barbosa-Cánovas, G. V., Vega-Mercado, H., Eds; International Thomson Publishing: New York, NY, **1996**; pp 1-7.

(13) Krokida, M.; Maroulis, Z. Quality changes during drying of food materials. In *Drying Technology in Agriculture and Food Sciences;* Mujumdar, A. S., Ed; Science Publishers, Inc.: Enfield, NH, **2000**; pp 61-97.

(14) Oakley, D. Contact driers. In *Industrial drying of foods*; Baker, C. G., Ed; Blackie Academic and Professional: London, UK, **1997**; pp 115-132.

(15) Snowman, J. W. Freeze driers. In *Industrial drying of foods*; Baker, C. G., Ed; Blackie Academic and Professional: London, UK, **1997**; pp 134-155.

(16) Abonyi, B. I.; Feng, H.; Tang, J.; Edwards, C. G.; Chew, B. P.; Mattinson, D. S.; Fellman, J. K. Quality retention in strawberry and carrot purees dried with refractance window™ system. *J. Food Sci*. **2002**, *67*, 1051-1056.

(17) Nindo, C. I.; Sun, T.; Wang, S. W.; Tang, J.; Powers, J. R. Evaluation of drying technologies for the retention of physical quality and antioxidants in asparagus (*Asparagus officinalis*, L.). *Lebensm. Wiss. Technol*. **2003**, *36*, 507-516.

(18) Desobry, S. A.; Netto, F. M.; Labuza, T. P. Comparison of spray-drying, drum-drying, and freeze-drying for β-carotene encapsulation and preservation. *J. Food Sci*. **1997**, *62*, 1158-62.

(19) Dewanto, V.; Wu, X; Liu, R. H. Processed sweet corn has higher antioxidant activity. *J. Agric. Food Chem*. **2002**, *50*, 4959-4964.

(20) Eberhardt, M. V.; Lee, C. Y.; Lui, R. H. Antioxidant activity of fresh apples. *Nature,* **2000**, *405*, 903-904.

(21) Swain, T.; Hillis, W. E. The phenolic constituents of *Prunus Domestica* I.—the quantitative analysis of phenolic constituents. *J. Sci. Food Agric.* **1959**, *10*, 63-68.

(22) Fuleki, T.; Francis, F. J. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *J. Food Sci*. **1968**, *33*, 72-77.

(23) Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC Int*. **2005**, *88*, 1269-1278.

(24) Britton, G. General carotenoid methods. *Meth. Enzymol*. **1985**, *111*, 113-149.

(25) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol*. **1995**, *28*, 25-30.

(26) Bendini, A; Cerretani, L; Carrasco-Pancorbo, A; Gómez-Caravaca, A; Segura-Carretero, A; Fernández-Gutiérrez, A; Lercker, G. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade. *Molecules*. **2007**, *12*, 1679-1719.

(27) Prior, R. L.; Wu, X. Anthocyanins: structural characteristics that result in unique metabolic patterns and biological activities. *Free Radic. Res*. **2006**, *40*, 1014-1028.

(28) Chew, B. P.; Park, J. Carotenoid action on the immune response. *J. Nutr*. **2004**, *134*, 257S-261S.

(29) Reddivari, L.; Hale, A. L.; Miller, J. C. Jr. Genotype, location, and year influence antioxidant activity, carotenoid content, phenolic content, and composition in specialty potatoes. *J. Agri. Food Chem*. **2007**, *55*, 8073-8079.

(30) Lingnert, H.; Waller, G. R. Stability of antioxidants from histidine and glucose by the Maillard reaction. *J. Agric. Food Chem*. **1983**, *31*, 27-30.

(31) Nicoli, M. C.; Anese, M.; Parpinel, M. T.; Franceschi, S.; Lerici, C. R. Loss and/or formation of antioxidants during food processing and storage. *Cancer Lett*. **1997**, *114*, 71-74.

(32) Andre, C. M.; Oufir, M.; Guignard, C.; Hoffman, L.; Hausman, J.; Evers, D.; Larondelle, Y. Antioxidant profiling of native Andean potato tubers (*Solanum tuberosum* L.) reveals cultivars with high levels of β-carotene, α-tocopherol, chlorogenic acid, and petanin. *J. Agric. Food Chem.* **2007**, *55*, 10839-10849.

(33) Brown, C. R; Culley, D.; Yang, C. P.; Durst, R.; Wrolstad, R. Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. *J. Amer. Soc. Hort. Sci*. **2005**, *130*, 174-180.

(34) Yurdugül, S. An evaluation of the retention quality characteristics in fresh and freeze-dried alpine strawberries. *Int. J. Food Sci. Technol*. **2008**, *43*, 865-870.

(35) Liaaen-Jensen, S. Basic carotenoid chemistry. In *Carotenoids in health and disease*; Krinsky, N. I., Mayne, S. T., Sies, H., Eds; Marcel Dekker: New York, NY, 2004; pp 1-28.

(36) Lachman, J.; Hamouz, K.; Orsák, M.; Pivec, V.; Dvořak, P. The influence of flesh colour and growing locality on polyphenolic content and antioxidant activity in potatoes. *Sci. Hort*. **2008**, *117*, 109-114.

(37) Reyes, L. F.; Miller, J. C. Jr.; Cisneros-Zevallos. Antioxidant capacity, anthocyanins and total phenolics in purple- and red-fleshed potato (*Solanum tuberosum* L.) genotypes. *Amer. J. Potato Res*. **2005**, *82*, 271-277.

CHAPTER 4

SENSORY EVALUATION OF PIGMENTED FLESH POTATOES (*SOLANUM TUBEROSUM* L.)

ABSTRACT

Pigmented potato cultivars were ranked for overall acceptance and acceptance of aroma, appearance, and flavor by a consumer panel. Potatoes were analyzed for total phenolics, anthocyanins and carotenoids. Concentrations of total phenolics in yellow and purple potato cultivars were 2 fold greater ($P < 0.001$) than in the white potato cultivar. Anthocyanins were low to non-detectable in white and yellow potatoes. The purple potatoes had a 20-fold greater ($P < 0.001$) concentration of anthocyanins than in yellow potatoes. For carotenoids, white and purple potatoes had similar concentrations, while yellow potatoes had a 45-fold greater carotenoids compared to white and purple. Consumers ranked the aroma and appearance of white and yellow potatoes higher than purple potatoes ($P < 0.05$). However, for overall acceptance, no significant differences were observed between the potato cultivars indicating that overall, all the pigmented potatoes (yellow and purple) were not significantly different from the widely-consumed white potatoes. More broadly, these results suggest that consumers may be willing to try pigmented potatoes that are beneficial to health due to their higher antioxidant content.

INTRODUCTION

High intake of fruits and vegetables rich in antioxidants has been linked to a decreased risk in the development of chronic diseases (Wilcox *et al*. 2004). Potatoes are the most commonly consumed vegetable in the U.S. Based on consumption patterns and antioxidant capacity, potatoes are an important source of dietary antioxidants (Chun *et al*. 2005). Potatoes have extensive genetic diversity, which has allowed for the cultivation of yellow-, orange-, red-, purple-, and blue-flesh cultivars (Brown *et al*. 2005a). These cultivars are rich sources of phenolic acids, anthocyanins and/or carotenoids (Brown 2005b; Andre *et al*. 2007).

Phenolic acids, anthocyanins and carotenoids are reported to have multiple beneficial properties, including anti-inflammatory (dos Santos *et al*. 2006; Rossi *et al*. 2003; Lee *et al*. 2004), anti-carcinogenic (Yi *et al*. 2005; Lazzé *et al*. 2004; Park *et al*. 1998) and cardio-protective (Natella *et al*. 2007; Chang *et al*. 2006; Milde *et al*. 2007) effects. Consumption of pigmented potatoes rich in these antioxidants may reduce the risk of chronic diseases.

The increased awareness of the health benefits of antioxidants found in fruits and vegetables has promoted the development of breeding programs designed to enhance flavor, diversify color and increase antioxidant concentrations in potatoes (Peabody 2007). Concentrations of phenolic acids in purple cultivars may be 14 times higher than in yellow cultivars and 33 times higher than in white (Andre *et al*. 2007). Yellow-flesh cultivars are rich in carotenoids, primarily lutein and zeaxanthin (Breithaupt and Bamedi 2002). Yellow-flesh potatoes may contain 10-fold higher concentrations of total carotenoids compared to white and 3 fold higher than purple-flesh potatoes (Brown *et al*. 2005a; Andre *et al*. 2007). Sensory studies suggest that color may affect perception of sensory attributes such as aroma, texture and flavor.

Consumers often associate color with healthy and appealing food products (Baker and Günther 2004). β-Carotene has long been added to products such as cheese to enhance color; more recently lutein has been added for its health benefits related to macular degeneration (Jones *et al*. 2005). Polyphenol antioxidants are presently being added to milk products (Axten *et al*. 2008) and soups (Llorach *et al*. 2005) as a functional food ingredient.

We recently reported (Kaspar *et al*. 2009) that consumption of yellow- or purple-flesh potatoes decreased inflammation and oxidative damage, and modulated immune response in humans. Therefore, pigmented potatoes can contribute significantly to total antioxidant intake, and potentially reduce certain chronic diseases. No studies are available on the sensory attributes of different pigmented potatoes. The purpose of this research is to determine the consumer acceptance of aroma, appearance and flavor of white-, yellow-, and purple-flesh potatoes through ranking. Results will provide potato breeders and consumers with important information on the sensory attribute preferences for pigmented potato cultivars.

MATERIALS AND METHODS

Potato Cultivars

White- (Ranger Russet), yellow- (PORO3PG6-3), and purple-flesh (PORO4PG82-1) potato (*Solanum tuberosum* L.) cultivars grown in Pomeroy, Washington were used in the study.

Antioxidant Composition

Whole potatoes (8 kg) were randomly selected, washed, abrasively peeled (1.5 min), cut into 6 mm thick slices, and steam blanched for 8 min at 133C. The potatoes were rapidly cooled in an ice-water bath for 8 min and mixed to a uniform consistency in a Hobart mixer (The Hobart

Mfg. Co., Troy, OH). The potato slurries were immediately frozen at -35C until assay. Potato slurries were analyzed in triplicate for total antioxidant activity, phenolic acid, anthocyanin and carotenoid content.

Total phenolic acids were quantified as previously described (Swain and Hillis 1959) with modifications. Briefly, phenolic acids were extracted by homogenizing (OMNI International, Waterbury, CT) 2.45 g potato slurry in 10 mL of a mixture of distilled water and methanol (1:1, v/v) and centrifuging at 16000×g for 20 min at 4C. The supernatant was collected and 0.5 mL was diluted with 8 mL water. Next, 0.5 mL Folin-Ciocalteu reagent (0.25 N; Sigma-Aldrich, St. Louis, MO) was added and vortexed. The mixture was allowed to react for 3 min, then 1 mL sodium carbonate (1 N) was added and incubated at RT for 2 h. Absorbance was measured at 725 nm (Beckman DU 640B, Seattle, WA) and total phenolic concentration expressed as gallic acid equivalent (GAE).

Total anthocyanin content was determined by the pH differential method as previously described with modifications (Fuleki and Francis 1968; Lee *et al.* 2005). Briefly, 2.45 g potato slurry was homogenized in 20 mL of a 95% ethanol and 1.5 N HCl mixture (85:15, v/v), incubated for 90 min at 4C, and centrifuged at 14000×g for 15 min at 4C. Five mL of the supernatant was added to 45 mL of 0.025 M potassium chloride buffer (pH 1.0) or 0.4 M sodium acetate buffer (pH 4.5) and equilibrated for 15 min. Absorbance was read at 535 and 700 nm and total anthocyanin concentration expressed as malvidin-3-*p*-coumarylglycoside equivalents.

Carotenoids were extracted by adding 15 mL acetone to 3 g of potato slurry, vortexing 30 min on a multi-tube vortexer (Scientific Manufacturing Industry model 2601, Emeryville, CA), and centrifuging at 1000×g for 15 min at 4C. This extraction was repeated twice. The acetone

was pooled and dried under nitrogen gas at 40C. White and purple potato dried residues were dissolved in 1 mL acetone and yellow potatoes in 3 mL acetone. Absorbance was read at 444 nm. Total carotenoids were calculated using the extinction coefficient for lutein, and expressed as μg total carotenoid/g dry matter (Britton 1985).

Sensory Evaluation

Freshly harvested potatoes were stored at 4°C, 95% RH until required for evaluations. On the day of the sensory study, potatoes were allowed to equilibrate to room temperature for 24h. Potatoes were wrapped in aluminum foil and baked at 204°C in a conventional oven for 105 min as previously described (Boylston *et al.* 2000). After baking, potatoes were divided into 15-20 g pieces and were placed in 3-digit random coded glass containers covered with poly wrap and an elastic band in order to retain moisture and aroma. Before sensory evaluation, potato samples were heated for 30 seconds in a microwave oven to serving temperature (50-55°C). Potato samples were served in random order.

For each attribute, potato cultivars were ranked by 60 untrained panelists (29 males and 31 females, ages 18-62). The Institutional Review Board of Washington State University approved all study procedures. Panelists were asked to rank cultivars in order of acceptance with 1 = most accepted and 3 = least accepted for aroma, appearance, flavor, and overall quality. Unsalted crackers and reagent grade water were provided for cleansing the palate between samples.

Statistical Analysis

Panelist ranking of potato cultivar aroma, appearance, flavor and overall quality were reported as rank sum using Compusense sensory software (version 4.6; Compusense Inc.,

Ontario, Canada). The most preferred samples were represented by the lowest value (sum of panelist responses). Statistical significance was analyzed by Friedman's analysis of ranked sums and means separation was determined by Tukey's HSD. Total phenolics, anthocyanins and carotenoids among cultivars were compared by ANOVA using the General Linear Models procedure of SAS (version 8; SAS Institute, Cary, NC). Differences in treatment means were compared using protected LSD. Statistical significance was established at $P < 0.05$.

RESULTS

The antioxidant concentrations of the potato cultivars are shown in Table 1. Yellow and purple cultivars had the highest concentration of total phenolics (3.2 and 3.1 mg/g, respectively), and concentrations were 2 fold greater ($P < 0.001$) than in the white cultivar (1.5 mg/g). Anthocyanins were not detectable in the white cultivar, and low in the yellow cultivar. The purple potatoes had a 20-fold greater ($P < 0.001$) concentration of anthocyanins than the yellow. White and purple cultivars had the same concentration of carotenoids (1.3 μg/g), while yellow potatoes had a 45-fold greater (58.1 μg/g; $P < 0.001$) concentration of carotenoids compared to both white and purple potatoes.

Sensory rank sum values for sensory attributes of the different potato cultivars are shown in Table 2. Based on aroma and appearance, white and yellow potatoes were ranked as significantly more acceptable ($P < 0.05$) compared to purple potatoes. No significant differences were observed between the potatoes based on flavor acceptance or overall acceptance.

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Table 4-1: Antioxidant concentrations of white-, yellow-, and purple-flesh potatoes

Cultivar	Total phenolics[1]	Total anthocyanins[1]	Total carotenoids[2]
White	1.5 [a]	0.0 [a]	1.3 [a]
Yellow	3.2 [b]	0.3 [a]	58.1 [b]
Purple	3.1 [b]	6.2 [b]	1.3 [a]
Overall SE	0.2	0.2	3.8

[1] g/kg

[2] mg/kg

[a, b] Different letters denote significant difference ($P < 0.001$).

Table 4- 2: Rank sum values for acceptance of aroma, appearance and flavor of baked white-, yellow-, and purple-flesh potatoes as evaluated by the consumer panel (n=60)[1]

Potato cultivar	Aroma	Appearance	Flavor	Overall acceptance
White	110 [a]	100 [a]	127	119
Yellow	108 [a]	109 [a]	115	109
Purple	142 [b]	151 [b]	118	132

[1] The more accepted cultivar is represented by the lower value.

[a, b] Different letters denote significant difference (P < 0.05).

DISCUSSION

The purpose of this study was to determine the acceptability of white-, yellow- and purple-flesh potatoes by consumers, compare the sensory evaluations of the pigmented potatoes to the commonly consumed white potato, and relate antioxidant concentrations to consumer acceptability. To our knowledge, this is the first study on sensory preferences for different pigmented potato cultivars and relating the antioxidant profiles to the sensory preferences.

Based on appearance and aroma, the purple potato was ranked as the least accepted ($P < 0.05$) potato compared to other potatoes. The purple-flesh potatoes had high ($P < 0.001$) concentrations of anthocyanins compared to white and yellow cultivars, which are responsible for purple skin and flesh color. The color of food plays a major role in perception of aroma, flavor and texture: color tends to give the perception of stronger odor intensity in foods when compared to non-colored counterparts (Christensen 1983). The lower ranking of aroma acceptance in the purple potatoes may be due to color perception or to a high concentration of volatile compounds compared to white or yellow potatoes. Volatile concentrations and corresponding aroma values have been shown to significantly differ among white potato cultivars (Oruna-Concha *et al*. 2001), and it is also possible that volatile concentrations differ among pigmented cultivars. However, the concentration of volatiles in the potatoes was not measured in this study. Although purple potatoes had the lowest ranking for appearance and aroma, panelists did not rank the purples potatoes as significantly lower in flavor compared to the white and yellow potatoes.

White potatoes were ranked as the most favorable in appearance. These results may be explained by the familiarity of consumers with white potatoes. Consumers would likely be more familiar with white potatoes compared to pigmented potatoes, thus the rankings of the white potato for aroma and appearance acceptance were more favorable. The pigmented potatoes (yellow and purple) were higher ($P < 0.001$) in total phenolics compared to the yellow potatoes. Yellow potatoes were higher ($P < 0.001$) in total carotenoids and purple potatoes were higher ($P < 0.001$) in total anthocyanins, compared to white potatoes. White Russet-like potatoes are the most commonly consumed potatoes in the U. S. (Chun *et al*. 2005). These data suggest that the aroma and the appearance of the pigmented potatoes may be a deterrent to their acceptability but if consumers actually consume the potatoes, flavor and overall acceptance are not significantly different from the white potato. This result was supported by panelist comments regarding purple potatoes that indicated that the dark purple coloring of potatoes was not considered desirable. This trend may be explained by the relatively recent introduction of colored potatoes into the U.S. market (Stelljes, 2001).

Panelist ranked the appearance of yellow potatoes between white and purple potatoes, with white and yellow potatoes not being significantly different. Comments from panelists suggested that the yellow color potato flesh was positively associated with sweet potatoes, and therefore many panelists found yellow potatoes more acceptable. Sensory research involving carrots has demonstrated that consumers consistently preferred orange and white carrots over purple carrots, when evaluated for sensory attributes similar to those used in this study (Surles *et al*. 2004).

Compared to purple-flesh potatoes, yellow-flesh were ranked as more acceptable for aroma and appearance but not significantly different from white potatoes. Yellow potatoes have the highest amount of carotenoids ($P < 0.001$) compared to the other cultivars, and this may have influenced aroma and flavor. Carotenoids are known to produce aromas via enzymatic and non-enzymatic pathways; volatile compounds have been found in carotenoid-containing fruits, vegetables, and white wines (Winterhalter and Rouseff, 2002). Panelists may have perceived carotenoid-derived aromas from yellow potatoes as more acceptable, thereby ranking them higher than purple potatoes.

CONCLUSION

This study showed that in terms of antioxidant concentration, including total phenolics, anthocyanins and carotenoids, large differences were observed between white, yellow and purple potatoes. When these potatoes were ranked for the acceptance of sensory attributes by a consumer panel, results showed that purple potatoes were ranked as least accepted for appearance and aroma compared to yellow and white potatoes. Since color is known to have an effect on sensory perception of different foods, this may have played a role in the favorable flavor perception of yellow and purple potatoes. However, no significant differences were observed between the three potatoes for flavor and overall acceptance. These results indicated that pigmented potatoes are acceptable to consumers. Therefore, consumption of pigmented potatoes can potentially provide additional health benefits due to the higher content of phenolic acids, anthocyanins and carotenoids.

REFERENCES

ANDRE, C.M., OUFIR, M., GUIGNARD, C., HOFFMANN, L., HAUSMAN, J., EVERS, D. and LARONDELLE, Y.2007. Antioxidant profiling of native Andean potato tubers (*Solanum Tubersosum* L.) reveals cultivars with high levels of β-carotene, α-tocopherol, chlorogenic acid, and petanin. J. Agr. Food Chem. *55*, 10839-49.

AXTEN, L.G., WOHLERS, M.W. and WEGRZYN, T. 2008. Using phytochemicals to enhance health benefits of milk: Impact of polyphenols on flavor profile. J. Food Sci. *73*, H122-26.

BAKER, R. and GÜNTHER, C. 2004. The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. Trends Food Sci. Technol. *15*, 484-88.

BOYLSTON, T.D., POWERS, J.R., WELLER, K.M. and YANG, J. 2001. Comparison of sensory differences of stored Russet Burbank potatoes treated with CIPC and alternative sprout inhibitors. Amer. J. Potato Res. 78, 99-107.

BRITTON, G. 1985. General carotenoid methods. Methods Enzymol. *111*, 113-149.

BREITHAUPT, D.E. and BAMEDI, A. 2002. Carotenoids and carotenoid esters in potatoes (*Solanum Tubersosum* L.): New insights into an ancient vegetable. J. Agric. Food Chem. *50*, 7175-81.

BROWN, C.R., CULLEY, D., YANG, C.P., DURST, R., and WROLSTAD, R. 2005a. Variation of anthocyanin and carotenoid contents and associated antioxidant values in potato breeding lines. J. Am. Soc. Hortic. Sci. *130*, 174-80.

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BROWN, C.R. 2005b. Antioxidants in Potato. Am. J. Potato Res. *82*, 163-172.

CHANG, Y., HUANG, K., HUANG, A., HO, Y. and WANG, C. 2006. *Hibiscus* anthocyanin-rich extract inhibited LDL oxidation and oxLDL-mediated macrophage apoptosis. Food Chem. Toxicol. *44*, 1015-23.

CHRISTENSEN, C.M. 1983. Effects of color on aroma, flavor and texture judgments of foods. J. Food Sci. *48*, 787-90.

CHUN, O.K., KIM, D.O., SMITH, N., SCHROEDER, D., HAN, J.T. and LEE, C.Y. 2005. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. J. Sci. Food Agr. *85*, 1715-24.

DOS SANTOS, M.D., ALMEIDA, M.C. LOPES N.P. and PETTO de SOUZA. 2006. Evaluation of anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol. Pharm. Bull. *29*, 2236-40.

FULEKI, T. and FRANCIS, F J. 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. J. Food Sci. *33*, 72-77.

JONES, S.T., ARYANA, K.J. and LOSSO, J.N. 2005. Storage stability of lutein during ripening of cheddar cheese. J. Dairy Sci. *88*, 1661-70.

KASPAR, K.L., PARK, J., BROWN, C.R., MATHISON, B.D., NAVARRE, R., CHEW, B.P. 2009. Potato consumption on oxidative stress, inflammatory damage and immune response in humans. Amer. J. Clin. Nutr. (submitted).

LAZZÉ, M.C., SAVIO, M., PIZZALA, R., CAZZALINI, O., PERUCCA, P., SCOVASSI, A.I., STIVALA, L.A. and BIANCHI, L. 2004. Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. Carcinogenesis *25*, 1427-33.

LEE, E.H., FAULHABER, D., HANSON, K.M., DING, W., PETERS, S., KODALI, S., GRANSTEIN, R.D. 2004. Dietary lutein reduces ultraviolet radiation-induced inflammation and immunosupression. J. Invest. Dermatol. *122*, 510-517.

LEE, J., DURST, R.W. and WROLSTAD, R.E. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J. AOAC Int. *88*, 1269-78.

LLORACH, R., TOMÁS-BARBERÁN, F.A. and FERRERES, F. 2005. Functionalisation of commercial chicken soup with enriched polyphenol extract from vegetable by-products. Eur. Food Res. Technol. *220*, 31-36.

MILDE, J., ELSTNER, E.F. and GRAβMANN, J. 2007. Synergistic effect of phenolics and carotenoids on human low-density lipoprotein oxidation. Mol. Nutr. Food Res. *51*, 956-61.

NATELLA, F., NARDINI, M., BELELLI, F. and SCACCINI, C. 2007. Coffee drinking induces incorporation of phenolic acids into LDL and increases the resistance of LDL to ex vivo oxidation in humans. Am. J. Clin. Nutr. *86*, 604-9.

ORUNA-CONCHA, M. J., DUCKHAM, S. C., AMES, J. M. Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. J. Agric. Food Chem. *49*, 2414-2421.

PARK, J.S., CHEW, B.P. and WONG, T.S. 1998. Dietary lutein from marigold extract inhibits mammary tumor development in BALB/c mice. J. Nutr. *128*, 1650-56.

PEABODY, E. 2007. Veggies reinvented: Breeding vegetables with more flavor and nutrients. Agr. Res. *55*, 4-7.

ROSSI, A., SERRAINO, I., DUGO, P., DI PAOLA, R., MONDELLO, L., GENOVESE, T.,

    MORABITO, D., DUGO, G., SAUTEBIN, L., CAPUTI, A.C. and CUZZOCREA, S.

    2003. Protective effects of anthocyanins from blackberry in a rat model of acute lung

    inflammation. Free Radic. Res. *37*, 891-900.

STELLJES, K.B. 2001. Colorful potatoes offer nutrition, variety. Agr. Res. *49*, 6.

SURLES, R.L., WENG, N., SIMON, P.W., TANUMIHARDJO, S.A. 2004. Carotenoid profiles

    and consumer sensory evaluation of specialty carrots (*Daucus carota* L.) of various

    colors. J. Agric. Food Chem. *52*, 3417-21.

SWAIN, T. and HILLIS, W.E. 1959. The phenolic constituents of *Prunus Domestica* I.—the

    quantitative analysis of phenolic constituents. J. Sci. Food Agric. *10*, 63-68.

WILLCOX, J.K., ASH, J.K., and CATIGNANI, G.L. 2004. Antioxidants and the prevention of

    chronic disease. Crit. Rev. Food Sci. *44*, 275-95.

WINTERHAULTER, P. and ROUSEFF, R.L. 2002. Carotenoid-derived aromatic compounds:

    an introduction. In *Carotenoid-Derived Aromatic Compounds*, (P. Winterhalter and R.L.

    Rouseff, eds.) pp. 1-13, American Chemical Society, Washington DC.

YI, W., FISCHER, J., KREWER, G. and AKOH, C.C. 2005. Phenolic compounds from

    blueberries can inhibit colon cancer cell proliferation and induce apoptosis. J. Agri. Food

    Chem. *53*, 7320-29.

CHAPTER 5

OVERALL SUMMARY AND CONCLUSION

The main objective of this study was to determine the physiological effects of consuming whole potatoes containing high concentrations of phenolic acids, carotenoids and anthocyanins. Overall, consumption of yellow and purple-flesh potatoes containing high concentration of phenolic acids, carotenoids, and anthocyanins lowered inflammation associated with elevated CRP and IL-6, and lowered oxidative DNA damage.

Potatoes are one of the most commonly consumed vegetables. Typically, potatoes are thought of as a starchy food. This study revealed that there are many physiological health benefits associated with regular pigmented potato consumption; these include decrease in inflammatory damage and oxidative stress that are associated with heart diseases and cancer. Long-term consumption of antioxidant-rich potatoes may decrease the development of these chronic diseases. Our sensory study showed that acceptability of pigmented potatoes by consumers will not be an issue.

Future research with pigmented potatoes may include studies on :1) the oxidative stress, inflammatory and immune parameters after supplementation in at-risk populations, 2) the bioavailability of antioxidants from different potato cultivars, 3) the effects of other bioactive compounds in potatoes, such as angiotensin-converting enzyme (ACE) inhibitors, and 4) the glycemic index of pigmented potato cultivars. **Figure 5-1, Figure 5-2** and **Figure 5-3** are proposed mechanisms of pigmented potato antioxidants on the prevention of chronic diseases.
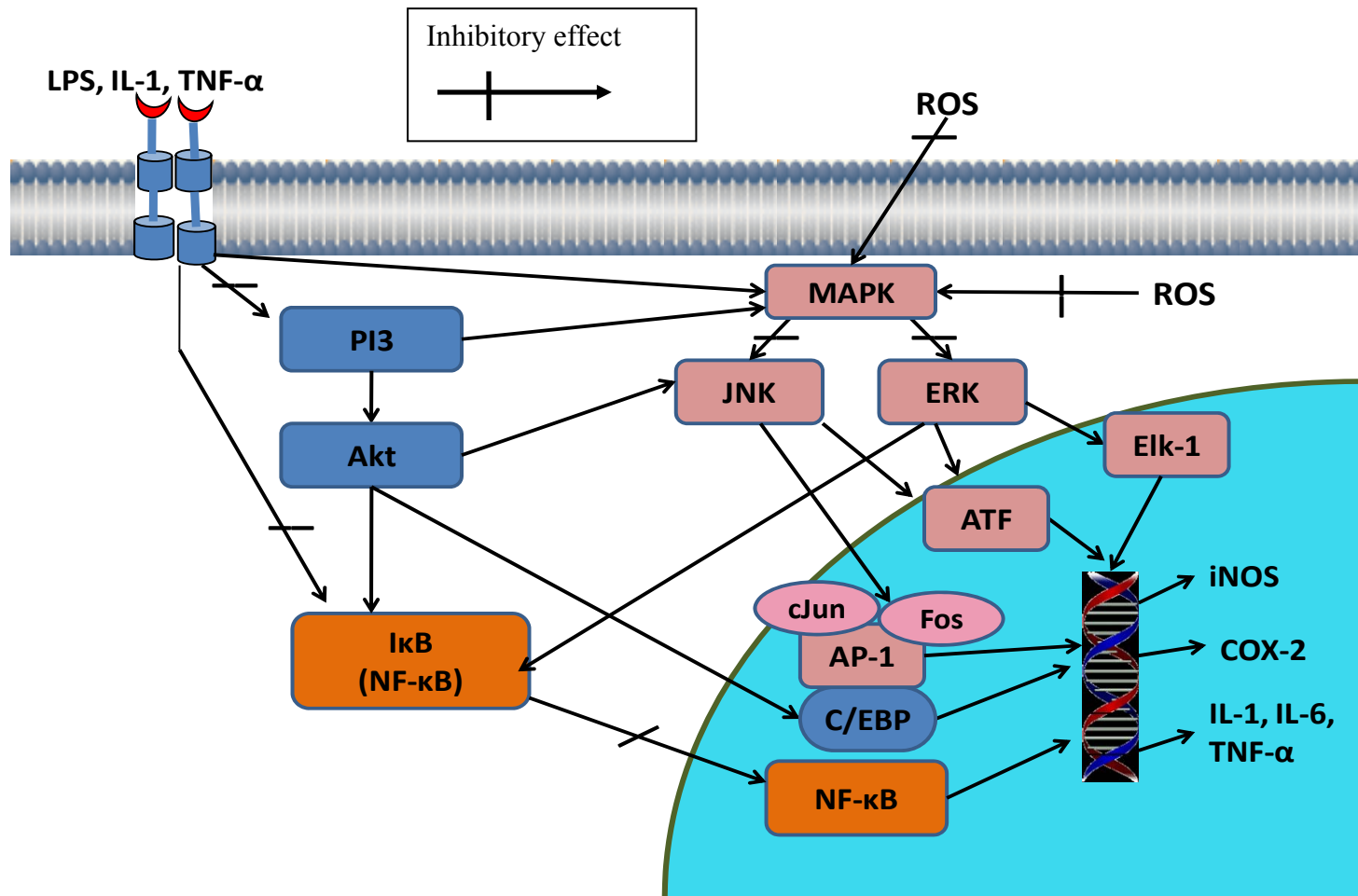
Figure 5-1. Proposed mechanism of yellow and purple potato antioxidants for the prevention of inflammation and oxidative stress.
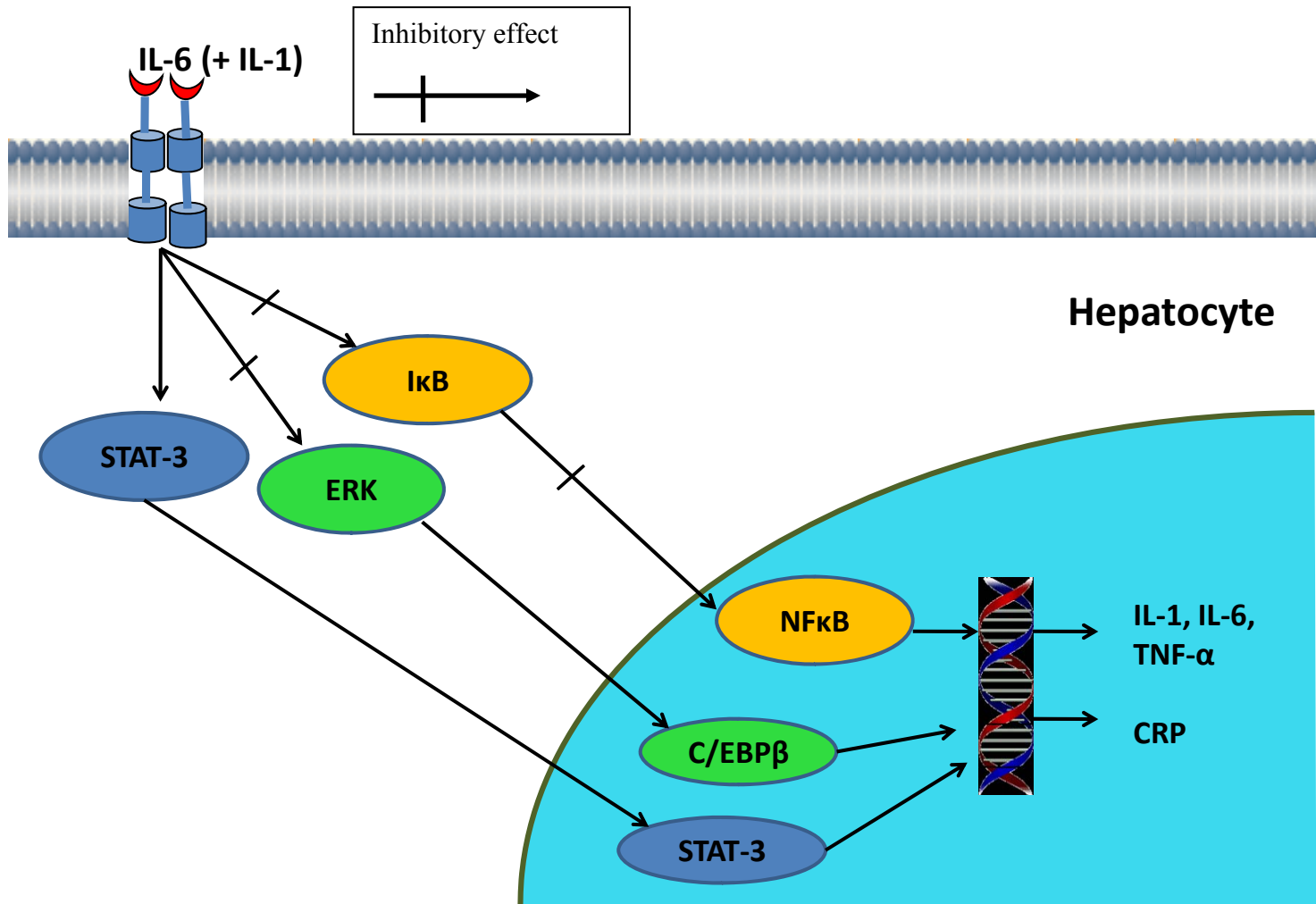
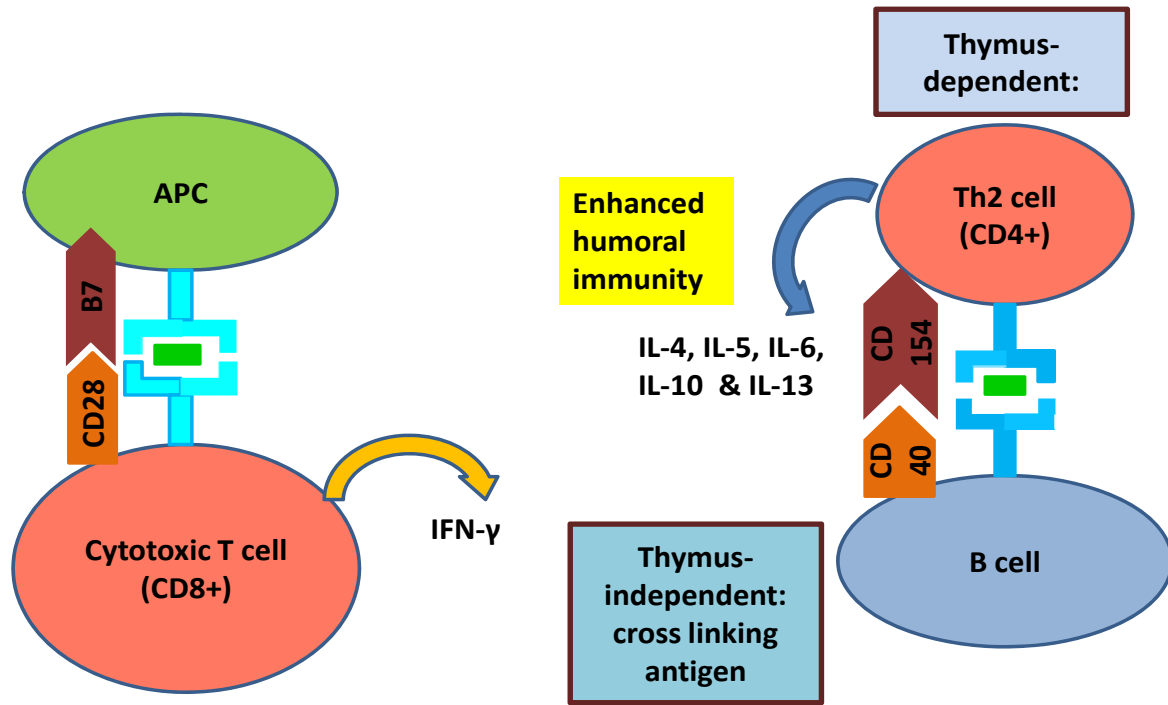**Figure 5-2.** Proposed mechanism of purple potato antioxidants for the prevention of CRP.

Figure 5-3. Proposed mechanism of purple potato antioxidants for the expansion of B cells.