# Chemical Constituents comprising the Antioxidant Activity of Fresh and Dehydrated Saskatoon Berries (*Amelanchier alnifolia* Nutt. cv. Smoky and Thiessen)

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## ABSTRACT

In recent years, numerous studies have shown that the polyphenolics present in fruit and vegetable products exhibit a wide range of protective effects against a variety of disease states, including lower incidences and mortality rates of cancer, reduction in blood pressure, stimulation of the immune system, detoxification of contaminants and pollutants; and reduction in inflammation. This protective effect has been thought to be associated with the antioxidant properties of these polyphenolics present in the fruits and vegetables.

This thesis research specifically dealt with characterizing the antioxidant capacity of a Canadian prairie small fruit, the Saskatoon berry (*Amelanchier alnifolia* Nutt.). Two specific Saskatoon berry varieties (Thiessen and Smoky) were investigated. In addition, differences among four different dehydration techniques (freeze-drying, vacuum-microwave drying, air drying, and combination drying of air and vacuum-microwave drying) were examined. Moreover, a partitioning study was performed to explore the berry constituents responsible for the proposed antioxidant activity. Investigation of the antioxidant capacities of this particular fruit and the characterization of the berry components contributing to the purported antioxidant activity was made. It was found that the Saskatoon berry fruit contained a high capacity for antioxidant activity that was associated with the total anthocyanin and phenolic composition of these berries. The reported antioxidant activity for the berry fruit may be attributed to the reducing property of the polyphenolics; in particular the anthocyanins and phenols which were suggested to act as donors of electrons or hydrogen and thereby terminating free radical chain reactions.

Results showed that a varietal difference existed between the Thiessen and the Smoky Saskatoon berry cultivars examined in this study. Thiessen berries were found to contain higher amounts of total anthocyanin and phenolic constituents and also associated with higher antioxidant capacities when compared to the Smoky variety. No statistically significant differences in total anthocyanin and phenolic content, and antioxidant activities were found for the three different harvest years (2000, 1999, 1998). As a result, the three harvest years were pooled for each variety.

The dehydration processing study performed with the Saskatoon berries showed that a lower retention of antioxidant activity was associated with the loss of anthocyanin pigments and phenolic contents. Greater losses of these compounds were found after those dehydration processes that used longer times and higher heating temperatures. Extracts from freeze-dried Saskatoon berries were found to retain the highest amounts of measured antioxidant activity and measured fruit parameters (anthocyanins, and total phenolics). Extracts from vacuum-microwave dried berries retained the second highest amounts of the measured parameters. The lowest retention of the anthocyanin pigments and phenolic contents, and associated antioxidant capacities, were found in extracts from air-dried berries. The combination method of air drying and vacuum microwave drying was found to provide results in between that of the individual vacuum-microwave dried and air-dried processes themselves.

The solvent fractionation study proved that the polar constituents (e.g. anthocyanins and phenolics) from the Saskatoon berry crude extract demonstrated higher antioxidant activities compared to less polar constituents. Possible synergistic effects of the different components in each of the fractions were also observed.

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| | |
|---|---|
| $a_w$ | Water activity |
| AAPH | 2,2'-azobis (2-amidinopropane) dichloride |
| ABTS | 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) |
| AD | Air dry |
| BBE | Blueberry extract |
| CAT | Catalase |
| CD | Combination dry |
| DPPH | 1,1-diphenyl-2-picrylhydrazyl |
| FF | Fresh frozen |
| FD | Freeze dry |
| GAE | Gallic acid equivalents |
| GSPx | Glutathione peroxidase |
| $L^{\cdot}$ | Allyl radical |
| LDL | Low-density lipoprotein |
| $LO^{\cdot}$ | Alkoxyl radical |
| $LOO^{\cdot}$ | Lipid peroxyl radical |
| LOOH | Lipid hydroperoxides |
| $NO^{\cdot}$ | Nitric oxide |
| $O_2^{\cdot}$ | Superoxide anion |
| $OH^{\cdot}$ | Hydroxyl radicals |
| $ONOO^{\cdot}$ | Peroxylnitrite anion |
| ORAC | Oxygen radical absorbance capacity |
| PE | R-phycoerythrin |
| $RO^{\cdot}$ | Alkoxyl radicals |
| $ROO^{\cdot}$ | Peroxyl radicals |
| ROS | Reactive oxygen species |
| SBE | Saskatoon berry extract |
| SOD | Superoxide dismutase |
| TBARS | Thiobarbituric acid reactive substances |
| VMD | Vacuum microwave dry |

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## INTRODUCTION

Reactive oxygen species (ROS) are essential to metabolic processes, but can also result in damage to cellular material at the molecular level (Rice-Evans and Diplock, 1993). At present, there is overwhelming evidence indicating that ROS species cause oxidative damage to lipids, proteins, and nucleic acids (Wang et al., 1996; Halliwell and Gutterbridge, 1990). Oxidation of proteins, lipids, lipid membranes, and DNA initiate degenerative diseases (Rice-Evans and Diplock, 1993; Yuan et al., 1996). Free radicals have been implicated as being the source of a number of diseases including cancer and atherosclerosis (Ames et al., 1993; Doll, 1990; Gey, 1990; Steinberg, 1991). Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these disease states (Wang et al., 1996). Moreover, in the case of foods, it is necessary to determine the efficacy of antioxidants in protection against oxidative damage, to avoid deleterious changes and loss of commercial and nutritional value (Halliwell, et al., 1995). Hence, the ability of natural antioxidants to react with free radicals is of special interest in both food and biological systems.

The consumption of fruits and vegetables has been associated with lower overall incidence and lower mortality rates from cancer in several human cohort and case-control studies for all common cancer sites (Halliwell and Gutterbridge, 1990; Doll, 1990; Gey et al., 1991; Ames et al., 1993). In addition, consuming fruits and vegetables as part of a daily diet has been reported to reduce blood pressure, stimulate the immune system, detoxify contaminants and pollutants, and reduce inflammation (Ascherio et al., 1992; Sacks and Kass, 1988). The protection that dietary intake of fruits and vegetables may provide against chronic diseases (e.g. cancer and cardio- and cerebrovascular diseases), has been associated with various natural antioxidant constituents commonly found in

many fruits and vegetables (Wang et. al., 1996). Fruits and vegetables contain many different antioxidant components and in some cases, the majority of the antioxidant capacity may be derived from phytochemical compounds other than the commonly known antioxidants such as vitamin C, vitamin E, and β-carotene (Wang et al., 1996; Cao et al., 1997). Some other phytochemicals present in plant tissues responsible for high antioxidant activity include phenolics, anthocyanins, and other flavonoid components (Cao et al., 1997). Flavonoids (including flavones, isoflavones, flavonones, anthocyanins, catechin, and isocatechin) are commonly ingested within a human diet (for example, soy, tea, berries, grains, and fruit sources) and have been demonstrated to possess strong antioxidant activities (Wang et al., 1996; Heinonen et al., 1998; Prior et al., 1998; Kalt et al., 1999a; Rapisarda et al., 1999).

Berries and dried fruits compose a relatively small part of the average diet of man, but represent potentially important sources of antioxidant materials. Highly pigmented berries have the highest antioxidant activity (Miller et al., 2000). Several of the common dehydration processes of berries employed by the industry include air drying, vacuum microwave drying, and freeze drying. Each process of dehydration has unique individual strengths and weaknesses.

Commercialization of Saskatoon fruit has resulted in increased research on various aspects of employment into food products that include production, handling, and processing. However, little information is available on the antioxidant capacity of Saskatoon berries in both fresh or processed dried form. There have been no previous comparative studies on the antioxidative capacity in different varieties of Saskatoon berries and the associated chemical components.

The purpose of this thesis research was to specifically determine the antioxidant capacities of Saskatoon berries and to identify the components of the berry that contribute to this property. In addition, two specific varieties will be assessed to determine any possible varietal differences. Moreover, this thesis will also focus on the effect of different drying processes on the content and stability of antioxidant phytochemicals in the Saskatoon berry extract and the purported antioxidant activities of such extracts. Finally, a partitioning study based on polarity differences was performed to investigate the individual fractions' antioxidant capacity.

## LITERATURE REVIEW

### Saskatoon Berries

#### *Appearance Qualities and Nutritional Content*

The Saskatoon berry (*Amelanchier alnifonia* Nutt.) is native to the southern Yukon and Northwest Territories, the Canadian prairies, and the northern prairie states of the United States (Harris, 1972). The fruit is referred to as a berry, but it is actually in the pome fruit family, closely related to apples and pears (Harris, 1972; Olson and Steeves, 1982). Saskatoon berries have traditionally been a major food source for native peoples and the early settlers of the North American prairies and, until recently, could only be picked in the wild (Mazza, 1986). Saskatoon berries have an appearance of and taste similar to blueberries, but they have a thicker skin and thicker juice. These berries can be used for table purposes or processed very much the same as the blueberry. In the last two decades, there has been increasing interest in the commercial cultivation and utilization of this fruit (Mazza, 1986). The most common cultivars used by growers include Honeywood, Martin, Northline, Pembina, Smoky and Thiessen. Commercialization of Saskatoon fruit has resulted in increased research on various aspects of these fruits, including production, handling, and processing. The estimated value of the 1996 Saskatoon crop of 70 000 kg (155,000 lb) was $0.4 million, up by 33 percent from 1995. (St. Pierre, 2000)

Fresh Saskatoon berries contain 78 to 81% moisture, up to 19% sugar, and small amounts of protein and fat, a fair amount of fiber and relatively large amounts of potassium, iron, magnesium, and aluminum content (Mazza, 1982) (Table 1). Compared to blueberries, Saskatoon berries contain significantly higher amounts of protein, fat,

fiber, calcium, magnesium, barium, and aluminum, but lower phosphorous and sulfur content. There are no significant differences in the content of iron, zinc, sodium, and carotene between the two fruits. Previous researchers in this field have suggested that Saskatoon berries are an excellent source of important micronutrients (e.g. manganese, magnesium, and iron and a relatively good source of calcium, potassium, copper, and carotene) (Mazza, 1986). However, the actual bioavailability of these minerals from Saskatoon berries has not been determined.

It is of special interest that Saskatoon berries do not contain detectable quantities of ascorbic acid (vitamin C) at any stages of maturity (Panther and Wolfe, 1972). This interesting fact of undectectable quantities of ascorbic acid in Saskatoon berries is attributed to oxidation of ascorbic acid to yield dehydroascorbic acid (the oxidized form of ascorbic acid), which is present in the order of 22 to 24 mg/ 100 g fresh weight. Previously, researchers have claimed that the rapid degradation of vitamin C is due to a very active ascorbic acid oxidizing enzyme system present in these berries. Thus, Saskatoon berries are not a good dietary source of vitamin C, as the efficiency of the dehydro form as an antiscorbutic factor (the ability to cure or prevent scurvy due to vitamin C deficiency) is low (Panther and Wolfe, 1972).

The major aroma component of Saskatoon berries is benzaldehyde (Mazza and Hodgins, 1985), which comprises 26 to 168 mg/kg of fresh berries and 76% to 96% of the essence. These results were derived from studies employing colorimetry and gas chromatography-mass spectrophotometry (GC-MS) from seven different cultivars of Saskatoon berries. The predominant acids present in Saskatoon berries are malic and citric acid (Wolfe and Wood, 1971).

**Table 1.** Chemical composition of Saskatoon berries and blueberries.

| Nutrient (% or ppm D.W.B. or mg) | Saskatoon Berry[x] | Blueberry [x, y] |
|---|---|---|
| Water (%) | 80.0 ± 1.5[a] | 83.3[x] - 84.6 [y] |
| Protein (%) | 9.7 ± 1.3 | 0.67[y] - 4.9[x] |
| Fat (%) | 4.2 ± .0.5 | 0.38[y] - 2.4[x] |
| Sugar (%) | 19.0 ± 3.0 | 8.8[x] 14.13[y] |
| Calcium (%) | 0.44 ± 0.06 | 0.006[y] - 0.08[x] |
| Phosphorous (%) | 0.16 ± 0.02 | 0.00017[y] - 1.44[x] |
| Potassium (%) | 1.22 ± 0.16 | 0.005[y] - 0.54[x] |
| Magnesium (%) | 0.20 ± .03 | 0.010[x] - 0.041[y] |
| Sulfur (%) | 0.059 ± .021 | 0.034[x] |
| Iron (ppm or mg) | 67.03 ± 11.65 | 50.15 ppm[x] – 0.017 mg[y] |
| Sodium (ppm or mg) | 31.83 ± 7.65 | 46.01 ppm[x] – 6 mg[y] |
| Manganese (ppm or mg) | 67.50 ± 11.79 | 35.2 ppm[x] – 0.282 mg[y] |
| Copper (ppm or mg) | 7.23 ± 0.70 | 11.4 ppm[x] – 0.061 mg[y] |
| Zinc (ppm) | 16.50 ± 2.78 | 20.6 ppm[x] – 0.11 mg[y] |
| Barium (ppm) | 34.76 ± 4.35 | 4.4[x] |
| Molybdenum (ppm) | 0.38 ± 0.01 | - [b] |
| Aluminum (ppm) | 74.45 ± 13.22 | 26.22[x] |
| Carotene (ppm) | 29.70 ± 5.00 | 20.30[x] |

[x]Adapted from Mazza, 1982
[y]Adapted from USDA
[a] Standard deviation
[b] Not detected

**Anthocyanins**

The anthocyanins (Greek: *anthos* = flower and *kyanos* = blue) include a very large and widespread group of plant constituents collectively known as flavonoids (Mazza and Miniati, 1993). Anthocyanins are water-soluble pigments, which are responsible for

nearly all the red, purple, and blue colours of flowers and fruits (Green and Mazza, 1988). The anthocyanin pigments of Saskatoon berries are important aesthetically and economically because they are located in the skin and flesh of the fruit. The stability of these compounds is of significance for the marketing of fresh berries, as well as the processed products.

Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (Figure 1). Chemical differences between individual anthocyanins consist of the number of hydroxyl groups on the molecule, the degree of methylation of these hydroxyl groups, the nature and number of sugars attached to the molecule and the position of the attachment, and the nature and number of aliphatic or aromatic acids attached to the sugars in the molecule (Mazza and Miniati, 1993).

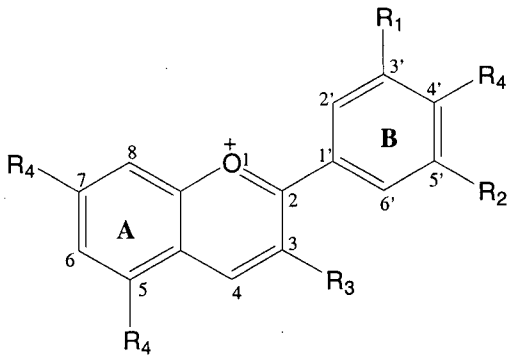



**FIGURE 1**. The flavylium cation. $R_1$ and $R_2$ are H, OH, or $OCH_2$; $R_3$ is a glycosyl or H; and $R_4$ is OH or a glycosyl (Mazza and Miniati, 1993).

Aglycone forms (containing no sugar compounds) of anthocyanins are referred to as anthocyanidins. Naturally occurring anthocyanidins are identified in Table 2. Of these, six occur most frequently in plants. They are pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (Mazza and Miniati, 1993). Since each anthocyanin can be glycosylated and acylated by different sugars and acids, at different positions, the

actual number of anthocyanins can vary fifteen to twenty times greater than the number of anthocyanidins. The sugars most commonly covalently linked to anthocyanins are glucose, galactose, rhamnose, and arabinose.

**TABLE 2.** Naturally Occurring Anthocyanidins. [1]

| | Substitution Pattern | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Name** | **3** | **5** | **6** | **7** | **3'** | **4'** | **5'** | **Color** |
| Apigeninidin (Ap) | H | OH | H | OH | H | OH | H | Orange |
| Aurantinidin (Au) | OH | OH | OH | OH | H | OH | H | Orange |
| Capensinidin (Cp) | OH | OMe | H | OH | OMe | OH | OMe | Bluish-red |
| Cyanidin (Cy) | OH | OH | H | OH | OH | OH | H | Orange-red |
| Delphinidin (Dp) | OH | OH | H | OH | OH | OH | OH | Bluish-red |
| Europinidin (Eu) | OH | OMe | H | OH | OMe | OH | OH | Bluish-red |
| Hirsutidin (Hs) | OH | OH | H | OMe | OMe | OH | OMe | Bluish-red |
| 6-Hydroxycyanidin (6 OHCy) | OH | OH | OH | OH | OH | H | | Red |
| Luteolinidin (Lt) | H | OH | H | OH | OH | OH | H | Orange |
| Malvidin (Mv) | OH | OH | H | OH | OMe | OMe | OMe | Bluish-red |
| 5-Methylcyanidin (5-Mcy) | OH | OMe | H | OH | OH | H | | Orange-red |
| Pelargonidin (Pg) | OH | OH | H | OH | H | OH | H | Orange |
| Peonidin (Pn) | OH | OH | H | OH | OMe | OH | H | Orange-red |
| Petunidin (Pt) | OH | OH | H | OH | OMe | OH | OH | Bluish-red |
| Pulchellidin (Pl) | OH | OMe | H | OH | OH | OH | OH | Bluish-red |
| Rosinidin (Rs) | OH | OH | H | OMe | OMe | OH | H | Red |
| Tricetinidin (Tr) | H | OH | H | OH | OH | OH | OH | Red |

[1] Mazza and Miniati, 1993

In aqueous media, most natural anthocyanins are present as red pigments at low pH, or bluish colored pigments at intermediate pH, and colorless at high pH (Mazza and Miniati, 1993). It has been demonstrated that four anthocyanin structures exist in equilibrium in acidic or neutral media; there being the flavylium cation, the quinoidal base, the carbinol pseudobase, and the chalcone structure (Figure 2). At pH values below 2, anthocyanins exist primarily in the form of the red or yellow flavylium cation. As the pH is raised, a rapid proton loss occurs to give the blue quinoidal forms. On standing, hydration of the flavylium cation occurs to give the colorless carbinol or pseudobase. This in turn, equilibrates to the open chalcone form, which is also colorless. The relative

amounts of cation, quinoidal forms, pseudobases, and chalcones at equilibrium vary with both the solvent pH and structure of the anthocyanins.

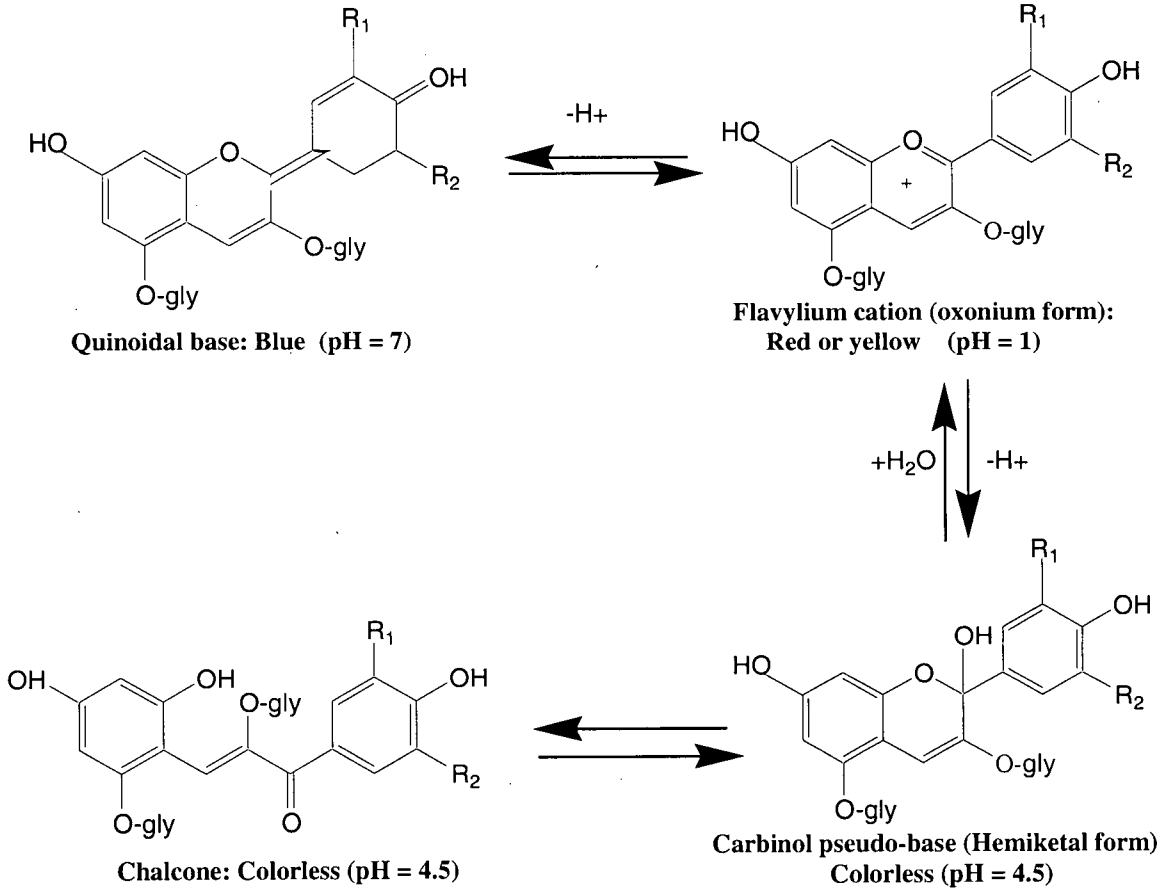



**FIGURE 2:** Predominant structural forms of anthocyanins present at different pH levels (Wrolstad, 2000).

The color of anthocyanin-containing media depends on a multitude of factors including concentration of the pigment, pH, temperature, presence of co-pigments, metallic ions, enzymes, oxygen, co-existing ascorbic acid, sugars and degradation products, sulfur dioxide, and the composition of the anthocyanin (Mazza and Miniati, 1993). Type of cultivar, maturity level of berries, and year of production are additional factors that have an appreciable effect on the content of total anthocyanins and total phenolics in the fruit (Green and Mazza, 1986).

Analytical methods for measuring anthocyanins have been reviewed in detail by many researchers (Francis, 1982; Jackman et al., 1987; Gross, 1987; and Strack and Wray, 1989). Total anthocyanins can be determined by the pH differential method of Fuleki and Francis (1968). The method relies on the structural transformations of the anthocyanin chromophore as a function of pH. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5 (Figure 2). The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds. In addition to measuring the $\lambda_{vis\text{-}max}$ of the sample, measurements were also made at 700nm, to allow for haze correction.

There are at least four anthocyanins in ripe Saskatoon berries, of which cyanidin 3-galactoside (Figure 3A) and cyanidin 3-glucoside (Figure 3B) account for about 61 and 21% of the total anthocyanins, respectively (Green and Mazza, 1986).

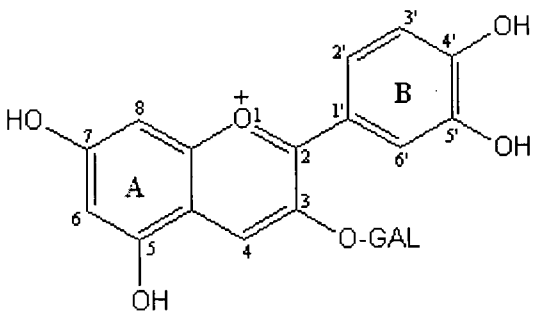



**FIGURE 3A:** Cyanidin 3-galactoside

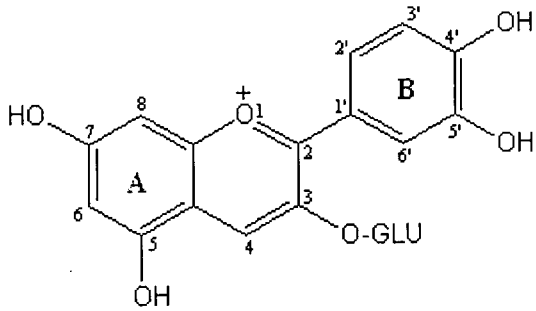



**FIGURE 3B:** Cyanidin 3-glucoside

High levels of anthocyanins in Saskatoon berries have been associated with many factors that correspond to factors known to stabilize anthocyanins in various fruits; these including high total phenolic, total acids and low pH, along with a low sugar:acid

ratio (Green and Mazza, 1986; Markakis, 1982; Daravingas and Cain, 1968; Simard et al., 1982).

Apart from the function of anthocyanins to impart a distinct color in plants including fruits, anthocyanins have also been reported to contribute to antioxidant activity against various free radicals and in oxidative stress (Sarma et al., 1996; Heinonen et al., 1998; Prior et al., 1998; Ghiselli et al., 1998; Kalt et al., 1999b; Wang et al., 1999; Gil et al., 2000; Miller et al., 2000; Connor et al., 2002).

In a recent study, the absorption of anthocyanins in humans has been investigated. These researchers showed that anthocyanins can be absorbed in their intact glycosylated and possibly acylated forms in human subjects and that consumption of blueberries, a small fruit reported to contain high anthocyanin levels and high *in vitro* antioxidant properties, is associated with a diet-induced increase in *ex vivo* serum antioxidant status (Mazza et al., 2002). The appearance of total anthocyanins in the serum in the human subjects was directly correlated with the increase in serum antioxidant capacity.

**Free Radicals and Oxidative Stress**

Free radicals are highly unstable, reactive and energized molecules containing unpaired electrons. Free radicals may be responsible for the etiology or natural history of a number of diseases including cancer, heart, vascular, and neurodegenerative diseases (Halliwell, 1994; Yu, 1994; Steinmetz and Potter, 1996; Garcia-Closas et al., 1999; Joseph et al., 1999; Dillard and German, 2000; Prior and Cao, 2000).

Reactive oxygen species (ROS), free radicals derived from oxygen that cause cellular damage, represent a side effect of normal oxidative metabolism which is essential

for the survival of cells (Halliwell, 1994). Highly reactive free radicals and oxygen species, such as hydroxyl ($OH^{\bullet}$) radicals, peroxyl ($ROO^{\bullet}$) radicals, alkoxyl ($RO^{\bullet}$) radicals, and the superoxide anion ($O_2^{\bullet}$), are continually produced in biological systems from a wide variety of sources (Halliwell and Gutterbridge, 1990). Other common reactive oxygen species produced by the body include nitric oxide ($NO^{\bullet}$) and the peroxylnitrite anion ($ONOO^{\bullet}$) (Prior and Cao, 2000). Free radicals react quickly with other compounds, trying to capture the electrons needed to gain stability. Generally, free radicals attack the nearest stable molecules, and sequester electrons, which results in electron loss in the native compound and its transformation to a free radical, thus beginning a chain reaction.

When an excess of free radicals are formed, endogenous protective enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSPx) are required to neutralize the otherwise causative, destructive and lethal cellular effects (e.g. apoptosis or cell death) of ROS on oxidized membrane lipids, cellular proteins, DNA and enzymes, thus shutting down cellular respiration (Yuan and Kitts, 1996; Halliwell and Gutterbridge, 1990).

Lipid oxidation can affect food quality, and it is a major cause of chemical spoilage (Colbert and Decker, 1991), resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture, and safety of foods (Shahidi et al., 1992).

A general mechanism for free radical oxidation reactions is given in Equations 1-8 (Antolovich et al., 2002). The process may be initiated (Eq 1) by the action of external agents such as heat, light, or ionizing radiation, or by chemical initiation involving metal ions or metalloproteins as well as their combination effects (Cuvelier et al., 1996). From

the mechanism equations LH represents the substrate molecule, for example, a lipid molecule and $R^{\cdot}$ is the initiating oxidizing radical. The oxidation of the substrate generates a highly reactive allyl radical ($L^{\cdot}$), which when reacting with oxygen ($O_2$) forms a lipid peroxyl radical ($LOO^{\cdot}$) (Eq 2). The lipid peroxyl radicals are the chain carriers of the reaction that further oxidize the lipid resulting in production of lipid hydroperoxides (LOOH) (Eq 3). The lipid hydroperoxides in turn break down to a wide range of compounds, including alcohols, aldehydes, alkyl formats, ketones, and hydrocarbons and radicals including the alkoxyl radical ($LO^{\cdot}$) (Eq 4, 5). The breakdown of lipid hydroperoxides often involves transition metal ion catalysis, in reactions analogous to that with hydrogen peroxide, yielding lipid peroxyl and lipid alkoxyl radicals. Termination reactions involve the combination of radicals to form non-radical products (Equations 6, 7, 8).

| | | |
|---|---|---|
| *Initiation:* | $LH + R^{\cdot} \rightarrow L^{\cdot} + RH$ | **(Equation 1)** |
| *Propagation:* | $L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$ | **(Equation 2)** |
| | $LOO^{\cdot} + LH \rightarrow L^{\cdot} + LOOH$ | **(Equation 3)** |
| *Branching:* | $LOOH \rightarrow LO^{\cdot} + HO^{\cdot}$ | **(Equation 4)** |
| | $2\ LOOH \rightarrow LOO^{\cdot} + LO^{\cdot} + H_2O$ | **(Equation 5)** |
| *Termination:* | $LO^{\cdot} + LO^{\cdot} \rightarrow$ | **(Equation 6)** |
| | $LOO^{\cdot} + LOO^{\cdot} \rightarrow$ non-radical products | **(Equation 7)** |
| | $LO^{\cdot} + LOO^{\cdot} \rightarrow$ | **(Equation 8)** |

**Antioxidants**

Antioxidants are agents that neutralize free radicals and therefore represent a central importance for prevention of many of the diseases that are purportedly initiated by oxidative damage. An antioxidant may be defined, as any substance that when present at

low concentrations, significantly inhibits, or delays, the oxidation of that substrate, compared with those of the oxidizable substrate (Gutterbridge, 1994). There are two main classes of antioxidants; there are primary or chain-breaking antioxidants, and secondary or preventative antioxidants (Jadhav et al., 1996). Secondary or preventative antioxidants are substances that retard the rate of oxidation chain reaction. This may be accomplished by removal of substrate or quenching of singlet oxygen. Primary antioxidants, AH, are compounds that when present in trace amounts, may either inhibit or delay the intiation step by reacting with a lipid radical (Eq 9) or the propagation step by reacting with peroxyl (Eq 10) or alkoxyl radicals (Eq 11) (Jadhav et al., 1996).

$R\cdot + AH \rightarrow RH + A\cdot$ **(Equation 9)**
$ROO\cdot + AH \rightarrow ROOH + A\cdot$ **(Equation 10)**
$RO\cdot + AH \rightarrow ROH + A\cdot$ **(Equation 11)**

The antioxidant radical intermediates may further interfere with chain-propagation reactions by forming peroxy antioxidant compounds (Eq 12, 13).

$A\cdot + LOO\cdot \rightarrow LOOA$ **(Equation 12)**
$A\cdot + LO\cdot \rightarrow LOA$ **(Equation 13)**

**Methods for Measuring Antioxidant Activity**

Protocols of assessing antioxidant activity in food systems focus on examining direct interaction between antioxidant and the ROS using a battery of *in vitro* assay systems. In the case of food systems, the results relate to the efficacy of an antioxidant(s) to provide protection against food related oxidative spoilage reactions (Lindley, 1998). In contrast, oxidative stress *in vivo* (e.g. tissue/organ systems) arises from an imbalance between the antioxidant defense and repair mechanisms *versus* the production of excessive ROS. Thus assessment of *in vivo* antioxidant stress can be made by measuring

the activity of antioxidant enzymes, such as SOD, CAT, and GSPx. These enzymes are key to reducing the extent of oxidative deterioration of tissue constituents, as well as preserving vitamin E, uric acid, and serum albumin (non-enzymatic antioxidants which otherwise would be spent in the absence of enzymatic antioxidants) (Moreno, 2002). Another important defense parameter is the consumption of dietary non-enzymatic antioxidants (vitamin E, vitamin C, carotenoid, etc).

In general, different assays are used in the assessment of antioxidant activity of the samples, according to specific free radicals or ROS in question. For each free radical or ROS, researchers have developed numerous different assays that can be used to assess antioxidant capacity. For example, one measure of antioxidant capacity is a test for the ability to scavenge free radicals in aqueous phases by the direct measurement of the inhibition of free radical generation upon addition of a potential antioxidant solution (Antolovich et al., 2001). The most commonly used antioxidant assay methods, for their ease, speed, and sensitivity, are those involving chromogen compounds of a radical nature to simulate ROS (Arnao, 2001). The presence of antioxidants leads to the disappearance of these radical chromogens and the activity can be subsequently calculated as the disappearance of colour. The two most widely used chromogens being the $ABTS^{+\cdot}$ and the $DPPH^{\cdot}$ radicals.

The odd electron in the $DPPH^{\cdot}$ radical displays a strong absorption band at the absorbance of 519nm, which is absent once the odd electron is paired off by a hydrogen- or electron-donating antioxidant (Figure 3). This decolorization assay quantifies the absorbance decrease at 519nm by the addition of the antioxidant to a $DPPH^{\cdot}$ solution in

ethanol. The results for the DPPH method are highly reproducible and comparable to other free radical scavenging methods such as $ABTS^{+\cdot}$ (Gil et al., 2000).

A more hydrophilic free radical, $ABTS^{+\cdot}$, has also been used to evaluate the free radical quenching activities of antioxidant compounds. The reduction of monocation, $ABTS^{+\cdot}$, accompanied by a reduction in absorbance at 734nm, thus the extent of discoloration of $ABTS^{+\cdot}$, when expressed as a percentage inhibition, is a function of the radical quenching ability of antioxidant compound.

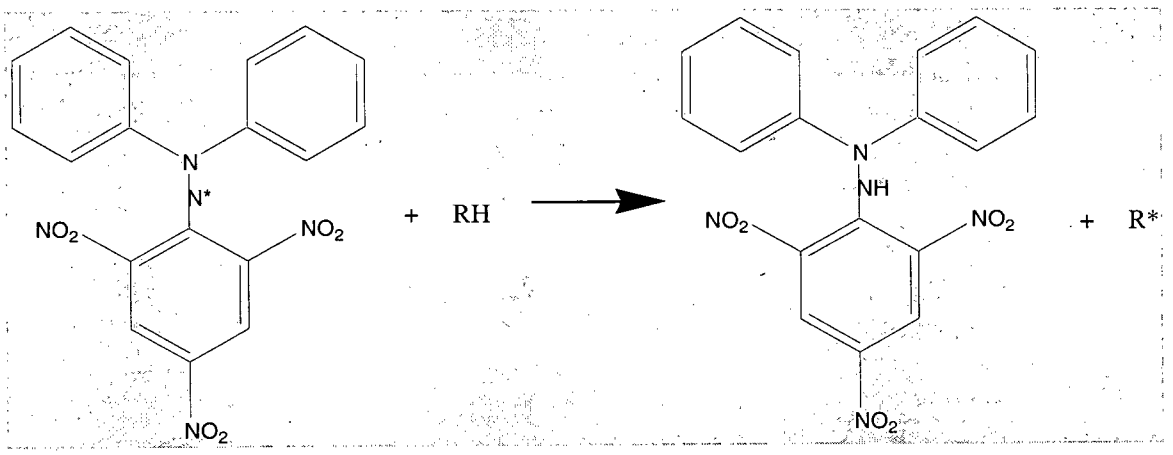



**Figure 4**. Scheme for scavenging the DPPH radical by an antioxidant, RH.

Results from the $ABTS^{+\cdot}$ assay should be comparable to results found in the $DPPH^{\cdot}$ assay and may be viewed as confirmation of findings from the $DPPH^{\cdot}$ assay. Both assays, from a methodological point of view, are recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable extracts (Antolovich et al., 2000). Results are highly reproducible and comparable to each other. Both radicals show the same stoichiometry with Trolox; 2 moles of $ABTS^{+\cdot}$ (Cano et al., 1998) or two moles of DPPH radicals (Leong and Shui, 2002) are scavenged by 1 mole of the hydrosoluble vitamin E analogue.

In addition to looking at protection against free radicals, a peroxyl radical system has also been performed by researchers. Protection against peroxyl radicals by reaction with AAPH [2,2'-azobis (2-amidinopropane) dichloride] through induction of DNA breakage could be monitored with electrophoresis. Antioxidant activity of the antioxidant compound could be examined for the relative affinity to prevent peroxyl radical-induced supercoiled DNA nicking, as compared to a positive control of just DNA, and a negative control of DNA plus AAPH. The retention percentages of the supercoiled DNA were calculated with respect to results from the two controls.

Researchers have also used a reduction power assay to assess the reduction potential of a substance to serve as an indicator for antioxidant activity. High reducing powers relate to electron and hydrogen donors and thus measure the samples' capacity to terminate the radical chain reactions by converting free radicals to more stable products. However, there may not always be a linear correlation between total antioxidant activity and reducing power activity.

**Natural Antioxidants**

Reports published in both popular and academic press have stressed in recent years the value and advantages of using natural ingredients as food preservatives. In recent years, the use of some synthetic antioxidants has been restricted because of reported possible toxic and carcinogenic effects (Frankel et al., 1995; Gazzani et al., 1998; Yen et al., 1998). This concern has led to an increased interest in the investigation of the effectiveness of naturally occurring compounds with antioxidant properties (Duh et al., 1992; Yen et al., 1996; Miyake and Shibamoto, 1997). Antioxidants may function in

one or more of the following ways. These are: (a) as reducing agents, (b) free radical scavengers, (c) complexers or sequestering agents of pro-oxidant metals, and (d) quenching agents that prevent the formation of singlet oxygen (Pratt and Hudson, 1990). As reducing agents, antioxidants function by transferring hydrogen atoms to the ROS, and thus stabilizing the radical from further chain reactions. However, reducing agents can also trigger peroxidation reactions when transition metal ions exist in the system, thus providing for prooxidant activity. As free radical scavengers, antioxidants donate an electron(s) to the ROS; thus neutralizing the free radical, and thereby ending the free radical chain-reaction. The antioxidants themselves do not become free radicals because they are stable in either form. As metal chelators, antioxidants bind the transition metal ions, such as copper and iron that would otherwise catalyze lipid autooxidation. As quenchers of the formation of singlet oxygen, antioxidants react with oxygen species in closed systems that would otherwise be used in oxidation of a compound, such as a lipid molecule (or substrate).

In recent years the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (Frankel et al., 1995; Gazzani et al., 1998; Yen et al., 1998), the affinity of natural antioxidants to react with free radicals makes this group of phytochemicals particularly interesting for both food and human health systems. Of particular interest is the research in the effectiveness of fruits and vegetables as sources of natural antioxidants.

**Antioxidants In Soft Fruits**

Amongst the research efforts conducted to seek natural antioxidants, great emphasis has been given to soft fruits. The term "soft fruits" describes a number of unrelated fruits that have been grouped together based on size and culinary properties, rather than for a specific structural or varietal reason (Taylor, 1996). Most of the work on antioxidant potential of fruits has been limited to grapes and berry fruits and juices.

Wang et al. (1996), investigated the total antioxidant activity of 12 fruits and 5 commercial fruit juices using the ORAC (oxygen radical absorbance capacity) assay. The ORAC assay measures the affinity of antioxidant components in test materials to inhibit the decline of R-phycoerythrin (PE) (a highly fluorescent protein species derived from red algae) fluorescence; that is otherwise induced by a peroxyl radical generator, AAPH (Cao et al., 1995). In their study, these researchers found that on the basis of dry weight (edible portion of the fruit), strawberries had the highest ORAC activity followed by plum (*Prunus domestia.)*, orange (*Citrus sinensis)*, pink grapefruit (*Citrus paradisi)*, tomato (*Lycopersicon esculentum)*, kiwi fruit (*Actinidia deliciosa)*, red grape (*Vitis vinifera)*, white grape (*Vitis vinifera)*, apple (*Malus domestica)*, honeydew melon (*Cucumis melo)*, pear (*Pyrus cammunis)*, and banana (*Musa spp.)*. They did not identify the specific substances responsible for the observed antioxidant capacity, since the principle objective was to determine the total antioxidant capacity and not the contribution of any individual antioxidant. Speculation was made that part of the antioxidant capacity of these fruits was derived from the flavonoids.

Heinonen et al. (1998), showed that extracts of several cultivars of blackberries (*Rubus ursinus)*, sweet cherries (*Prunus avium)*, black and red currants (*Ribes nigruum;*

*Ribes sativum)*, blueberries (*Vaccinium corymbosum)*, and black and red raspberries (*Rubus occidentalis; Rubus idalus)* showed remarkably high scavenging activity toward chemically generated superoxide radicals in both human low-density lipoprotein (LDL) and liposome oxidation model systems. In the liposome system, lipid oxidation was induced by AAPH and the extent of inhibition by antioxidants in each extract was determined in the form of measured TBARS (Thiobarbituric acid reactive substances) at 532 nm (Antolovich et al., 2002). For the LDL system, the effect of antioxidant in extracts on lag time of the copper (II)-mediated oxidative modification of LDL was measured by monitoring conjugated diene formation at 234 nm (Antolovich et al., 2002). Relative antioxidant activity of the berry extracts was found to be different, as determined by the two different oxidation model systems used. In the LDL oxidation system, blackberry extracts were significantly more active than those of red raspberries, sweet cherries, or blueberries. In the liposome oxidation model, extracts of sweet cherries were more active compared to those of other berries. In both systems, strawberries exerted the weakest antioxidant activity. When interpreting these results, researchers associated the antioxidant activity for LDL oxidation directly with anthocyanin content (of which blackberries had the highest amount) and indirectly with the flavonol content. For the liposome assay, a correlation was determined that described the associated antioxidant activity with the hydroxycinnamate content (of which sweet cherries had the highest amount). The finding that strawberries had the weakest antioxidant activity was in contradiction to the results of Wang et al. (1996), where the researchers found strawberries had the highest antioxidant activity compared to other fruits. The contradicting results are most likely due to the different methods used in assessing

antioxidant capacity, thus providing for varying relative antioxidant activities in these fruits.

Kalt et al. (1999a), reported antioxidant capacities in strawberries (*Fragaria ananassa*), raspberries (*Rubus sp.*), and blueberries (*Vaccinium corymbosum*). In this study, blueberries had a stronger antioxidant capacity than either the strawberries or raspberries using the ORAC method. These researchers also correlated the antioxidant capacity with the phenolic and anthocyanin content in these fruits.

Wang and Lin (2000), tested the total antioxidant capacity using the ORAC method on different cultivars of blackberry (*Rubus ursinus*), red and black raspberry (*Rubus idalus; Rubus occidentalis*), and strawberry (*Fragaria ananassa*). They also reported a linear relationship between ORAC values and anthocyanin content. For the ripe fruits tested, black raspberry and blackberries were the richest sources of antioxidants, on the basis of the wet weight of the fruit. But when analyzed on the basis of dry weight of fruit, strawberries had the highest ORAC activity followed by black raspberries, blackberries, and red raspberries. Researchers concluded a linear correlation between total phenolic content and ORAC activity for fruits and leaves.

Even within the same genus of fruit, there can be a great deal of difference in the antioxidant potentials among species and varieties. For example, Prior et al. (1998), found different antioxidant capacities in various species and cultivars of blueberries (*Vaccinium sp.*). They also reported that increasing blueberries maturity and harvest yield resulted in higher antioxidant anthocyanin, and total phenolic contents. With their study, a linear relationship between ORAC and anthocyanin or total phenolic content was identified.

In summary, many research papers that have delved into studying the antioxidant capacities of soft fruits produce a common general set of conclusions. For example, there is definitely a great potential for the use of soft fruits as sources of natural antioxidants derived from anthocyanins and phenolics; however, the results will largely depend on the antioxidant assay method of choice, the species and cultivars of the fruits being used, and the method of calculation (wet *versus* dry weight basis).

Edible berries are a rich source of fiber and various phenolic compounds. The majority of berries studied have similar or higher levels of flavonoids and phenolic acids than the commonly consumed fruits such as grapes (*Vitis* vinifera) and oranges (*Citrus sinensis*)(Torronen, 2000). Blueberries are one of the richest sources of antioxidants studied so far with antioxidant capacity as high as 45.9 μmol Trolox equivalent per gram, using the ORAC method. Differences in antioxidant activity of these fruit in the different studies may be because of differences in activities of phenolic compounds and their antagonistic and synergistic reactions with other antioxidants.

Side group attachments to the anthocyanin structure are also important in its associated antioxidant properties. Pratt and Hudson (1990) reported that the position and degree of hydroxylation of flavonoids, especially of the B ring, has a substantial role in the extent of antioxidant activity with all flavonoids; with the 3', 4'-dihydroxy configuration having higher antioxidant activity than the dehydroxylated forms. Fukumoto and Mazza (2000) found that for the benzoic and cinnamic acid derivatives, flavonols, and anthocyanidins, an increase in the number of hydroxyl groups led to higher antioxidant activity. Dzeidzic and Hudson (1983), similarly suggested that at least two hydroxyl groups were required for antioxidant activity of phenolic acids.

Addition of a sugar moiety decreased antioxidant activity of the aglycon, and the addition of a second moiety decreased activity further, probably due to steric hindrance by addition of sugar moieties (Fukumoto and Mazza, 2000).

**Antioxidants in Saskatoon Berries**

Research is very limited in terms of the antioxidant capacity of Saskatoon berries. Fukumoto and Mazza (2000) have compared the antioxidant and prooxidant activities of the phenolic compounds amongst Saskatoon berries, blackberries, blackcurrants, and blueberries using the β-carotene bleaching method and the DPPH (diphenylpicrylhydrazyl) radical discoloration method for assessing the antioxidant potentials for the various fruits. The β-carotene bleaching method is a coupled oxidation of β-carotene and linoleic acid that estimates the relative ability of antioxidant compounds in the sample to scavenge the radical of linoleic acid peroxide ($LOO^{\bullet}$) that oxidizes β-carotene. The DPPH radical absorbs at 519 nm and antioxidant activity can be determined by monitoring the decrease in this absorbance (Antolovich et al., 2002). The results obtained by these researchers suggest that all extracts had both prooxidant and antioxidant activity. All berry extracts were found to have similar phenolic contents, with variations in type and composition among the berry type. Berry extracts were also found to contain a large proportion of anthocyanins. Saskatoon berries and blackberries contain mainly glycosylated cyanidin (Mazza, 1986); blueberries and blackcurrants contain a mixture of glycosylated anthocyanidins (Gao and Mazza, 1994). They further reported that the slight variation in the activities among the berry extracts were accounted for by the differences in anthocyanins found in these berries.

In a study by Wang and Mazza (2002), it was reported that the anthocyanin-rich crude extracts of phenolic compounds from several berries (Saskatoon berries, blueberries, blackberries, and black currants) had an inhibitory effect on nitric oxide ($NO\cdot$) production. This effect was correlated to total phenolic and anthocyanin contents in the crude extracts. Nitric oxide is a diatomic free radical produced from L-arginine in numerous mammalian cells and tissues that may be generated in excess during the host response against viral and bacterial infections, and is known to contribute to some pathogenesis by promoting oxidative stress, tissue injury, and even cancer (Maeda and Akaike, 1998; Akaike et al., 2000).

**Dehydration Processing**

Many changes occur with fruits and vegetables during harvesting, preparation, and handling, and many of these changes potentially impact on antioxidant capacities (Lindley, 1998). Preservation is a convenient method of storing fruit for use in periods when the fresh products are not available. If processed and stored properly, the nutritive value of the processed fruit is comparable to that of fresh products (Fourie, 1996).

Dehydration is, perhaps, the oldest and the most effective method for preserving fruits, of which the oldest typeS is sun-drying (Woodroof, 1986). Modern procedures of lyophilizing (drying from the frozen state) yield more acceptable products compared to the traditional methods (Hsu and Beuchat, 1986).

The term "dehydrated" usually is considered to imply virtually complete water removal to a range of 1-5% moisture (Hsu and Beuchat, 1986). Because of the low moisture content, dried fruits are not as susceptible to microbial spoilage and undesirable

enzymatic reactions can occur. Removing water from fruit lowers water activity ($a_w$) and thus the availability of water to reach the microorganisms that cause fruit deterioration (Hsu and Beuchat, 1986). Water activity is defined as the ratio of water vapor pressure of a food with the water vapor pressure of pure water at the same temperature (Fennema, 1996). Most bacteria will not grow if $a_w$ is below 0.9, and most yeasts and molds will be inhibited at $a_w$ below 0.7 (Heidelboug and Karel, 1975).

The lowering of the $a_w$ of a fruit has an extremely microbiostatic effect (Fennema, 1996). Depending on the duration of the drying operation and the actual $a_w$ reached, the microbial species, and microbial growth phase, the reduction of microbial population can be significant (Hsu and Beuchat, 1986). Dried fruits contain two common features: high proportions of sugar, together with low proportion of water, resulting in given $a_w$ value on the order of 0.75, or less (Hsu and Beuchat, 1986). This degree of physiological dryness completely prevents the growth of the common microorganisms (Cook, 1958). Products with low water content can be stored at room temperature for periods well over two years with no significant changes in quality (Hsu and Beuchat, 1986).

### *Conventional Hot-Air Drying*

Of the many dehydration techniques available, air-drying is the cheapest and easiest to accomplish. Dehydrators and convection ovens are equipped with heating elements and a fan which blow hot, dry air at moderate speeds around the food. The air serves two purposes. First is supplying the heat needed to evaporate the water, and the dry air that is blown across the food serves to carry away the water vapor. Food dehydrators and convection ovens differ in range of temperature settings and available

square feet of drying space. The major concern in hot air drying is the tremendous energy consumption and low drying efficiency (Yongsawatdigul and Gunasekaran, 1996a). Furthermore, loss of volatile compounds inevitably occurs during hot air drying. Since the products are exposed to a high temperature for a long period, these volatile compounds are vaporized and lost with the water vapor. Moreover, the high temperature and long time associated with conventional hot air drying adversely affects texture, color, flavor, and nutritional value of the dried product (Schadle et al., 1983; Yang and Atallah, 1985; Yongsawatdigul and Gunasekaran, 1996b).

***Freeze Drying***

Freeze drying (FD) is another method for dehydration of foods. In this process, the fruit is frozen and then dried under vacuum to around 2% moisture (Hsu and Beuchat, 1986). The ice sublimes as water vapor without melting. Water vapor is removed from the frozen food, which is achieved by reducing the pressure in a sealed drying chamber by a vacuum pump (Salunkhe et al., 1991). The low processing temperatures and the rapid transition minimize the extent of various degradative reactions, such as non-enzymatic browning, protein denaturation, and enzymatic reactions that are associated with other drying methods (Salunkhe et al., 1991). FD has been reported to be the better method for retaining moist food structure, appearance, rehydration, and nutrients. This is because heat damage does not occur and the effect of thermal damage imposed on the food products is reduced. This process is, however, technically complicated, slow and demands high capital costs, thus it is only suitable for high-value products. FD also

usually causes large losses of flavor volatiles (Flink, 1975), resulting in the FD foods often being described as being tasteless.

### *Vacuum Microwave Drying*

A third method for dehydration of fruits is with vacuum microwave drying (VMD). VMD is as the name suggests, a combination of vacuum (which keeps the temperature low) and microwave (for the ultra-rapid energy transfer).

When fruit is dehydrated in a heated vacuum chamber, moisture can be removed at a lower temperature than if it was dehydrated using heat at atmospheric pressure. This is because, at an atmospheric pressure of 760 mm Hg, water boils at 100°C (212°F); but at pressures below 760 mm Hg, boiling water will occur at a lower temperature (Hsu and Beuchat, 1986). Moreover, the absence of air during vacuum drying may inhibit oxidation. Thus, with this system, the dehydrated products dried at lower temperatures and in the absence of oxygen, will have less degradation in color, flavor, and texture.

The use of microwave energy allows for ultra-rapid energy transfer. Microwaves are the portion of electromagnetic spectrum between far infrared and the conventional radio frequency region (Salunkhe et al., 1991). The electromagnetic spectrum ranges from 0.3 to 300 GHz, corresponding to wavelengths ranging from 1 mm to 1 m (between radio waves ($10^2$ to $10^4$) and infrared radiation ($10^{-2}$) (Salunkhe et al., 1991; Drouzas and Schubert, 1996). For microwave heating or drying of foods, a frequency around 2456 MHz is used (Drouzas and Schubert, 1996). Microwaves are similar to light waves and can pass through, be reflected by, or are absorbed into materials; all of which will depend on the nature of the material. As the energy passes through the food material, the

molecules within the food attempt to align themselves with the electric field direction. As they oscillate around an axis, heat is produced by the intermolecular friction. The amount of heat produced will vary depending on structure, shape, composition, and mass of the product. Thus, with microwave energy, heat is generated within the food product thereby contributing to the rapid increased temperature of the product. As a consequence, the rate of water removal is faster with VMD than with conventional drying or FD.

Combining the technology and principles of both vacuum and microwave, the major advantages of using the vacuum microwave drier is that it is a low temperature and short time process; thus the process produces high quality products with little loss of nutrients and flavor. Color changes are minimized which retains the natural appearances of the fruit. The process allows for improved energy efficiency and product quality. However, fast drying is also one of the weaknesses for this process, since the potential for "overdrying" may occur because the process is so fast, and thus must be precisely timed and monitored. There is also a high capital cost associated with the vacuum microwave drier. Vacuum-microwave drying technique has been successfully applied to numerous food materials such as cranberries (*Vaccinium macrocarpon*), apples (*Malva domestica*), grapes (*Vitis vinifera*), peanuts (*Arachis hypogaea*), rice (*Oryza sativa*), asparagus (*Asparagus officinalis*), mushrooms, shrimp, soybeans (*Glycine max.*), potato chips, and bananas (*Musa spp.*) (Yongsawatdigul and Gunasekaran, 1996a, 1995b; Anon, 1990; Sham et al., 2001; Petrucci and Clary, 1989; Delwiche et al., 1986; Wadsworth et al., 1990; Lin et al., 1999a; Slater, 1975; Durance and Liu, 1996; Drouzas and Schubert 1996).

***Comparison of Drying Techniques***

Yousif and colleagues (2000), looked into the effect of headspace volatiles and physical characteristics of oregano (*Origanum vulgare*) using the three drying techniques (AD, FD, and VMD). In their study, it was concluded that the level of thymol (the major volatile compound of the plant) after VMD was comparable to that of fresh or FD sample. They also noted that the AD oregano samples were darker, were less green, and exhibited lower rehydration rates than that from VMD or after FD of the sample. Moreover, the VMD and FD oregano appeared to be quite similar in physical structure.

Lin et al. (1999b), reported that VMD carrot slices had higher rehydration potential, higher α-carotene and vitamin C content, lower density, and softer texture than carrots prepared from AD. They also report that FD carrot slices had improved rehydration potential, appearance, and greater nutrient retention than either AD or VMD methods. However, the VMD product was rated as equal to or better than FD samples by a sensory panel for color, texture, flavor and overall preference in both the dry and rehydrated state.

Yongsawatidigul and Gunasekaran (1996b), reported that AD cranberries had less color and a harder texture as compared to the VMD cranberries. Petrucci and Clary (1989) indicated that the nutrient content, including vitamin A, vitamin C, riboflavin, thiamin, and niacin, in dried grape (*Vitis vinifera*) were largely preserved during VMD.

Kim and colleagues (2000), reported that individual alkamide concentrations in roots and leaves of Canadian grown *Echinacea purpurea* were affected by the different drying methods used. They showed that the FD method was best for preservation of the alkamides.

No processing studies have been performed to investigate the dehydration effects for Saskatoon berries.

### *Effects of Processing*

Berry fruits contain high amounts of anthocyanins that are responsible for a distinguished and appealing color. Anthocyanins are relatively stable during frozen storage but are degraded upon heating (Shewfelt, 1986). Processing with heat will degrade the anthocyanin pigments to some extent, but for most dehydration processes, this cannot be avoided because a certain amount of heat is necessary to ensure that the food product has a longer shelf-life after processing undergoing dehydration (Francis, 1982).

Kalt and colleagues (2000), studied the effect of lowbush blueberry processing products in terms of anthocyanin, phenolic contents, and antioxidant capacity. The yield of total anthocyanins, total phenolics, and antioxidant capacity was markedly affected by the extraction time, temperature, and pH. Extracts at pH 1 had the greatest anthocyanin content as compared to extracts normalized to pH 4 and 7. In addition, increased temperature (from 25°C to 60°C) yielded higher antioxidant phytochemical results. Oxygenation was found to be detrimental to both anthocyanin and antioxidant capacity. They also reported that products that underwent less processing, in general, had higher antioxidant capacity as measured by ORAC values. In particular, fresh blueberries had the highest antioxidant capacity over the dried blueberry fruit or juice concentrate. Moreover, it was suggested that in spite of processing losses, anthocyanins make up a significant contribution to the total antioxidant capacity of blueberry food products.

Overall, they found that antioxidant capacity of processed products was positively correlated with anthocyanin (R=0.92) and phenolic content (R=0.95), and negatively correlated with % polymeric color (R=-0.64).

Skrede and colleagues (2000), measured the changes in anthocyanins and polyphenolics during juice processing of highbush blueberries. They reported that substantial losses of anthocyanins and polyphenols occurred when the blueberries were processed into juice and concentrate, respectively, and that the different classes of compounds had varying degrees of susceptibility to degradation. The anthocyanin profile changes drastically because of the varying stability of individual pigments. Their finding that processed products generally had lower degrees of anthocyanin and phenolic content agreed with those obtained from Kalt et al., (2000).

In summary, the quality changes that occur during fruit dehydration are primarily dependent on the method of drying. Processing is considered to be detrimental to fruit quality and the goal of a fruit processor is to minimize flavor and color changes while producing a safe, acceptable product (Shewfelt, 1986). Fruits that are properly dehydrated, particularly to a moisture level below 5% have several advantages. These include, having almost unlimited shelf-life under proper storage conditions; providing a consistent product, in terms of antioxidant content (and other secondary metabolites); providing maximum convenience, flexibility, and economics as industrial or foodservice ingredients; hardly affecting the micronutrient content (e.g. minerals and vitamin losses are not greater with dehydration than with other preservation methods); and finally utilizing a more economical and disposable means of packaging (Hsu and Beuchat, 1986).

## RESEARCH HYPOTHESES AND OBJECTIVES

### Hypotheses

$H_A1$. Saskatoon berries possess antioxidant constituents that are comparable to commercial antioxidants (e.g. ascorbic acid, Trolox).

$H_A2$. Antioxidant activity of Saskatoon berries is influenced mainly by varietal differences, as opposed to year of harvest.

$H_A3$. Different dehydration methods for processing Saskatoon berries will result in different antioxidant activity associated with the different retention of anthocyanin pigments and phenolic contents.

$H_A4$. Constituents of Saskatoon berries, which exhibit antioxidant activity, are contained in the medium polar to polar fractions that can be fractionated by different solvents.

### Objectives

The first objective of this research project was to assess whether Saskatoon berries possess antioxidant properties. If antioxidant activity exists, the next question was to determine how the antioxidant characteristics compare to that of known commercial antioxidants (e.g. ascorbic acid and Trolox).

Following confirmation of antioxidant properties of Saskatoon berries, subsequent experiments were designed to investigate whether varietal differences have significant impacts on fruit and antioxidant characteristics.

This research also compared different drying methods, to include freeze-drying, vacuum microwave drying, air-drying, and a combination drying technique on the retention of fruit characteristics and antioxidant activity in Saskatoon berries.

The last objective of this study was to fractionate and examine the relative partitioning, according to polarity, of the antioxidant constituents present in Saskatoon berries according to polarity.

# EXPERIMENT 1: CHEMICAL CHARACTERISTICS AND THE ASSOCIATED ANTIOXIDANT ACTIVITIES FOR TWO VARIETIES OF SASKATOON BERRY

## INTRODUCTION

Studies have shown that the consumption of fruits and vegetables is related to a reduced risk of cancer and cardio- and cerebro-vascular diseases (Ames, 1993; Gey, 1990; Steinberg et al., 1989, 1991). Research has shown that fruits and vegetables contain many different phytochemicals having antioxidant properties. Flavonoids (including compounds such as flavonols, flavones, isoflavones, flavonones, anthocyanins, and catechins) of fruits and vegetables are a major dietary factor responsible for this protective effect (Hertog et al., 1992, 1993, 1994; Knedt et al., 1996, 1997).

There is overwhelming evidence to indicate that reactive oxygen species (ROS) cause oxidative damage, especially to lipids, proteins, nucleic acids, and pigments. ROS have been implicated to be involved in a number of chronic diseases including cancer, heart, vascular, and neurodegenerative disorders (Halliwell, 1994; Yu, 1994). Therefore, antioxidants that can neutralize free radicals may have a central importance in the prevention of chronic disease states.

The Saskatoon berry (*Amelanchier alnifolia* Nutt.) is native to the southern Yukon and Northwest Territories, the Canadian prairies and the northern prairie states of the United States (Harris, 1972). It has traditionally been a major food source for the native peoples and early settlers in these regions, but recently, has been cultivated and grown in orchards, and no longer can be picked only in the wild. There has been an increase in the number of people interested in its cultivation and utilization of this fruit. With increased

market interest in the fruit, follows increased research interest and potentialities for beneficial functionalities of the fruit.

Significance correlations have been reported with high levels of anthocyanins and high total phenolics and acids and low pH and sugar-acid ratios in Saskatoon berries (Green and Mazza, 1986). Environmental conditions are known to affect the production of anthocyanins in plant material (Ribereau-Gayon, 1972; Ballinger et al., 1972; Karppa, 1984), but the degree of influence by other internal physico-chemical parameters is unclear.

The aim of this experiment was to assess the chemical characteristics and the association with the antioxidant capabilities of Saskatoon berry extracts. In addition, this information on Saskatoon berries was from two varieties (*Amelanchier alnifolia* Nutt. cv. Smoky and Thiessen) measured over three different years. Antioxidant activities were compared with known commercial antioxidants (e.g. Trolox).

## MATERIALS AND METHODS

Saskatoon berries (*Amelanchier alnifolia* Nutt. cv. Smoky and Thiessen varieties) were kindly supplied by Riverbend Plantation Gourmet Foods (Saskatoon, Saskatchewan, Canada). The berries were frozen after harvest and stored at –25°C, packed into separate boxes and delivered to the Food Science Department of UBC where the berries were also stored at –18°C until analysis. The berries harvested in 1998 were held in frozen storage for approximately 25 months, and the berries from 1999 were held in frozen storage for approximately 13 months, while the berries from 2000 were stored for only 1 to 2

months. Blueberries (*Vaccinium corymbosum.* cv. Blue Crop) from 2000 were purchased from a local producer.

Metaphosphoric acid, sodium hydroxide, potassium dihydrogen orthophosphate ($KH_2PO_4$), were from BDH Inc. (Toronto, ON). Sodium bicarbonate ($NaHCO_3$), sodium acetate ($CH_3CO_2Na_3 \cdot H_2O$), sodium chloride (NaCl), sodium phosphate ($Na_2HPO_4$), potassium ferricyanide ($K_3Fe(CN)_6$, trichloroacetic acid (TCA), and glycerol were obtained from Fisher Scientific (Fair Lawn, NJ). Methanol, acetic acid, and hydrochloric acid were from Fisher Scientific (Nepean, ON). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (diammonium salt, ABTS), ferric chloride ($FeCl_3$), potassium chloride (KCl), Folin-Ciocalteau's reagent, gallic acid, ethidium bromide, ethylenediaminetetraacetic acid (EDTA) disodium salt, and xylene cyanol were from Sigma Co. (St. Louis, MO). Agarose, sodium dodecyl sulfate (SDS) and bromophenol blue was obtained from Bio-Rad Laboratories (Richmond, CA). 96-well flat-bottom EIA microtitration plates were from Corning Inc. (Corning, NY). 2, 2'-azobis(2-amidino-propane (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Trolox was obtained from Aldrich Chemical Co. (Milwaukee, WI).

**Berry Extracts**

Fresh frozen Saskatoon berries (25 g) from 1998, 1999, and 2000 harvest years of both Smoky and Thiessen varieties were weighed and transferred into a Waring blender. To each 25g berry sample, 250 ml of homogenization solution was added [80% methanol, 0.3% metaphosphoric acid (w/v), and 0.8% acetic acid (v/v)] (Wang and Mazza, 2002). Berries were homogenized for 2 minutes and the slurry was then

transferred to a 500ml Erlenmeyer flask. The glass vessel (blender) was washed with three washings of 25ml of homogenization solution and washings were pooled into the 500ml flask containing the Saskatoon berry slurry. The combined slurry and washings were then sonicated (Bransonic 220, SmithKline Company, Shelton, Conn. USA) for 10 minutes. After sonication, the solution was then passed through a 5.5cm No.1 Whatman filter paper in a Buchner funnel under vacuum. The vessel was washed again three times with 25ml homogenization solution and passed through the filter. After filtration, the collected organic solvent was dried by rotary evaporation (Rotovap, Fort Lane, New Jersey, USA) under vacuum below 35°C to approximately 1/8 volume (e.g. approximately 30 ml). This extract was made to a final volume of 50ml with distilled water. These final Saskatoon berry extracts (SBEs) were then stored in 50ml plastic centrifuge tubes, containing 0.3ml of 1N HCl. The oxygen in the extract was then removed by nitrogen infusion for 30 seconds, and was kept frozen at –25°C until further analysis. SBEs were expressed as fruit weight/volume. Saskatoon berries from 1998, 1999, and 2000 of both the Thiessen and Smoky varieties were extracted.

An identical extraction procedure was used for extracting three subsamples of Bluecrop blueberry (BBE).

## Saskatoon Fruit Characterization

### *Moisture Content*

Moisture content of the Saskatoon berries was determined and averaged (n=3) using the vacuum oven approach for each year and for each variety (Sham et al., 2001). Samples, in triplicate, were weighed into pre-weighed, labeled aluminum weighing pans

and then dried in vacuum oven at 70°C and a vacuum level of 27 inches of Hg for 4 days. Upon completion of the drying, weight pans were cooled and weighed. The moisture content of the sample was then calculated from the difference between the wet and dry weights divided by the dry weight of the sample.

$$\% \text{ Moisture Content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

***Total Monomeric Anthocyanin Content***

Total Saskatoon berry monomeric anthocyanin content was determined according to a method described by Fuleki and Francis (1968). The method is based on a pH differential of anthocyanins at pH 1.0 and 4.5. The two buffers used were, potassium chloride (KCl) buffer (pH 1.0, 0.025M) and sodium acetate buffer ($CH_3CO_2Na \cdot 3H_2O$) (pH 4.5, 0.4M). A dilution factor (DF) for the sample was first determined; using KCl buffer (pH 1.0) to obtain an absorbance reading of less than 1.200 (the detection limit of the spectrophotometer) at 520nm ($\lambda_{vis\text{-}max}$). The DF was calculated by dividing the final volume of the sample by the initial volume. The sample did not exceed 20% of the total volume in order to prevent exceeding buffer capacity. Using the pre-determined dilution factor, two samples, at two different pH's, with the same dilution strength were prepared (e.g. one with a KCl buffer at pH 1.0 and another with a sodium acetate buffer at pH 4.5. The mixtures were left to stand for 15 minutes to allow the solutions to equilibrate. Measurements of each dilution were made at 520nm and at 700nm ($\lambda_{vis\text{-}max}$) against a blank cell of deionized distilled water ($ddH_2O$). Absorbance readings were made between 15 minutes to 1 hour after preparation. Longer standing times were found to

unnecessarily increase the absorbance readings. The calculation used for absorbance for sample (A):

$$A = (A_{\lambda vis\text{-}max} - A_{700nm})_{pH\ 1.0} - (A_{\lambda vis\text{-}max} - A_{700nm})_{pH\ 4.5}$$

The monomeric anthocyanin pigment concentration in the original sample was calculated using the following equation:

$$\text{Monomeric anthocyanin pigment (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

MW = molecular weight (corresponds to the predominant anthocyanin in sample)
DF = dilution factor
ε = Molar absorptivity (corresponds to the predominant anthocyanin in sample)

Due to the fact that the pigment composition in the two SBE varieties was unknown, the pigment content was calculated using ε (molar absorptivity) for cyanidin 3-glucoside, with MW = 449.2 and ε = 26,900. The same coefficient of cyanidin 3-glucoside was also used in the anthocyanin calculation in BBE. Final results were expressed as milligrams of anthocyanin per 100 g of fresh berry weight.

***Total Phenolic Content***

To assess the phenolic content of SBE that may have an effect on the overall antioxidant activity, total phenolics assessment was performed. The total phenolics content of the Saskatoon berries was determined using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965) with slight modifications, using gallic acid as a standard. This assay uses the Folin Ciocalteau reagent to oxidize the phenolic compounds. Once oxidized, sodium bicarbonate was added and the phenolics were converted to compounds that absorb light at 725nm. A spectrophotometer was used to measure the absorbance @725nm.

For the modified procedure, 100 µl berry extract (e.g. SBE or BBE) was mixed with 750µl Ciocalteau reagent (10x dilution with distilled water) and allowed to stand at 22°C for 5 min. A volume of 750µl of a sodium bicarbonate (60g/L) solution was added to the mixture, and incubated for 90 minutes at room temperature. A small aliquot (150µl) of the mixture was transferred to each well in a 96-well flat-bottom EIA microtitration plate and absorbance reading was taken at 725nm (ThermoLabsystems Multiskan Spectrum, Thermolabsystem, Chantilly, VA, USA). Results were calculated according to a standard curve obtained from gallic acid and expressed as a gallic acid equivalent (GAE) (µg of gallic acid/mg fruit). A standard curve of gallic acid was prepared by plotting the absorbance at various concentrations of gallic acid (e.g. 0, 15.6, 31.3, 62.5, 125, 250, 500 µg/ml).

### *Soluble Solids Content*

To complete the indices for comparison of the SBE, a soluble solids content was determined by means of a refractometer (MARK II digital refractometer, Cambridge Instruments Inc., Buffalo, N.Y.) (AOAC, 1990). Soluble solids of berry extracts content were measured using the refractometer at 20°C and expressed as °Brix.

## Antioxidant Assays

### *DPPH Radical Scavenging Test*

The activity of Saskatoon and blueberry fruit extracts to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured according to principle of Blois (1958) with some modifications reported by Hu and Kitts (2000a). SBE or BBE were mixed

with a 0.1mM DPPH solution in ethanol and incubated at 20 minutes at room temperature. Discoloration as an indication for scavenging activity was monitored by measuring the absorbance at 519 nm after the incubation period. Measurements were made using a UV-visible spectrophotometer (Shimadzu UV-160, Tekscience, Oakville, ON) at room temperature. Scavenging capacity was represented as a percentage of DPPH radical inhibition and calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \times 100$$

$\text{absorbance}_{\text{control}}$ = absorbance of the control
$\text{absorbance}_{\text{sample}}$ = absorbance of the sample

***ABTS Radical Scavenging Test***

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation model (Pellegrini *et al*, 1999) was used to evaluate the free radical scavenging effect of berry extracts, with slight modifications (Hu and Kitts, 2002a). Discoloration was determined by comparing the U.V. absorbance at 734 nm of the treatment groups with a control after an 8 minute incubation period at room temperature. Measurements were made in a 96-well flat-bottom EIA microtitration plate, using a microplate spectrophotometer (ThermoLabsystems Multiskan Spectrum, Thermolabstystem, Chantilly, VA, USA). Scavenging capacities of ABTS was expressed as the percent inhibition of ABTS radical, calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{absorbance}_{\text{control}} - \text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}} \times 100$$

$\text{absorbance}_{\text{control}}$ = absorbance of the control
$\text{absorbance}_{\text{sample}}$ = absorbance of the sample

**Reducing Power Assay**

Berry extracts from both SBE and BBE were analyzed for reducing power using the method of Yen et al., (1995). Briefly, 0.2 ml of berry extract was incubated with 0.5 ml, 0.2 M phosphate ($KH_2PO_4$) buffer (pH 6.6), and 0.5 ml (1%) ferricyanide ($K_3Fe(CN)_6$) at 50°C for 20 minutes. Following incubation, 0.5 ml (10%) TCA was added and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, 0.5 ml of the upper layer was transferred to a test tube, along with the addition of 0.5 ml de-ionized distilled water and 0.1 ml (0.1%) ferric chloride ($FeCl_3$). After vortexing the mixture, 0.15 ml was added to a 96-well flat-bottom EIA microtitration plate. Absorbance was then made at 700 nm with a microplate spectrophotometer (ThermoLabsystems Multiskan Spectrum, Thermolabsystem, Chantilly, VA, USA). The reducing power was calculated according to a standard curve obtained from ascorbic acid and expressed as ascorbic acid equivalent (AAE) (μg of ascorbic acid equivalent/mg of fruit).

**Statistical Analysis**

Experiments were performed in triplicates and results were expressed as mean ± S.D. One-way ANOVA was used to evaluate the differences between samples with significance set at $p<0.05$ (Minitab for Windows, version 13.32, State College, PA). A correlation statistic was used to evaluate statistical differences between the different methods and assays used in this experiment, among the years and varieties measured (significance set at $p<0.01$, $p<0.001$). A pooled average was also collected over the three year harvest period for both varieties for each assay.

## RESULTS

### Total Anthocyanins, Total Phenolics, Total Soluble Solids

Results of total anthocyanin content, total phenol content, total soluble solids content, and moisture content of the berries are listed in Table 3. The soluble solids content of Saskatoon berries averaged 4.5 °Brix for Thiessen berries, and 3.7 °Brix for Smoky berries. There was no statistically significant difference between varieties ($p<0.05$). The moisture contents of the berries ranged from 77.5% to 81.5%, as compared to the moisture content of blueberries of 82%.

The anthocyanin content for the Saskatoon berries averaged 60.5mg/100g fresh weight for the Thiessen variety, and 41.3mg/100g fresh weight for the Smoky berries. The two varieties are significantly different from one another ($p<0.05$) with respect to average total anthocyanin content. The Thiessen SBE was significantly higher ($p<0.05$) in total monomeric anthocyanin content as compared to the experimental BBE in this study, containing 38.7mg/100g fresh weight. However, the Smoky SBE was not significantly different ($p<0.05$) from the BBE. Bluecrop blueberries have been reported by other researchers to contain total monomeric anthocyanin contents ranging from 82.3mg/100g (Kalt et al., 1999) to 182mg/100g of fresh weight (Ehlenfeldt and Prior, 2001). The lower value of 38.7mg/100g of fresh weight for the Bluecrop variety obtained in this study may have been due to many factors, e.g. differences in maturity, location and time of harvest, and storage period before analysis may have contributed to the difference. Highbush blueberries have been reported to contain total anthocyanin levels between 25-495 mg/100 g (Mazza and Miniati, 1993).

Total phenol content for SBE averaged 3.62mg/g and 2.52mg/g fresh weight for the Thiessen and Smoky varieties, respectively. This was calculated in a different manner to that reported by Fukumoto and Mazza (2000), in which they reported Saskatoon berries to contain a total phenol content for Smoky variety to be around 405 mg chlorogenic acid/100g of berry. However, as observed with the total anthocyanin content, the Thiessen variety contained a significantly higher ($p<0.05$) phenolic content as compared to the Smoky variety. When compared to the BBE, the Thiessen variety was also significantly higher ($p<0.05$) in total phenolic contents. The Smoky variety, however, was not significantly different ($p<0.05$) to the BBE. For the BBE, the total phenol content was measured to be at 2.14mg/g fresh weight. Bluecrop blueberries have been reported to contain total phenolic contents between 0.48mg/g to 1.9mg/g (Prior et al., 1998) using gallic acid as a standard.

**Table 3.** Total anthocyanin, total phenolic, and total soluble solids content of fresh frozen Saskatoon berry extract and blueberry extract.[1]

| Variety | Year | Moisture (% wb) | Total Anthocyanin (mg/100g fresh wt) | Total Anthocyanin (mg/100g dry wt) | Total Phenol (mg/g fresh wt) | Total Phenol (mg/g dry wt) | Total Soluble Solids (°Brix) |
|---|---|---|---|---|---|---|---|
| Thiessen | 2000 | 77.5 | 48.0±0.3 | 213.4±1.3 | 3.32±0.08 | 17.31±0.43 | 4.87±0.23 |
| | 1999 | 79.5 | 68.4±0.4 | 333.9±1.0 | 4.28±0.02 | 20.86±00.8 | 4.80±0.06 |
| | 1998 | 81.5 | 65.1±0.4 | 351.9±2.3 | 3.25±0.02 | 17.57±0.10 | 3.97±0.25 |
| | **Average** | **79.5±2.0$^{a}$** | **60.5±9.5$^{a}$** | **300.7±65.4$^{a}$** | **3.62±0.58$^{a}$** | **18.58±1.98$^{a}$** | **4.5±0.5$^{a}$** |
| Smoky | 2000 | 80.8 | 35.9±0.1 | 187.2±0.6 | 2.68±0.03 | 11.89±0.14 | 3.83±0.15 |
| | 1999 | 78.7 | 51.3±0.1 | 241.0±0.5 | 2.52±0.20 | 11.82±0.96 | 3.36±0.46 |
| | 1998 | 81.3 | 36.8±0.1 | 196.6±0.5 | 2.37±0.09 | 12.67±0.47 | 3.93±0.15 |
| | **Average** | **80.3±1.4$^{a}$** | **41.3±7.5$^{b}$** | **208.2±25.2$^{b}$** | **2.52±0.16$^{b}$** | **121.3±0.47$^{b}$** | **3.7±0.3$^{a}$** |
| Blueberry | **Average** | **82.1±1.9$^{a}$** | **38.7±0.7$^{b}$** | **216.3±3.9$^{b}$** | **2.14±0.01$^{c}$** | **11.97±0.05$^{b}$** | |

[1] Values for each year represent a mean of 3 sub-samples. Values within each column of the table followed by a different letter are statistically different at $P< 0.05$.
Moisture contents were measured and calculated as percent wet basis.
Total anthocyanins were calculated as mg anthocyanins per 100 gram of fresh or dry weight of berries.
Total phenol contents were calculated based on a standard curve of gallic acid, as is expressed as mg gallic acid equivalents (GAE) per gram of fresh or dry weight of berries.

## Antioxidant Activity

### *DPPH Radical Scavenging*

The free radical scavenging activity for various concentrations of SBE towards the stable DPPH radical is shown in Table 4. There was a concentration dependent response, where increased scavenging activities were observed as the amount of the SBE increased. All SBE samples investigated, exhibited scavenging affinity to the DPPH radical, even at the lowest concentration tested (0.25mg/ml). At the highest concentration (4.0mg/ml) tested, all Thiessen varieties almost completely inhibited DPPH activity (greater than 91% inhibition). This extent of activity was not observed for the Smoky variety, which only registered 72 to 81% inhibition. At the same concentration, the Thiessen always exhibited higher ($p<0.05$) scavenging ability than the Smoky variety. An illustration of the varietal differences is shown in Figure 5, which depicts an average percent inhibition for the two varieties, as measured over the three harvest years. In addition, for the Thiessen variety, at all concentrations, exhibited significantly higher ($p<0.05$) capability to scavenge the DPPH radical than BBE. The Smoky variety, at all concentrations, was not significantly different ($p<0.05$) to the BBE in scavenging ability of the DPPH radical.

**Table 4.** DPPH scavenging activity of various concentrations of fresh frozen SBE and BBE. Values are expressed as % inhibition of DPPH radical. [1]

| | | Concentration (mg/ml SBE) | | | | |
|---|---|---|---|---|---|---|
| Variety | Year | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 |
| Thiessen | 2000 | 13.66±0.21 | 22.00±1.80 | 41.85±2.37 | 69.08±2.16 | 91.73±1.87 |
| | 1999 | 15.30±0.86 | 26.54±1.37 | 47.83±2.41 | 73.48±0.95 | 94.15±0.76 |
| | 1998 | 13.91±0.51 | 26.78±2.03 | 44.50±1.51 | 73.83±1.45 | 93.27±0.35 |
| | **Avg** | **14.3±0.9$^{ax}$** | **25.1±2.7$^{bx}$** | **44.7±3.0$^{cx}$** | **72.1±2.6$^{dx}$** | **93.1±1.3$^{ex}$** |
| Smoky | 2000 | 6.82±0.38 | 15.71±1.08 | 28.55±1.90 | 50.07±2.33 | 72.34±0.98 |
| | 1999 | 8.58±0.04 | 17.52±1.9 | 31.87±0.2 | 54.05±2.07 | 81.95±2.03 |
| | 1998 | 5.38±0.27 | 10.19±0.79 | 24.26±2.76 | 44.69±1.63 | 72.59±1.63 |
| | **Avg** | **6.9±1.6$^{ay}$** | **14.5±3.8$^{by}$** | **28.2±2.8$^{cy}$** | **49.6±5.0$^{dy}$** | **75.6±5.5$^{ey}$** |
| Blueberry | **Avg** | **7.1±0.8$^{ay}$** | **14.2±2.9$^{by}$** | **25.8±2.6$^{cy}$** | **49.9±3.5$^{dy}$** | **81.4±2.8$^{ey}$** |

[1] Values represent mean±SEM of 3 sub-samples.
Average of SBE for each variety calculated as pooled averages for the three harvest years, mean±SEM of averages per harvest year.
$^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ within a row.
$^{x, y}$: represent statistical difference at $P<0.05$ within a column.

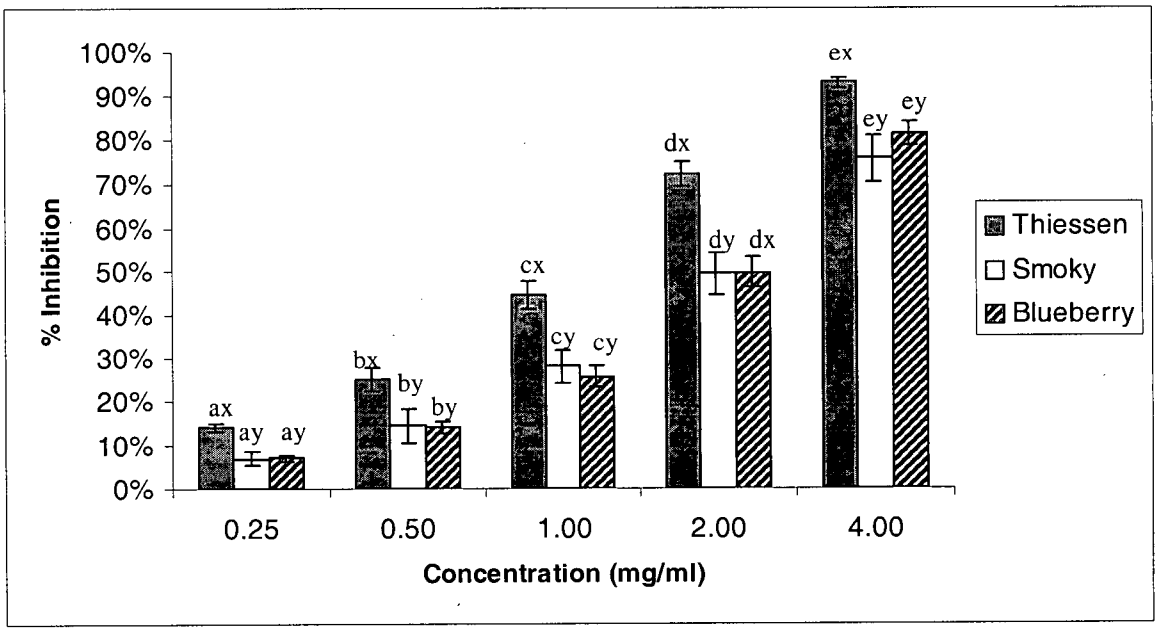



**Figure 5** Average DPPH scavenging activity of BBE and fresh frozen SBE. Calculated as pooled averages over the three harvest years. Activity is calculated as percent inhibition of the free radical.
[a, b, c, d, e]: represent statistical difference at $P<0.05$ among the different concentrations for each berry extract
[x, y, z]: represent statistical difference at $P<0.05$ between the different berry extracts

### *ABTS Radical Scavenging*

The free radical scavenging activity for SBE towards the stable ABTS radicals is shown in Table 5. There was also a concentration dependent response, whereby increased ABTS scavenging activities were observed as the concentration of the SBE increased. All SBE samples investigated exhibited scavenging activity, even at the lowest concentration tested (0.25mg/ml). At the highest tested concentration (4.0mg/ml), the Thiessen varieties showed almost complete inhibition of the ABTS radical (e.g. greater than 90% inhibition), while the Smoky variety had a lower inhibition at around 87% inhibition. Moreover, Thiessen SBE had significantly higher ($p<0.05$) affinity to scavenge the ABTS radical when compared to a blueberry extract (Figure 6). While, the Smoky SBE was not significantly different ($p<0.05$) from the BBE.

**Table 5.** ABTS scavenging activity of various concentrations of fresh frozne SBE and BBE. Values are expressed as % inhibition of ABTS radical. [1]

| | | Concentration (mg/ml SBE) | | | | |
|---|---|---|---|---|---|---|
| Variety | Year | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 |
| Thiessen | 2000 | 23.08±0.14 | 42.05±0.56 | 66.69±1.54 | 86.41±1.08 | 91.18±0.37 |
| | 1999 | 24.43±0.07 | 44.38±2.71 | 71.31±2.70 | 87.68±0.50 | 90.52±0.36 |
| | 1998 | 24.99±0.93 | 46.24±1.27 | 72.77±1.51 | 88.10±0.25 | 90.45±0.40 |
| | **Avg** | **24.3±1.0** $^{ax}$ | **44.4±2.1** $^{bx}$ | **70.6±3.4** $^{cx}$ | **87.4±0.9** $^{dx}$ | **90.7±0.4** $^{ex}$ |
| Smoky | 2000 | 13.04±1.44 | 24.84±1.12 | 44.54±2.36 | 72.19±1.84 | 87.74±0.57 |
| | 1999 | 14.97±1.21 | 28.27±0.94 | 51.90±2.21 | 78.69±1.24 | 88.53±0.121 |
| | 1998 | 12.87±2.77 | 26.64±4.81 | 45.85±2.72 | 73.26±2.06 | 86.56±1.36 |
| | **Avg** | **13.6 ±1.2** $^{ay}$ | **26.6±1.7** $^{by}$ | **47.4±3.9** $^{cy}$ | **74.7±3.5** $^{dy}$ | **87.6±1.0** $^{ey}$ |
| Blueberry | **Avg** | **13.4±0.4** $^{ay}$ | **25.7±1.5** $^{by}$ | **47.0±0.5** $^{cy}$ | **74.4±1.0** $^{dy}$ | **86.4±0.1** $^{ey}$ |

[1] Values represent mean±SEM of 3 sub-samples.
Average of SBE calculated as pooled averages for the three harvest years, mean±SEM of averages per harvest year.
$^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ within a row.
$^{x, y,}$: represent statistical difference at $P<0.05$ within a column.

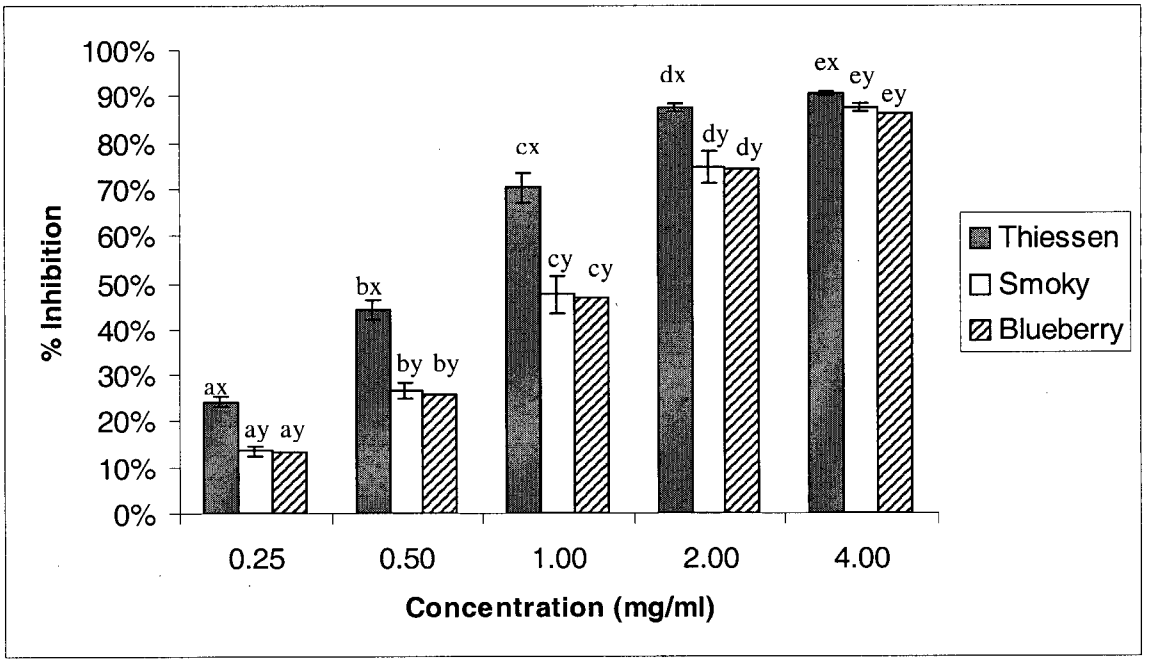



**Figure 6**. Average ABTS scavenging activity of BBE and fresh frozen SBE as pooled averages calculated over the three harvest years. Activity is calculated as percent inhibition of the free radical.

[a, b, c, d, e]: represent statistical difference at $P<0.05$ among the different concentrations for each berry extract

[x, y, z]: represent statistical difference at $P<0.05$ between the different berry extracts

## Reducing Power

Saskatoon berry extract reducing power at various concentrations was evaluated and presented in Table 6. There was a concentration dependent response in reducing power with high activities especially prevalent with higher extract concentration. All SBE had reducing activity, even at the low concentration of 0.5mg/ml of extract. At this concentration, reducing power was only 8.53μg AAE for the Thiessen variety, and 4.78μg AAE for the Smoky variety. At the highest concentration tested (4.0mg/ml), the Thiessen variety had reducing power of 47.54 μg AAE, as compared to the 29.49 μg AAE of reducing activity for the Smoky variety. The Thiessen variety had significantly higher ($p<0.05$) reducing power than the Smoky variety at all concentrations observed

(Figure 7). Reducing activities for Thiessen BBE were significantly higher ($p<0.05$) compared to BBE. The Smoky SBE showed no significant differences ($p<0.05$) in reducing activities compared to the BBE.

**Table 6.** Reducing power of various concentrations of fresh frozen SBE and BBE. Values are expressed as ascorbic acid equivalents (AAE).[1]

| | | Concentration (mg/ml SBE) | | | |
|---|---|---|---|---|---|
| Variety | Year | 0.5 | 1.0 | 2.0 | 4.0 |
| Thiessen | 2000 | 7.27±2.41 | 10.65±0.55 | 21.16±2.67 | 36.91±2.43 |
| | 1999 | 9.34±0.35 | 15.21±0.43 | 27.55±0.56 | 51.44±1.09 |
| | 1998 | 8.98±0.69 | 15.88±0.68 | 28.98±1.51 | 54.26±2.98 |
| | **Avg** | **8.53±1.11** $^{ax}$ | **13.91±2.85** $^{bx}$ | **25.89±4.17** $^{cx}$ | **47.54±9.31** $^{dx}$ |
| Smoky | 2000 | 4.67±1.56 | 8.13±0.75 | 15.13±2.50 | 26.78±1.98 |
| | 1999 | 5.11±0.98 | 9.08±0.94 | 16.78±1.52 | 32.25±1.39 |
| | 1998 | 4.55±0.74 | 7.08±0.56 | 15.43±1.67 | 29.43±1.10 |
| | **Avg** | **4.78±0.29** $^{ay}$ | **8.10±1.00** $^{by}$ | **15.78±0.88** $^{cy}$ | **29.49±2.74** $^{dy}$ |
| Blueberry | **Avg** | **5.81±0.45** $^{ay}$ | **9.89±0.21** $^{by}$ | **16.92±0.27** $^{cy}$ | **30.7±0.30** $^{dy}$ |

[1] Values represent mean±SEM of 3 sub-samples.
Average of SBE calculated as pooled averages for the three harvest years, mean±SEM of averages per harvest year.
$^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ within a row
$^{x, y}$: represent statistical difference at $P<0.05$ within a column

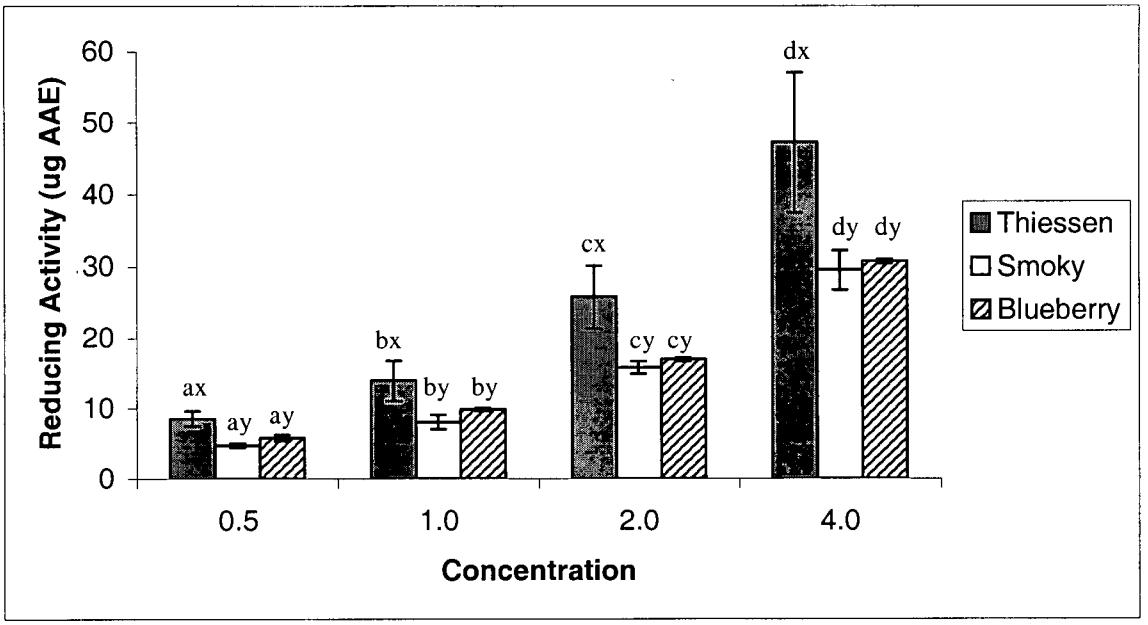



**Figure 7.** Reducing activity of fresh frozen BBE and SBE. Calculated as pooled averages over the three harvest years (2000, 1999, and 1998)).
[a, b, c, d, e]: represent statistical difference at $P<0.05$ among the different concentrations for each berry extract
[x, y, z]: represent statistical difference at $P<0.05$ between the different berry extracts

**Correlation Coefficients**

Correlation coefficients for all berry extract parameters measured are shown in Table 7. A significant correlation ($r=0.671$, $p<0.01$) was observed with the total anthocyanin concentration and the total phenolic content in the samples. Also, highly significant correlations ($p<0.001$) were observed for total anthocyanin concentration and antioxidant activities, for both DPPH ($r=0.769$) and ABTS ($r=0.823$) radicals. A highly significant correlation ($p<0.001$) was also observed with the total anthocyanin content and reducing power of the berry extracts ($r=0.946$). Moreover, significant correlations ($p<0.001$) were found between total phenolic content of berry with both antioxidant activity against DPPH ($r=0.884$) and ABTS ($r=0.843$) radicals, and with the reducing power ($r=0.673$, $p<0.01$). Between the DPPH and ABTS assays, the two are highly

correlated (r=0.941, p<0.001). The reducing power of the extracts was also highly correlated with results from the DPPH (r=0.723, p<0.01) and ABTS (r=0.740, p<0.001) radical scavenging activity.

**Table 7.** Correlation coefficients between antioxidant activity (DPPH and ABTS scavenging), reducing activity, total anthocyanin, total phenolic concentrations in fresh frozen Saskatoon berries for triplicate measurements in the two varieties (Thiessen and Smoky) over the three harvest years (2000, 1999, 1998) (n=18).

| | **Total Anthocyanin (c3g/100 g dry fruit)** | **Total Phenolics (GAE/g dry fruit)** | **Antioxidant Activity: DPPH scavenging (% Inhibition)** | **Antioxidant Activity: ABTS scavenging (% Inhibition)** |
|---|---|---|---|---|
| **Total Phenolics** | 0.671** | | | |
| **DPPH** | 0.796*** | 0.884*** | | |
| **ABTS** | 0.832*** | 0.843*** | 0.941*** | |
| **Reducing** | 0.946*** | 0.673** | 0.723** | 0.740*** |

**, *** designate significance at P<0.01 and P<0.001, respectively.

## DISCUSSION

Cultivar, source, relative maturity of berries at harvest, and year of production are important factors with an appreciable effect on the total anthocyanin and total phenolic content in the Saskatoon berry fruit (Green and Mazza, 1986). The general fruit characteristics measured for the Saskatoon berry extracts (SBE) in this study indicated that the Thiessen variety contained a higher total anthocyanin and total phenolic content when compared to the Smoky variety for all the years tested. The varietal difference was maintained in values expressed on a dry weight basis, as well as a fresh weight basis.

There was no identifiable trend in terms of harvesting year for both the total anthocyanin or total phenol content measurements. Thus it can be suggested that the harvest year has relatively little effect on prediction of compositional parameters for

Saskatoon berries; or more correctly stated, has an unpredictable effect on the compositional parameters. This finding is possibly due to the fact that year to year changes occur on so many levels (including weather conditions, time of harvest, fertilizer, soil conditions, etc) and therefore different varieties may respond differently to these changes. In light of the fact that the year of harvest was not a good indicator for estimation of berry constituent composition; a decision was made to collectively pool the data over the three year harvest period.

The SBE from the Thiessen variety had significantly higher ($p<0.05$) total anthocyanin and total phenol contents, compared to the Smoky variety. Values of total anthocyanins for Saskatoon berries were within the range found in the literature (25.1 to 178.7 mg/100g of fruit) (Green and Mazza, 1986; Mazza and Miniati, 1993). But because total phenolics for Saskatoon berries were calculated differently in literature sources, using different standards of assessment (Green and Mazza, 1986), comparison for total phenol content in Saskatoon berries could not be carried out. Moreover, higher anthocyanin and phenolic content was noticed for Thiessen SBE but not Smoky SBE compared to an experimental blueberry extract (BBE). This indicates that Saskatoon berries, in this study, depending on variety, can have either comparable (Smoky) or higher (Thiessen) total anthocyanin and total phenolic content when compared to that of the experimental BBE. Fukumoto and Mazza (2000) on comparison of several berry extracts also found similar characteristics between the blueberry and Saskatoon (cv. Smoky) extracts. Mazza (1982) also reported similar chemical compositions between these two berries. This is an important observation as most of the research in fruit antioxidant activity to date had been performed mostly with blueberries and other soft

fruit berries (Cornelia and Wrolstad, 1995; Bridle and Garcia-Viguera, 1997; Wang et al., 1997; Kalt et al., 1999a). If the compositional data and fruit characteristics are similar for the Saskatoon berries and blueberries, then there may also be potential comparisons with the purported beneficial effects that have been investigated with blueberries concerning human disease prevention. Considerable evidence has indicated that high anthocyanin and phenol content of soft fruits is associated with their antioxidant capacity (Wang et al., 1996; Heinonen et al., 1998; Prior et al., 1998; Ghiselli et al., 1998; Kalt et al., 2000; Rapisarda et al., 1999) similar to the results found in this study.

The SBE investigated in this study scavenged free radicals of DPPH and ABTS in a concentration dependent manner (Figure 5, 6). The two antioxidant assays gave very similar results in terms of the affinity of SBE to scavenge these individual free radicals ($r=0.941$). Other researchers have reported these two assays as being relatively easy to conduct, highly reproducible and accurate to evaluate the antioxidant activity of fruit and vegetable juices or extracts (Sanchez-Moreno, 2002). These assays have been applied to anthocyanin-based natural colorants from berries (Espin et al., 2000b), grape pommace (Larrauri et al., 1998), and fruits (Leong and Shui, 2002). In terms of berry variety, the Thiessen variety, in addition to having higher total anthocyanin and total phenolic contents, also had a relatively higher scavenging capacity for the two free radicals as compared to the Smoky berries. This is the first time that different varieties of Saskatoon berries have been shown to have different antioxidant capacities. When compared to the scavenging ability of the Saskatoon berries with an experimental blueberry extract (BBE), it was also found that the Thiessen SBE was more superior. However, the Smoky

SBE was not significantly different to the BBE in scavenging either of the DPPH or ABTS radicals.

The phytochemicals found to be linked to the antioxidant activity in SBE were primarily the phenolic acids, anthocyanins, and other flavonoid compounds (Cao et al., 1997). It is important to point out that vitamin C is not present in any appreciative amounts in Saskatoon berries (Panther and Wolfe, 1972). Thus the associated antioxidant capabilities found in Saskatoon berries does not come from ascorbic acid; contrary to findings found in other similar soft fruits. In this experiment, a high correlation was found between antioxidant activities and both the total anthocyanin and total phenolic contents. Similar conclusions with blueberries have been reported in a study by Prior and colleagues (1998). A linear relationship existed between the antioxidant capacity and anthocyanin or total phenolic content for blueberries. Kalt and colleagues (1999a; 2000) also reported a strong correlation between the total antioxidant capacity with the content of total phenolic content and anthocyanin content in blueberries, strawberries, and raspberries.

Reducing activity was found in all berry extracts tested as measured by the reducing power. Again, the reducing power was found to be higher in the Thiessen berries compared to the Smoky berries over the three year period. Similar to the total anthocyanin, total phenolic, and free radical scavenging assays of DPPH and ABTS, the Thiessen SBE was found to be significantly more superior when compared to the BBE; whereas, the Smoky SBE was not significantly different. Saskatoon berry extracts, with the highest reducing power correlated well with antioxidant activity ($r=0.723$, $p<0.01$ and $r=0.740$, $p<0.001$ for the DPPH and ABTS assays respectively). Reducing power also

correlated well with the total anthocyanins content ($r=0.946$, $p<0.001$) and with the total phenolic content ($r=0.673$, $p<0.01$) in the berry extracts. Along with findings from both the DPPH and ABTS assays, it was suggested that SBE could act as a hydrogen- or electron donating antioxidant (Antolovich, et al. 2002). Thus, it can be suggested the anthocyanins and phenolics present in SBE, are mostly the constituents that contribute to the electron and hydrogen donor property and thereby terminating the radical chain reaction by converting free radicals to more stable products.

It is important not to overlook the fact that, the reducing power may also contribute to a prooxidant activity when in the presence of free transition metal ions. Cupric ions have been shown to mediate the peroxidation of liposome through a decomposition of trace amounts of lipid peroxide in phospholipid (Coupland and McClements, 1996). Studies have shown many examples of where reducing power can elicit a prooxidant effect from phytochemicals of origin from *Echinacea* (Hu and Kitts, 2001), ginseng (Hu and Kitts, 2001b), and bamboo (Hu et al., 2000). The reducing power of SBE enabled the conversion of $Cu^{2+}$ to $Cu^{+}$, which favors the Fenton reaction; and this in turn promotes the peroxidation of lipids by generation of hydroxyl radicals. Hu and Kitts (2001a) also demonstrated that epigallocatechin gallate reduced cupric ions and showed prooxidant activities in an LDL oxidation model. Ascorbic acid in vitro provides similar results, which manifests either antioxidant or prooxidant activity (Otero et al., 1997; Gerster, 1999; Paolini et al., 1999). Wang and Mazza (2002) have shown that Saskatoon berries possess prooxidant effect as well as its antioxidant properties.

Mazza (1986) identified the major anthocyanins in the Smoky variety of Saskatoon berries to include cyanidin 3-galactoside (61%) and cyanidin 3-glucoside

(21%) of the total anthocyanins. Thus, it could be suggested that the associated antioxidant activities of Saskatoon berries may originate from these anthocyanin sources. Due to the existence of di-ortho-hydroxy groups, cyanidin is more likely to contribute to the antioxidant activity (Pratt and Hudson, 1990). A more detailed analysis of the anthocyanin composition of the Saskatoon berries investigated in this study would be required, to attribute associated antioxidant activity more precisely to the specific phytochemical component(s) responsible, as the different cultivars will most contain varying concentrations or profiles of the anthocyanins.

There has been no previous phenolic profile performed with Saskatoon berries, and thus a more detailed analysis of the phenolic composition of the Saskatoon berries will also be required in future studies.

Based on results from this study, Saskatoon berries can be considered as a good source of anthocyanins and phenolics with potential antioxidant activity *in vitro*. Extrapolation of this finding to *in vivo* conditions has not been attempted. A study performed by Wang and Mazza (2002) reported the inhibitory effects of anthocyanins and berry phenolic compounds (from Saskatoon berries, blackberries, blueberries, and black currants) on nitric oxide (NO) production. NO production has been associated with oxidative stress and effects cardiovascular diseases and chronic inflammatory diseases (Maeda et al., 1998; Akaike et al., 2000). This preliminary finding that some anthocyanins and berry phenolics contribute to the inhibition of NO production *in vitro* provides promising suggestions towards possible *in vivo* benefits associated with these compounds. Results from a blueberry study has shown that anthocyanins can be absorbed in their intact glycosylated and possibly acylated forms in human subjects and

that consumption of these berries was associated with a diet-induced increase in *ex vivo* serum antioxidant status (Mazza et al., 2002).

Based on the results from this study, Saskatoon berries can be considered as good sources of anthocyanins and phenolics and also as antioxidants. The potential beneficial antioxidant activities of fruits such as cranberries and blueberries are actively exploited and emphasized by producers and processors of these fruits. The results of the Saskatoon berry study offer similar opportunities for the Saskatoon berry industry. The information in this respect can lead to the use of Saskatoon berries and Saskatoon berry extract in functional foods, nutraceuticals and natural health products.

## CONCLUSION

Saskatoon berry extracts obtained from two varieties (Thiessen and Smoky) for all three harvest years (2000, 1999, and 1998) were tested in this experiment. Fruit characteristics, for Saskatoon berries, when expressed as an average of the three harvest years are comparable to that of blueberries in terms of total anthocyanin, and total phenol contents. All berry extracts possessed antioxidant activities as measured by free radical scavenging activities to DPPH and ABTS radicals. The relative antioxidant properties for the berry extracts were accounted for by notable differences in anthocyanin and phenolic contents. A varietal difference between SBE was noticed for all parameters measured, in which the Thiessen berries consistently showed higher total anthocyanins, total phenols content and greater free radical scavenging, reducing activities when compared to the Smoky variety. The reducing powers of these extracts was investigated and correlated highly with the antioxidant capacity. The antioxidant activity of the SBE

was suggested to be due to an affinity to act either as a good electron or hydrogen donor, thereby effectively terminating the radical chain reactions by converting free radicals to more stable products.

# EXPERIMENT 2: COMPARISON OF DIFFERENT DEHYDRATION TECHNIQUES ON THE FRUIT CHARACTERISTICS AND ASSOCIATED ANTIOXIDANT ACTIVITIES OF SASKATOON BERRIES

## INTRODUCTION

Dehydration is one of the oldest and efficient methods of food preservation in chemical and food processing industries. The basic objective in drying food products is the removal of water from solids to a level at which microbial spoilage is avoided. The amount of thermal damage endured by a product during dehydration processes is directly proportional to the temperature and time involved. Conventional air drying has the major disadvantage of imparting thermal degradations of important flavor and nutritional substances due to thermal degradation. The high temperature and long time processes associated with conventional hot-air drying most often adversely affects texture, color, flavor, and nutritional value of the product (Schadle et al., 1983; Yang and Atallah, 1985; Yongsawatdigul and Gunasekaran, 1996b). Although freeze drying can be applied to avoid heat damage and produce products with excellent structural retention, it is a costly process and is only suitable for high-value products. Moreover, freeze drying usually causes large losses of flavor volatiles (Flink, 1975).

Vacuum microwave drying has been successfully used to prevent significant losses in a product due to thermal degradation. Due to the absence of convection, the removal of moisture is accelerated and heat transfer to the solid phase is slowed down significantly. The low temperature and fast mass transfer obtained by vacuum (Yongsawatigul and Gunasekaran, 1996a), combined with rapid energy transfer by microwave heating, generates very rapid, low temperature drying (Lin et al., 1999b). Successful applications of microwave vacuum technology in drying of food products

have been reported with many food products including: potato chips (Durance and Liu, 1997), cranberries (Yongsawatidigul and Gunasekaran, 1996a,b), grapes (Petrucci and Clary, 1989), carrots (Lin et al., 1999b), oregano (Yousif et al., 2000), and Echinacea (Kim et al., 2000a,b).

Many of the beneficial biological properties of fruits and vegetables are believed to be associated with the antioxidant activity of anthocyanins pigments, flavonoids, and other phenolic compounds (Rice-Evans et al., 1996; Wang et al., 1997). Saskatoon berries (*Amelanchier alnifolia Nutt.*) have been shown to contain high anthocyanin and phenolic contents (Green and Mazza, 1986). Knowledge of the changes that the anthocyanin pigments undergo with processing is important with respect to the role in color quality. Anthocyanins as well as other polyphenols are readily oxidized because of their antioxidant properties and, thus, susceptible to degradative reactions during various processing unit operations. Because of possible beneficial roles, it is critical that changes in polyphenols during processing be measured and evaluated to better assess the dietary value of the processed products. Moreover, the changes in polyphenols during processing will be associated with changes in the antioxidant properties. Thus, a measure of the antioxidant capacity of the processed products must also be addressed. The aim of this experiment was to evaluate and compare the effects of the different drying methods, such as freeze-drying, vacuum microwave drying with full vacuum, air-drying, and a combination of air-drying with vacuum microwave drying on the retention of the fruit characteristics and the associated antioxidant activity in Saskatoon berries.

## MATERIALS AND METHODS

Saskatoon berries were supplied by the Riverblend Plantation Gourmet Foods (Saskatoon, Saskatchewan, Canada). Metaphosphoric acid, sodium hydroxide, and potassium dihydrogen orthophosphate were from BDH Inc. (Toronto, ON). Methanol, sodium bicarbonate ($NaHCO_3$), sodium acetate, and trichloroacetic acid (TCA) was obtained from Fisher Scientific (Fair Lawn, NJ). Methanol, acetic acid and hydrochloric acid were obtained from Fisher Scientific (Napean, ON). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (diammonium salt, ABTS), AAPH (2,2'-azobis (2-amidinopropane) dichloride), AAPH (2,2'-azobis (2-amidinopropane) dichloride), ferric chloride ($FeCl_3$), potassium chloride, Ciocalteau's reagent, and gallic acid were purchased from Sigma Co. (St. Louis, MO). Agarose was obtained from Bio-Rad Laboratories (Richmond, CA). Flat-bottom, 96-well EIA microtitration plates were obtained from Corning Inc. (Corning, NY).

### Moisture Content

Moisture contents were measured according to methods previously described on page 37.

### Drying of Saskatoon Berries

#### *Freeze-Drying*

Saskatoon berries were placed in 8" pans, covered with foil and stand in a –35°C cold room for three days. After they were completely frozen, berries were freeze dried for 48 hours. The dried berries were vacuum packed in sealed polyethylene bag. The

final water activities of the berries were determined using an AquaLab®, Model Series 3 (Decagon Devices, Inc.; Pullman, WA).

### *Air-Drying*

Saskatoon berries were placed in 8" pans, lined with aluminum foil, and placed in the convection dryer that was set at 75°C. Berries were dried for approximately 3 days until the final water activities reached a range value of 0.330 to 0.420 (AquaLab®, Model Series 3; Decagon Devices, Inc.; Pullman, WA).

### *Vacuum Microwave Drying*

One kilogram of Saskatoon berries was packaged in a sealed polyethylene bag. At least one kilogram in quantity of berries was required for the vacuum microwave drying process because at least 100 grams of dried products must remain in the vacuum microwave basket after drying. Damages to the basket and vacuum microwave dryer would occur if less than 100 grams of product is in the basket during drying. The packaged berries were put inside a polyethylene bag and then were thawed in cold water. When completely thawed, berries were transferred to the basket for drying in the vacuum microwave dryer. The basket was weighed prior to putting in the berries. Then the basket with the berries was weighed and recorded. The basket was placed into the vacuum microwave dryer to begin the drying process. Microwave power was set at 1.77 kilowatts, with a vacuum set at 30 Torr, and an RPM of 3. The drying process continued for approximately 20-25 minutes until the final water activities of the berries lie within a

range of 0.330 to 0.420 (AquaLab®, Model Series 3; Decagon Devices, Inc.; Pullman, WA).

### *Combination Drying*

A combination drying method which combined both air and microwave vacuum was performed, in order to assess whether a significant drip loss occurred during the vacuum microwave drying of the Saskatoon berries. Berries were first air-dried to remove approximately 50 to 60% of the moisture content. After air-drying, further drying of the berries was done using a vacuum microwave dryer until water activities reached a range of 0.330 to 0.420 (AquaLab®, Model Series 3; Decagon Devices, Inc.; Pullman, WA).

## Saskatoon Berry Extracts

Fractions of Saskatoon berries were extracted according to method as previously described on page 36.

## Berry Characterization

### *Total Monomeric Anthocyanin Content*

Total monomeric anthocyanin content was determined according to the method described previously on page 38.

### *Total Phenolic Content*

Total phenol content was determined according to the method previously described on page 39.

### *Soluble Solids Content*

Soluble solids of berry extracts content were measured using the refractometer at 20°C and expressed as Brix° amounts (AOAC, 1990).

## Reducing Power

The reducing power of berry extracts were analyzed as previously described on page 42.

## Antioxidant Assays

### *Free Radical Scavenging Activity*

The activity of SBE to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured according to the method previously described on page 40.

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation model was used to evaluate the free radical scavenging effect of SBE, as previously described on page 41.

The comparison of the scavenging effects of SBE with the DPPH and ABTS radicals were transformed to Trolox (a vitamin E analogue) equivalents (TE) (μmol of TE/mg of SBE), calculated from to a standard curve for Trolox. Triplicate measurements were made to produce the curve at concentrations of Trolox at 0, 12.5, 25, 50, 75 μmol.

*DNA breaking activity*

The SBE's were analyzed for antioxidant activity by the method of AAPH induced DNA breaking activity assay, based on Wijewickreme and Kitts (1998) with modifications by Hu and Kitts (2001). 2 µl of plasmid supercoiled strand DNA (pB322 DNA, Sigma), 2 µl SBE sample, 2 µl AAPH (30mM), and 6 µl PBS buffer (10 mM, pH 7.4) were mixed and vortexed in a PBS buffer (prepared using 0.2 g KCl, 0.2 g $KH_2PO_4$, 1.43 g $Na_2HPO_4$, and 8 g NaCl made up to one liter and adjusted to pH 7.4 with NaOH, and then autoclaved). The reaction mixture was incubated at 37°C for 2 hours at 20 rpm. After the incubation period, 2 µl of loading buffer was added to each sample and the resulting mixture (14µl) was loaded onto an agarose gel [0.35g agarose, 1x TAE (Tris-acetate acid-EDTA, 50mM) buffer (pH 8.5) 2.5µl ethidium bromide] set in a horizontal electrophoresis chamber (E-C apparatus Corp., St. Petersburg, FL, USA), and filled with running buffer (1x TAE) at 72V for 1 hr. Image analysis was visualized under ultraviolet light and a picture was taken using a Polaroid instant camera. Electrophoresis images were analyzed by an image densitometer (GS-670, Bio-Rad) with Molecular Analyst software (version 1.3, Bio-Rad). The protective effect of test antioxidants were compared by the retention percentage (% Ret), calculated by the following equation:

$$\%Ret = (concentration_{sample}/concentration_{control}) \times 100$$

where, $concentration_{sample}$ and $concentration_{control}$ represent the supercoiled DNA with the oxidative radical AAPH and without oxidative radical, respectively

**Statistical Analysis**

Experiments were performed in triplicates and results were expressed as mean ± SD. One-way ANOVA was used to compare the differences between treatments with significant level set at $p<0.01$ (Minitab for windows, version 13.32, State College, PA)

## RESULTS

### Berry Characteristics

Fruit characteristics, including total anthocyanin, total phenol content, and total soluble solids content for each berry extract, which had undergone different dehydration procedures, are presented in Table 8. Data have been grouped by Saskatoon berry variety for each processing method and expressed as mean values over three consecutive harvest years (2000, 1999, and 1998). In addition, amounts over the three years were averaged for all samples measured, combining the two Saskatoon varieties. The overall average for the total soluble solid contents were not significantly different among all dehydrated berry extracts and ranged between 11.2 to 12.5 Brix °.

The anthocyanin content was found to be consistently higher ($p<0.05$) in the Thiessen berries, as compared to the Smoky berries regardless of the dehydration process used. For example, total anthocyanin content for the fresh frozen, unprocessed berries, were 300.7mg/100g and 208.8mg/100g for the Thiessen and Smoky varieties, respectively. Dehydration processing contributed to a marked loss ($p<0.05$) of total anthocyanin content in these berries, as seen with the lower amounts of the anthocyanins obtained after specific dehydration processing methods. Total anthocyanin content in freeze dried (FD) berries were 203.5mg/100g and 161.2mg/100g berry for the Thiessen and Smoky varieties, respectively. Relatively lower anthocyanin concentrations were found in the vacuum microwave dried berries, where the Thiessen variety contained 148.9mg/100g versus 96.9mg/100g for the Smoky variety. The combination dried (CD) Saskatoon berries contained even lower amounts of anthocyanins relative to freeze dried berries for the Thiessen and Smoky varieties; averaging only 115.4 and 77.2mg/100g

respectively ($p<0.05$). The lowest retention of total anthocyanins among all methods employed occurred after air drying (AD) Saskatoon berries; where the Thiessen had 49.9mg/100g and Smoky variety had 25.8 mg/100g berries.

**Table 8.** Total anthocyanin, total phenolic, and total soluble solids content of Saskatoon berry for the five different processing methods.

| Processing Method | Variety | Total Anthocyanin (mg/100 g dry wt) | Total Phenol (μg GAE/g dry wt) | Total Soluble Solids (°Brix) |
|---|---|---|---|---|
| Fresh Frozen | Thiessen* | 300.7±65.4 | 18.58±1.98 | 4.5±0.5 |
| | Smoky* | 208.2±25.2 | 12.13±0.47 | 3.7±0.3 |
| | ***Avg*** | **254.5±67.72[a]** | **15.35±3.76[a]** | **4.1±0.4[a]** |
| Freeze Dried | Thiessen* | 203.5±5.2 | 12.38±0.47 | 12.2±0.3 |
| | Smoky* | 161.2±3.4 | 10.96±0.65 | 12.8±0.5 |
| | ***Avg*** | **182.3±27.8[b]** | **11.67±0.83[b]** | **12.5±0.5[b]** |
| Vacuum Microwave Dried | Thiessen* | 148.9±4.0 | 8.94±0.48 | 11.7±0.5 |
| | Smoky* | 96.9±20.8 | 7.73±0.26 | 11.8±0.5 |
| | ***Avg*** | **122.9±29.7[c]** | **8.33±0.71[c]** | **11.7±0.5[b]** |
| Air Dried | Thiessen* | 49.9±5.0 | 6.40±0.39 | 11.9±1.1 |
| | Smoky* | 25.8±3.6 | 5.27±0.76 | 10.4±0.4 |
| | ***Avg*** | **37.8±12.9[d]** | **5.83±0.78[d]** | **11.2±1.1[b]** |
| Combination Dried | Thiessen* | 115.4±18.7 | 8.10±0.42 | 12.0±0.5 |
| | Smoky* | 77.2±6.3 | 6.87±0.64 | 12.0±0.5 |
| | ***Avg*** | **96.3±21.8[c]** | **7.68±1.13[c]** | **12.0±0.5[b]** |

Total anthocyanins were calculated as mg anthocyanins per gram dry weight of berries.
Total phenol contents were calculated based on a standard curve of gallic acid, as is expressed as μg gallic acid equivalents (GAE) per gram of dry weight of berries.
Average values within each column for the individual dehydration methods followed by a different letter are statistically different at $P < 0.05$.
*Average pooled data are reported from samples collected over the three harvest years (2000, 1999, and 1998).

Looking at the total anthocyanin content in Saskatoon berries following the different processing methods (Figure 8), it can be noted that the trend in loss of this particular fruit characteristic occurred as follows. Freeze drying produced the greatest retention of anthocyanins (least loss was observed with the FD samples at, e.g. 182.3mg/100g, or 73% retention of the original unprocessed extract). This was followed by berries from the VMD and CD processing methods at 122.9mg/100g and 96.3mg/100g, respectively. Finally, the use of AD proved to yield the worst retention of anthocyanins (e.g. only 37.8mg/100g), which was statistically ($p<0.05$) much lower than any of the other processing methods examined. The retention of anthocyanins in berries processed by AD was only about 15% of the unprocessed extract.

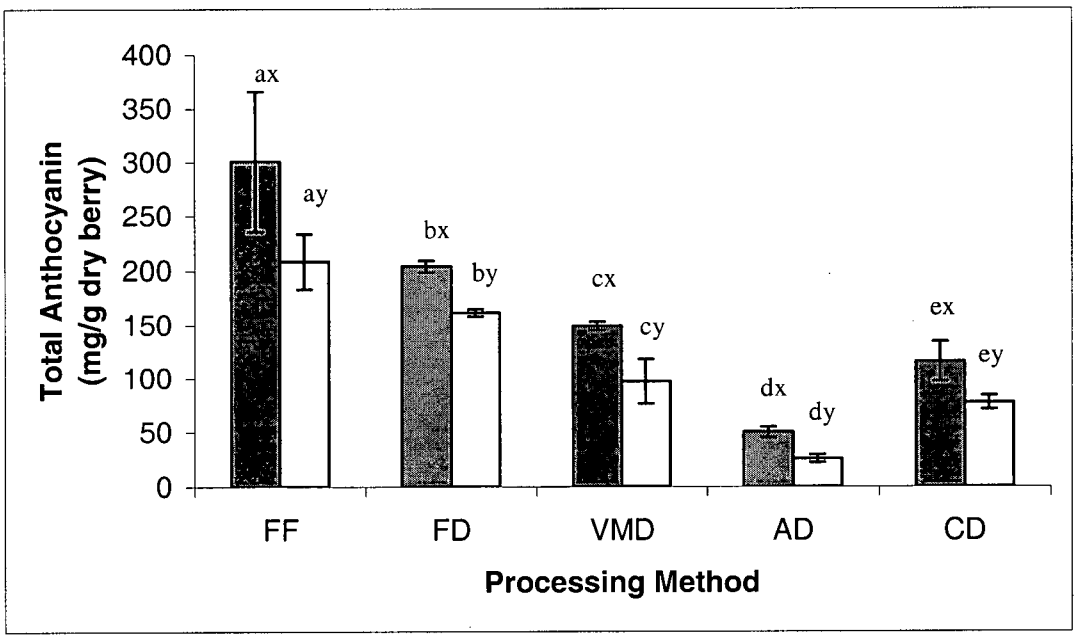



**Figure 8.** Total monomeric anthocyanin content of Saskatoon berry extracts from five different processing methods. Results are expressed as Mean ± SD, n = 6 (3 years, 2 varieties). ■ represents Thiessen variety; □ represents Smoky variety. FF- fresh frozen; FD – freeze dried process; VMD – vacuum microwave dried process; AD – air dried process; CD –combination dried process (which is a combination of air drying and vacuum microwave drying. $^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ among the different processing methods for each individual variety. $^{x, y}$: represent statistical difference at $P<0.05$ between the two varieties.

The results for total phenolic content in all berry samples produced similar trends to that obtained for the total anthocyanin content. A significant ($p<0.05$) decrease in the phenol content was observed in processing berries, as compared to the unprocessed berries. Total phenolic contents in FD berries were 12.38 μg GAE/g and 10.96 μg GAE/g berry for the Thiessen and Smoky varieties respectively. In berries processed using VMD, the phenolic content of the Thiessen variety was 8.94 μg GAE/g, and the Smoky variety was 7.72μg GAE/g. The CD processed berries had lower ($p<0.05$) phenolic content for both Thiessen and Smoky varieties, averaging only 8.10 and 6.87μg GAE/g respectively. The lowest retention of phenolics was observed in the air dried (AD) berries, where the Thiessen had only 6.40μg GAE/g and Smoky variety had 5.21μg GAE/g berries. A varietal difference was also noted for the total phenolic content, regardless of dehydration method, with the Thiessen variety containing the greater phenolic content ($p<0.05$) (Figure 9).

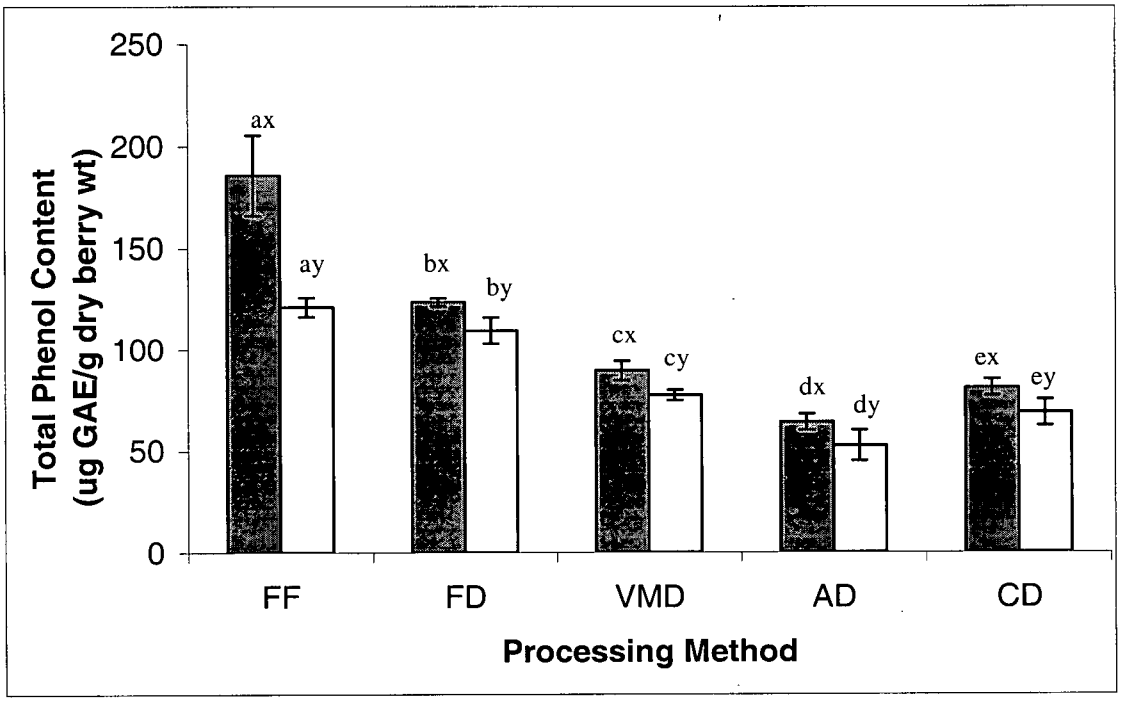



**Figure 9.** Total phenolic content of Saskatoon berry extracts from five different processing methods. Results are expressed as Mean ± SD, n = 6 (3 years, 2 varieties). ■ represents Thiessen variety; □ represents Smoky variety. FF- fresh frozen; FD – freeze dried process; VMD – vacuum microwave dried process; AD – air dried process; CD – combination dried process (which is a combination of air drying and vacuum microwave drying). $^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ among the different processing methods for each individual variety. $^{x, y}$: represent statistical difference at $P<0.05$ between the two varieties.

From the overall average for individual dehydration methods for the two varieties, it can be seen that the total phenol content averaged 15.35μgGAE/g dry weight for the frozen, unprocessed, extracts. For FD berry extracts, the phenolic content averaged 11.67μg GAE/g. This represented the highest retention of phenolics compared to all other processing methods, retaining total phenolics of the unprocessed FF extract of about 76%. VMD and CD processing methods retained approximately 50% (8.33μg GAE/g and 7.68 μg GAE/g) total phenolics respectively. These two processes were not significantly different to one another from the standpoint of retention of berry phenolics. The lowest retention of total phenolics in processed Saskatoon berries was found with the

AD method, retaining only 5.83 μg GAE/g. This was statistically ($p<0.05$) much lower other dehydration processing methods. The AD processed berries contained only 38% of the amount of phenolics as compared to the unprocessed FF berries.

**Reducing Power**

The reducing power of SBE derived from berries processed by the various dehydration techniques was evaluated and is presented in Table 9. A standard concentration of 1mg/ml of SBE was used for all samples to measure reducing capacity. All dehydrated berries showed reducing activities that were significantly ($p<0.05$) lower than that of the unprocessed (FF) extract. Of the four different dehydration methods employed, the FD method produced berries that had the highest ($p<0.05$) reducing ability; FD berries registered about 58% (30.4μg AAE) the reducing power of the unprocessed FF extract. All other methods were indifferent to one another in terms of their reducing activity (VMD at 19.7μg AAE, AD at 17.5μg AAE, and CD at 19.2μg AAE). These methods gave reducing activities at around 33-38% as compared to the unprocessed FF extract. The Thiessen berries had higher reducing power than the Smoky varieties, as seen across all processing methods. The relations between the different processing methods and the reducing power of the Saskatoon berry extracts are depicted in Figure 10.

**Table 9.** Reducing power, DPPH radical scavenging, and ABTS radical scavenging capacities of Saskatoon berries undergone the various dehydration methods.

| Processing Method | Variety | Reducing Power (μg AAE/mg) | DPPH radical scavenging (μmol TE/mg) | ABTS radical scavenging (μmol TE/mg) |
|---|---|---|---|---|
| Fresh Frozen | Thiessen* | 65.5±9.4 | 158.1±11.3 | 149.8±5.6 |
| | Smoky* | 38.9±3.4 | 92.8±2.6 | 91.7±1.7 |
| | ***Avg*** | **52.2±15.9$^{a}$** | **125.4±36.9$^{a}$** | **120.8±32.8$^{a}$** |
| Freeze Dried | Thiessen* | 32.6±1.5 | 92.8±3.6 | 85.9±7.5 |
| | Smoky* | 28.3±0.5 | 84.1±3.0 | 72.2±1.9 |
| | ***Avg*** | **30.4±2.9$^{b}$** | **88.4±6.5$^{b}$** | **79.1±8.7$^{b}$** |
| Vacuum Microwave Dried | Thiessen* | 22.0±1.2 | 56.9±3.3 | 51.8±3.8 |
| | Smoky* | 17.4±0.7 | 42.6±3.2 | 39.2±6.6 |
| | ***Avg*** | **19.7±2.7$^{c}$** | **49.7±8.2$^{c}$** | **45.5±8.2$^{c}$** |
| Air Dried | Thiessen* | 20.5±1.3 | 41.5±0.5 | 35.7±2.7 |
| | Smoky* | 14.5±0.7 | 24.1±1.6 | 23.2±3.6 |
| | ***Avg*** | **17.5±3.3$^{c}$** | **32.8 ±9.1$^{d}$** | **29.4±7.3$^{d}$** |
| Combination Dried | Thiessen* | 20.7±0.6 | 47.8±2.3 | 43.8±29.0 |
| | Smoky* | 17.8±1.0 | 35.5±8.1 | 27.8±1.7 |
| | ***Avg*** | **19.2±1.8$^{c}$** | **41.6±8.0$^{cd}$** | **36.4±9.0$^{d}$** |

* designates pooled data collected over the three harvest years (2000, 1999, and 1998). Reducing power measured as micrograms of ascorbic acid equivalence (AAE) at 1mg/ml of extract. DPPH and ABTS radical scavenging assays measured as umol of Trolox equivalent (TE) at 1mg/ml of extract. Average values within each column for the individual dehydration methods followed by a different letter are statistically different at $P< 0.05$.

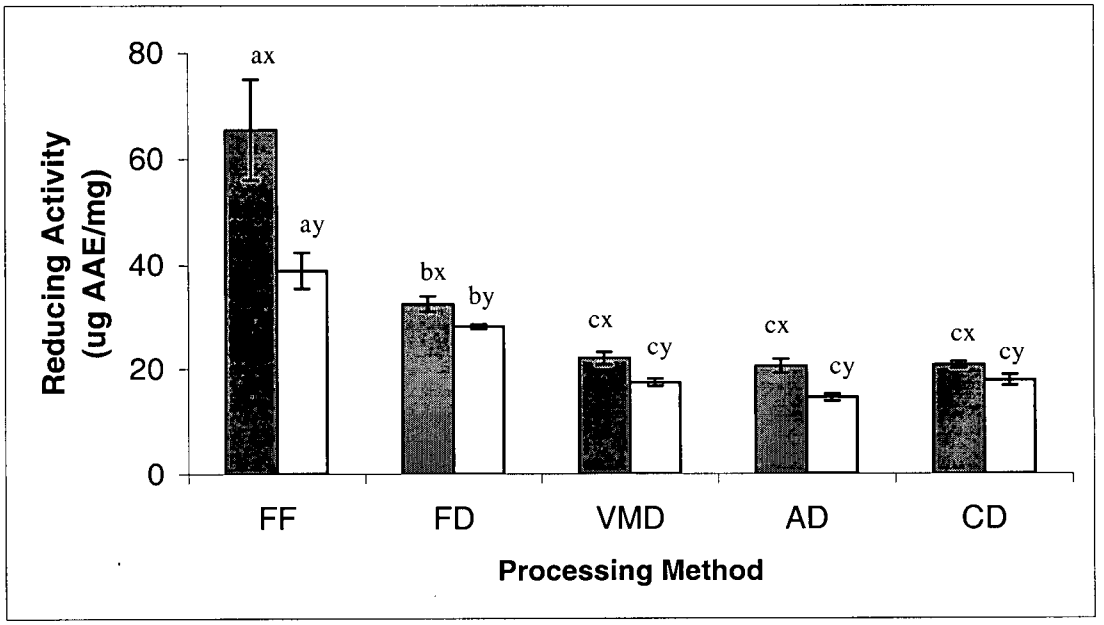



**Figure 10**. Reducing power of the Saskatoon berry extracts from five different processing methods. Results are expressed as Mean ± SD, n = 6 (3 years, 2 varieties). FF- fresh frozen; FD – freeze dried process; VMD – vacuum microwave dried process; AD – air dried process; CD –combination dried process (which is a combination of air drying and vacuum microwave drying). $^{a, b, c, d, e}$: represent statistical difference at P<0.05 among the different processing methods for each individual variety. $^{x, y}$: represent statistical difference at P<0.05 between the two varieties.

## Antioxidant Activity

### DPPH radical scavenging

The scavenging activities for the DPPH radical by Saskatoon berry extracts obtained from berries that underwent different dehydration processes are presented in Table 9. All dehydrated samples had significantly ($p<0.05$) lower DPPH radical scavenging capacities than the non-processed FF samples. FD processed samples produced significantly ($p<0.05$) higher DPPH scavenging activities (at 88.4μmol TE) than the different dehydration methods, which accounted to 70% of the original activity found in unprocessed FF extract. The VMD and CD processed berries were not significantly ($p<0.05$) different in respective affinities to scavenge free radical DPPH, but

both were significantly ($p<0.05$) lower than the scavenging activity found from berries processed using the FD method. The VMD method provided 49.7μmol TE, and the CD method provided 41.6μmol TE, these two methods retained approximately 40% and 33% scavenging activity, respectively. The AD processed Saskatoon berries had the lowest affinity to scavenge the DPPH radical, at only 32.8μmol TE, or 26% of the activity exhibited by the FF extract. The AD process was also found to be not significantly different than the CD process in respect to the DPPH radical scavenging activity. Regardless of the processing method employed, the Thiessen berries tend to contain the greatest scavenging capability, as compared to the Smoky variety. The relations between the different processing methods and the DPPH radical scavenging capacities of the Saskatoon berry extracts are depicted in Figure 11.

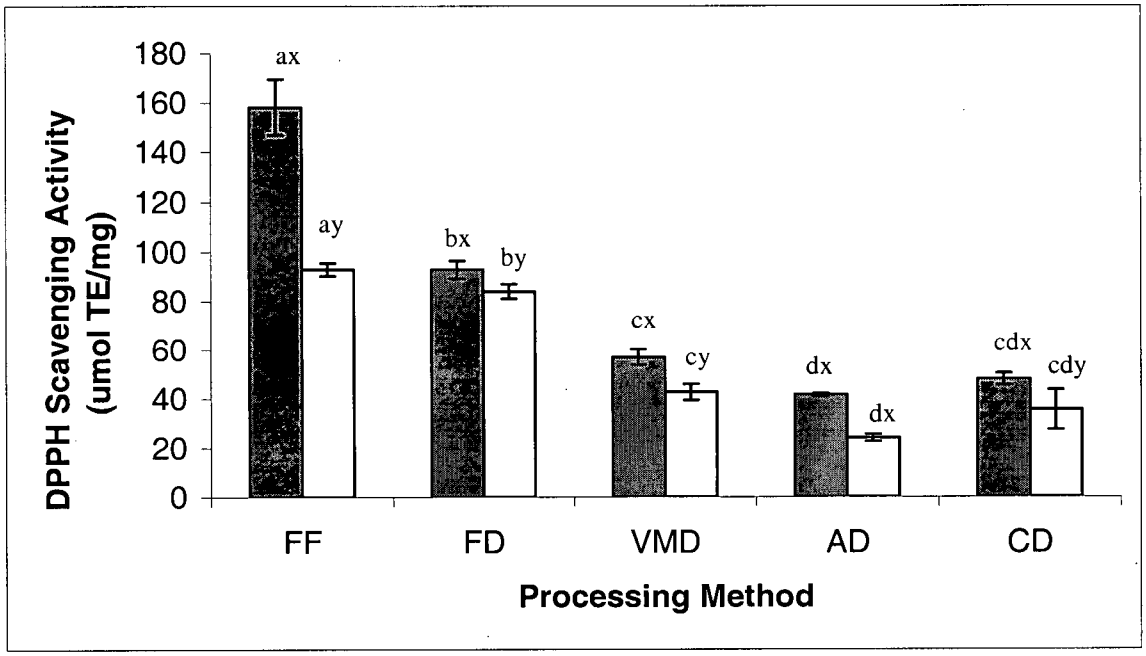



**Figure 11.** Efficiency of Saskatoon berries on DPPH radical scavenging after different drying procedures. Activity was measured as micromoles of Trolox equivalents (TE). Results are expressed as Mean ± SD, n = 6 (3 years, 2 varieties). ■ represents Thiessen variety; □ represents Smoky variety. FF- fresh frozen; FD – freeze dried process; VMD – vacuum microwave dried process; AD – air dried process; CD –combination dried process (which is a combination of air drying and vacuum microwave drying). $^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ among the different processing methods for each individual variety. $^{x, y}$: represent statistical difference at $P<0.05$ between the two varieties.

**ABTS radical scavenging**

The scavenging activity towards the ABTS radical by Saskatoon berries following various processing methods is presented in Table 9. FD processed samples had significantly ($p<0.05$) higher scavenging activities compared to other dehydration methods, providing 79.1μmol TE, or approximately 65% retention of the original unprocessed FF extract. The VMD process method (45.5μmol TE) provided similar ABTS radical scavenging activity as the CD processed berries (36.4 μmol TE); both methods retained only about 30-37% scavenging activity. The AD processed berries were significantly lower in ABTS scavenging activity than all other dehydration methods,

showing only 29.4μmol TE (24% of the original FF extract activity). The trend of hierarchical order of the different processing method in respect to the ABTS assay results were similar to the trend found from the DPPH scavenging assay. Moreover, as with the DPPH radical scavenging assay, the Thiessen variety usually was superior to scavenging the ABTS radical, as compared to the Smoky variety. The relations between the different processing methods and the ABTS radical scavenging capacities of the Saskatoon berry extracts are depicted in Figure 12.

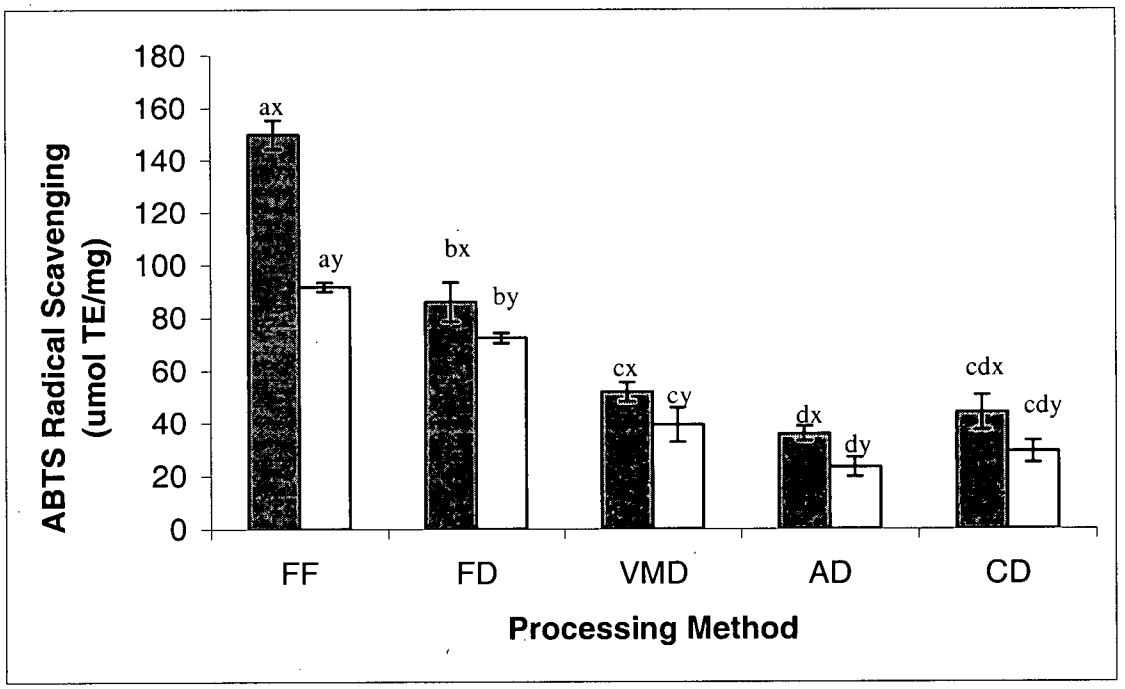



**Figure 12.** Efficiency of Saskatoon berries on ABTS radical scavenging after different drying procedures Activity was measured as micromoles of Trolox equivalents (TE). Results are expressed as Mean ± SD, n = 6 (3 years, 2 varieties). FF- fresh frozen; FD – freeze dried process; VMD – vacuum microwave dried process; AD – air dried process; CD –combination dried process (which is a combination of air drying and vacuum microwave drying). $^{a, b, c, d, e}$: represent statistical difference at $P<0.05$ among the different processing methods for each individual variety.

**Supercoiled strand DNA scission by peroxyl radical**

The average retention percentages of the various Saskatoon berry extracts (SBE) collected over the three harvest years 2000, 1999, and 1998 are shown in Tables 10. Data were collected from scanning Polaroid pictures of stained agarose gels using a densitometer. A typical example of one of the scanned picture, which depicts DNA nicking protection by the various extracts of Saskatoon berries at both the 50μg/ml and 500μg/ml of berry extract is shown in Figure 13A and 13B. Positive control (lane 1) containing DNA only with no added of peroxyl radical induced strand nicking that was set at 100% retention percentages. The negative control containing DNA plus the peroxyl radical from AAPH, to induce strand nicking, was thus was set at 0% in retention percentage. The densities of lanes 3 through 7 were then calculated as compared to the two control lanes. The affinity of each SBE to protect DNA from strand nicking was measured at two concentrations of 50 μg/ml and 500 μg/ml, respectively.

Figure 13A show that there was essentially no protection at the low concentration of 50 μg/ml for all SBE tested from the year 2000. While at the higher concentration of 500 μg/ml, all extracts exhibited protection against the peroxyl radical, at varying degrees with respect to the dehydration method (Figure 13B). All other harvest years and varieties exhibited very similar results (Data not shown).

From Table 10, the degree of protection of the DNA strand is presented as an average for the three harvest years. Following different dehydrated processes, FD processed berries had the highest of DNA nicking protection at around 86.3%, followed by the VMD process (79.5%), the CD process (73.5%), and the AD processed berries

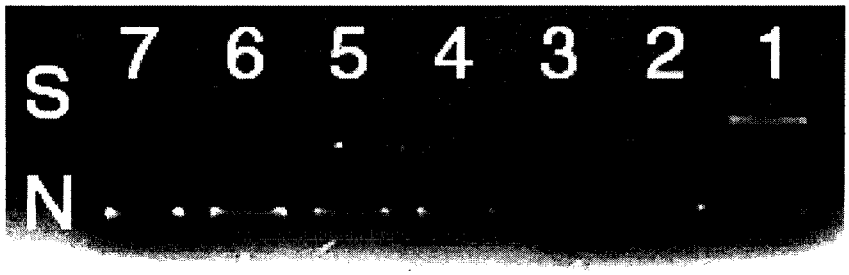
(68.3%). Fresh frozen extracts (fresh weight basis) were also measured and found to contain about 50.6% varietal average retention percentage (Data not shown).

**Table 10.** Average retention percentage (%) of supercoiled DNA strand with the addition of 500μg/ml SBE for three consecutive harvest years (2000, 1999, 1998), in peroxyl radical induced supercoiled DNA strand nicking.

| | **Rentention Percentage (%)** | | |
|---|---|---|---|
| **Processing Method** | **Thiessen** | **Smoky** | **Varietal Average** |
| AD | 70.1±3.7 | 66.5±6.6 | **68.3±5.5** [a] |
| FD | 88.1±2.7 | 84.5±3.0 | **86.3±3.4** [b] |
| VMD | 80.7±3.7 | 78.3±4.3 | **79.5±4.1** [c] |
| CD | 74.3±4.3 | 72.7±5.5 | **73.5±4.9** [ac] |

Values represent data from two replicates. Lanes represent DNA + AAPH + SBE.
AD=air dried; FD= freeze dried; VMD=vacuum microwave dried; CD=combination dried (AD+VMD).
Values within column followed by a different letter are statistically different at $P< 0.01$.

A




B

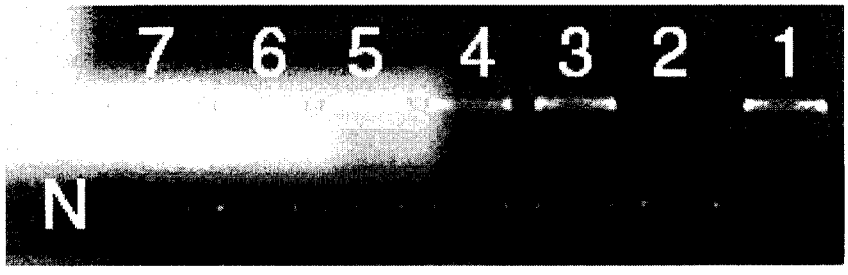



**Figure 13.** Effect of Year 2000 Thiessen Saskatoon berry extracts (SBE) in preventing peroxyl radical induced DNA nicking at 37°C. **(A)** represents concentrations of SBE at 50 μg/ml. **(B)** represents concentrations of SBE at 500 μg/ml. "S" represents supercoiled DNA, "N" represents nicked DNA. Lane 1=DNA+PBS; lane 2=DNA+2ul (30mM) AAPH; lane 3=DNA+air dried (AD) SBE; lane 4=DNA+freeze dried (FD) SBE; lane 5=DNA+fresh frozen (FF) SBE; lane 6=DNA+vacuum microwave dried (VMD) SBE; and lane 7=DNA+combination dried (CD) SBE

## DISCUSSION

In this chapter, the use of different dehydration processes resulted in varying degrees of anthocyanin and total phenolic retentions in the Saskatoon berries. Dehydration processes often affect the quality attribute, reflecting a loss of anthocyanins (which are responsible for the color pigments in these berries), as seen also for phytochemicals in other fruits, vegetables and herbs (Lin et al., 1999b; Yousif et al., 2000).

Anthocyanins are easily susceptible to heat damage, as seen with blueberries where researchers have suggested that heat labile factors may accelerate anthocyanin pigment destruction (Skrede, et al., 2000). Polyphenolics, including anthocyanins are also readily oxidized because of inherent antioxidant properties and, thus, susceptible to degradative reactions during various processing operations (Skrede et al., 2000). This fact was confirmed in this study, as all the dehydrated extracts of SBE investigated had lower degrees of total anthocyanins and total phenolics as compared to FF unprocessed counterparts (Table 8).

Thermal damage resulting from drying of the product is directly proportional to the temperature and the time involved in the process (Lin et al., 1999b). Extracts of FD Saskatoon berries that had not undergone heat treatment, and therefore were not subject to thermal losses, were observed, as expected, to contain the highest anthocyanin and phenolic content as compared to the other dehydration methods. In contrast, the use of higher temperature and longer drying time, associated with the conventional hot air drying, caused greater heat damage and adversely affected the overall quality of the product. This method rendered the worst retention in the anthocyanins content and phenol content as confirmed in this study, producing only 15% of the anthocyanins and 38% of the phenolics remaining in the berries. VMD is a low temperature and fast time dehydration method such that absence of air during drying that otherwise would be involved in oxidation reactions, and therefore higher retention of fruit characteristics as compared to the AD method is obtained.

In this study, a combination drying (CD) method, which employed both AD and VMD principles, was also used. The temperature and time involved with the CD method

was in between the AD and VMD methods. We see from the data that the retention of anthocyanins and total phenols, when employing the CD method was higher than the AD method, since less time was spent in the drier due to the use of the vacuum microwave. The CD method was found to be lower, but not different significantly to the VMD method, thus suggesting that the combination of AD and VMD was as effective as the VMD method alone. Similar findings from other researchers have also suggested the relative abilities of these dehydration methods in the retention of color and quality indices.

A study performed with carrot slices by Lin and colleagues (1999b), reported that AD and VMD samples were darker than the FD samples. They report that this was due to the exposure of heat during drying, where higher temperatures caused darker colors, due to browning reactions. They also suggested that the possibility that the AD and VMD samples were darker was because the density of carrot slices from these processes were greater than FD product, thus yielding a higher concentration of pigment per volume of tissue. In their study, the researchers also reported that losses of α- and β-carotene during drying of the carrot slices were greatest for AD samples, as compared to the VMD samples. Thus a decreased nutritional value of the product was due to the heating rate and oxygen offered by these different dehydration methods. For example, AD processes require high temperature and relatively long times which would contribute to severe losses of the nutrients. In contrast, the VMD carrot slices had higher α -carotene and vitamin C content than samples from AD. VMD method was therefore deemed the better procedure for the retention of these nutrients, because of the rapid heating rate and depletion of oxygen offered by the vacuum microwave. However, VMD processed

carrots were found to have lower nutritional content than that found with the FD samples. While for FD samples, no loss in α- and β-carotene or vitamin C content was observed. The reason being that temperature was not a factor with FD compared to the other two dehydration methods.

In a study with oregano, by Yousif and colleagues (2000), researchers showed that AD samples were darker, and contained a weaker green pigment than those prepared by VMD or FD. These researchers showed that the combination of heat and atmospheric oxygen in AD facilitates enzymatic activity of polyphenol oxidase, which resulted in an enzymatic browning effect that characterizes many AD food materials. Less browning was observed for VMD samples, due to the shorter periods of VMD and the resulting reduced exposure to oxygen. A color analysis of the herb also showed that VMD samples were significantly greener in color than the AD samples.

Drying studies performed with *Echinacea purpurea* have shown a detrimental effect for preserving the chicoric acid and the caftaric acid contents (caffeic acid derivatives present in these roots) from the different dehydration methods (Kim et al., 2000b). These researchers reported highest retentions of chicoric acid and caftaric acid following VMD of Echinacea flower with low moisture contents to be similar to the FD flowers. Moreover, FD was the best method to preserve higher levels of total alkamides in the roots, and VMD was a superior method for drying roots than AD at 70°C (Kim et al., 2000a). These researchers also proposed that the different susceptibility to the degradation was caused by the different drying methods.

The results in my study are therefore very similar to the findings of other researchers on the effects of different dehydration methods for retention of color and

quality indices. Moreover, a comparison of these extracts of dehydrated Saskatoon berries to impart antioxidant properties was measured in this study.

All dehydrated SBE registered much lower retention in antioxidant capacities, as compared to their FF counterparts. Research by others and results from this study have shown that antioxidant activity to be significantly correlated with the content of total phenolics and anthocyanins (Prior et al., 1998). A loss in the fruit parameters (anthocyanins and phenolics) in turn could relate to losses in antioxidant activities. In this study, we noted that the extracts from the dehydrated Saskatoon berries showed reduced total phenolics and total anthocyanins content, which in turn also registered losses in antioxidant capacity, as measured by the affinity to scavenge both the DPPH radical and ABTS radicals, and the ability to protect the supercoiled DNA peroxyl radical induced strand nicking.

With the free radical antioxidant assays (as measured by the scavenging activity towards DPPH and ABTS radicals), FD processed berries provided the greatest scavenging ability as compared to the other dehydration methods. This was followed by the VMD and the CD method. In the case of the DPPH radical scavenging assay, these two methods of drying were actually not significantly different to one another. Moreover, the AD processed berries were insignificantly different than the CD method in providing scavenging activity for these radicals. To summarize these results, it can be said that besides the much higher scavenging activity as reported for the FD processed berries, all other dehydration methods (VMD, AD, and CD) provided very similar abilities to scavenge the free radicals DPPH and ABTS. However, AD processed berries still provide the weakest scavenging activity as compared to other processing methods.

The difference in the scavenging activities between the different dehydration methods is most likely due to losses of total anthocyanin and phenolic content associated with each dehydration process.

Peroxyl radicals have been shown to induce DNA breakage, which involves both base modification and strand scission (Rodriguez et al., 1999). The presence of peroxyl radicals is most relevant to biological systems as they are a very abundant source of free radicals (Prior and Cao, 1999). DNA treated with peroxyl radical in this study resulted in the formation of supercoiled DNA strand scission. DNA singly-strand breakage has also been reported in other free radical involved circumstances, such as γ-radiation (Kumar et al., 1999). In this study, SBE was shown to effectively prevented peroxyl radical-induced DNA strand breakage at 500μg/ml when exposed to APPH induced peroxyl radicals. At the low concentration of 50μg/ml, no detectable retention percentage of supercoiled DNA was observed for all samples investigated. The different dehydration methods led to varying degrees of protection against the radical-induced DNA damage, with SBE from the FD process providing the greatest protection, followed by the VMD process, and then the CD process. The dehydration method that provided the poorest protection against the radical-induced DNA damage was the SBE derived from the AD process. From the results, it can be suggested that direct free radical scavenging mechanisms of SBE may lead to the prevention of peroxidation reactions in DNA scission, which was affected by the dehydration method of choice.

Reducing activity was found in all dehydrated SBE extracts tested, as measured by its reducing power. However reducing power of the dehydrated extracts showed considerably lower reducing potential as compared to FF counterparts. This finding may

again correspond to the fact that the dehydrated samples contained lesser total anthocyanin content or total phenolics as compared to the FF samples, which in fact imparts the reducing power. From the previous study (Experiment 1), reducing activity was highly correlated to the total anthocyanin content and total phenolics content. Thus the loss in these fruit parameters following dehydration may have contributed to the loss in reducing power. Highest reducing activities were reported for extracts from the FD Saskatoon berries, which also contained the highest fruit parameters, free radical scavenging capability, and DNA protection. All other dehydration methods exhibited either equal or no difference in regards to final reducing activity.

## CONCLUSION

Frozen Saskatoon berries were dehydrated using various methods, including freeze drying (FD), vacuum microwave drying (VMD), air drying (AD), and a combination drying (CD) method [in which the berries first underwent partial air drying and were then completely dried using vacuum microwave]. The changes in total anthocyanin and total phenolic contents were monitored, as well as associated antioxidant activity and reducing powers. All dehydration processes resulted in substantial losses in both the total anthocyanin and total phenolic content. The FD process rendered the least loss, followed by the VMD and CD methods, and with the AD process having the greatest loss in both of these fruit characteristics. Due to the substantial loss in total anthocyanin and phenolic content, sample extracts also registered substantial losses in antioxidant capacities, as measured by scavenging activity towards the free radicals

DPPH and ABTS, and by affinity to protect DNA strand breakage from the peroxyl radical. All dehydrated extracts also registered losses in reducing activities.

# EXPERIMENT 3: FRACTIONATION STUDY OF FREEZE DRIED SASKATOON BERRIES

## INTRODUCTION

High intakes of fruits and vegetables have been associated with lower incidences of chronic diseases such as cancer and heart disease (Steinmez and Potter; 1996; Ames et al., 1993). It has been suggested that the phytochemicals (e.g. flavonoids and other phenolics) present in fruits and vegetables, in addition to the vitamins and minerals, may contribute to this protective effect. Many of these phytochemicals have antioxidant activity and may aid in protection of cells against the oxidative damages caused by free radicals. Recently, there has been increasing interest in the health benefits of consuming berries such as blueberries, cranberries, blackberries, etc. Researchers have reported the high antioxidant activity of many berries to be attributable in part to their anthocyanin content (Prior et al., 1998; Heinonen et al., 1998; Wang and Lin, 2000).

The Saskatoon berries (*Amelanchier alnifolia* Nutt.) are highly pigmented berries that are native to the southern Yukon and Northwest Territories, the Canadian prairies and the northern prairie states of the United States (Harris, 1972). High levels of anthocyanins in these berries have been suggested to be associated with high total phenolics and acids and low pH and sugar-acid ratios (Green and Mazza, 1986). Extracts from this berry have been observed *in vitro* to be a potent antioxidant or radical scavenger (Fukumoto and Mazza, 2000; Wang and Mazza, 2002). However, more in-depth research is required for identification of the berry components responsible for this antioxidant activity in Saskatoon berries.

The aim of this study was to examine the structure-function relationship that characterizes antioxidant activity of Saskatoon berries relative to partitioning of active components in different polar states. Fractionations of the extracts of freeze dried Saskatoon berry (SBE) was performed.

## MATERIALS AND METHODS

Saskatoon berries were kindly supplied by the Riverblend Plantation Gourmet Foods (Saskatoon, Saskatchewan, Canada). Metaphosphoric acid, sodium hydroxide, and potassium dihydrogen orthophosphate were purchased from BDH Inc. (Toronto, ON). Methanol, sodium bicarbonate ($NaHCO_3$), sodium acetate, trichloroacetic acid (TCA), ethyl ether, ethyl acetate, and butanol was obtained from Fisher Scientific (Fair Lawn, NJ). Methanol, acetic acid and hydrochloric acid were from Fisher Scientific (Napean, ON). 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (diammonium salt, ABTS), ferric chloride ($FeCl_3$), potassium chloride, Ciocalteau's reagent, and gallic acid were from Sigma Co. (St. Louis, MO). Agarose was obtained from Bio-Rad Laboratories (Richmond, CA). Formic acid was from EM Science (Gibbstown, NJ). 96-well flat-bottom EIA microtitration plates were from Corning Inc. (Corning, NY).

### Saskatoon Berry Extracts

Saskatoon berries were first freeze dried according to the method previously described on page 61. Fractions of Saskatoon berries were extracted according to method as previously described on page 36.

**Liquid/liquid Extraction of SBE**

In order to investigate the water-soluble constituents that are responsible for the associated antioxidant capacity, a solvent fractionation of the SBE was performed (Hung and Yen, 2002; Lim et al., 2002). Twenty milliliters of the SBE sample was added to twenty milliliters of a mixture of 70% methanol and 1% formic acid. The mixture was added to a separatory funnel and was first extracted with 3 x 20ml of ethyl ether. The ether phase (top organic phase) was collected from all three separations and combined and concentrated by rotary evaporator. After evaporation, the ether fraction was dissolved in distilled water and stored in a volumetric tube with 0.3ml of 1N HCl. The sample was deoxygenized with a stream of nitrogen gas, capped, foiled, and stored in a freezer at –25°C.

The aqueous phase was extracted again with 3 x 20ml of ethyl acetate. The ethyl acetate phase (top organic phase) was collected from each separation and concentrated by rotary evaoporator. After evaporation, the acetate fraction was reconstitued in distilled water and stored in a volumetric tube with 0.3ml of 1N HCl. This fraction was deoxygenized under a stream of nitrogen gas, capped, foiled, and stored in the freezer at -25°C.

The remaining aqueous phase was finally extracted with 3 x 20ml butanol. The butanol phase (top organic phase) was collected from each separation and the bottom phase (last fraction) was collected from each separation and concentration by rotary evaporator. The butanol fraction was then reconstituted in distilled water and stored in a volumetric tube containing 0.3ml of 1N HCl. Samples were also deoxygenized with nitrogen gas, capped, foiled, and stored in the freezer at -25°C.

The final remaining water fraction following butanol extraction was labeled as fraction four of the sample. This fraction was also roto-evaporated and the final residue was reconstituted with distilled water.

## Saskatoon Fruit Characterization

### *Total Mnomeric Anthocyanin Content*

Total monomeric anthocyanin content of the fractions were analyzed according to the method previously described on page 38.

### *Total Phenolic Content*

The total phenolics content of the fractions were determined according to the method previously described on page 39.

## Antioxidant Assays

## Free Radical Scavenging Activity

### *DPPH radical scavenging test*

The DPPH radical scavenging activity of the fractions were measured according to the method previously described on page 40.

### *ABTS radical scavenging test*

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical radical scavenging activity of the fractions were measured according to the method previously described on page 41.

### Reducing Power Assay

Reducing power of the fractions were analyzed according to the method described previously on page 42.

### Statistical Analysis

Experiments were performed in triplicate and results were expressed as mean ± S.D. One-way ANOVA was used to evaluate the differences between samples with significance set at $p<0.05$ (Minitab for Windows, version 13.32, State College, PA). A correlation statistic was used to evaluate statistical differences between the different methods and assays used in this experiment (significance set at $p<0.01$, $p<0.001$).

## RESULTS

### Total Anthocyanins and Total Phenolic Contents

Total anthocyanin content for the non-fractionated sample (NFS) and the four individual fractions of the extracts of freeze-dried SBE are tabulated in Table 11. The data represent a mean measurement collected over the three harvesting years (2000, 1999, and 1998). Very low amounts of anthocyanins (11mg/100g berry for the Thiessen variety and ~8mg/100g for the Smoky berries) were found in Fraction I (the ether fraction). Highest total anthocyanin contents were contained in Fraction III (the butanol fraction). In this fraction, the Thiessen variety contained approximately 95mg/100g of anthocyanins, and the Smoky variety 81mg/100g. Fraction II (the acetate fraction) contained the second highest concentration of anthocyanins, with the Thiessen berries containing approximately 55mg, while the Smoky berries contained approximately

35mg/100g. Fraction IV (the remaining water fraction) contained very low amounts of anthocyanin contents (e.g. Thiessen berries had about 28.3mg/100g and the Smoky variety contained only 17.3mg/100g). From the fractionation, it was a common finding that the Thiessen variety always contained the highest total anthocyanin content compared to the Smoky variety. The only exception to this was Fraction IV, where no difference was noted between the two varieties. When both varieties were analyzed together, Fractions I, II, III, and IV contained a total anthocyanin content of 9.3, 45.2, 87.9, and 22.8mg/100g respectively. When the anthocyanin contents for the four fractions were collectively summed, almost (e.g. >87%) complete recovery of the total anthocyanin content of samples was made.

**Table 11.** Total anthocyanin content, and total phenol content of fractionated freeze dried Saskatoon berry extracts (FD-SBE). Calculated as pooled measurements from three harvesting years (2000, 1999, and 1998) for each variety of Thiessen and Smoky berries. NFS=non-fractionated samples for the individual sample group.[1]

| Sample Group | Fractions | Total Anthocyanin (mg/100 g) | Percentage of Unfractionated Sample | Total Phenolics (μg GAE/mg) | Percentage of Unfractionated Sample |
|---|---|---|---|---|---|
| Thiessen | **NFS** | **203.5±5.2** [a] | | **12.38±0.19** [a] | |
| | I | 10.9±0.9 [b] | 5.4% | 1.55±0.37 [b] | 12.5% |
| | II | 55.4±6.4 [c] | 27.2% | 3.17±0.07 [c] | 25.6% |
| | III | 94.8±1.4 [d] | 46.6% | 3.87±0.27 [d] | 31.3% |
| | IV | 28.3±4.0 [e] | 13.9% | 2.82±0.36 [c] | 22.8% |
| | **Sum of Fractions** | **189.4±12.7** [a] | **92.9%** | **11.41±1.20** [a] | **92.2%** |
| Smoky | **NFS** | **161.2±3.4** [a] | | **10.96±1.76** [a] | |
| | I | 7.7±1.7 [b] | 4.8% | 1.52±0.30 [b] | 13.9% |
| | II | 35.0±4.3 [c] | 21.7% | 2.71±0.38 [c] | 24.7% |
| | III | 81.0±7.8 [d] | 50.2% | 3.50±0..8 [d] | 31.9% |
| | IV | 17.3±9.0 [e] | 10.7% | 2.30±0.43 [c] | 21.0% |
| | **Sum of Fractions** | **141.0±22.8** [a] | **87.5%** | **10.04±1.50** [a] | **91.6%** |
| Average Of Both Varieties | **NFS** | **182.3±23.5** [a] | | **11.67±1.36** [a] | |
| | I | 9.3±2.1 [b] | 5.1% | 1.54±0.37 [b] | 13.2% |
| | II | 45.2±12.2 [c] | 24.8% | 2.94±0.38 [c] | 25.2% |
| | III | 87.9±9.1 [d] | 48.2% | 3.50±0.38 [d] | 30.0% |
| | IV | 22.8±8.7 [e] | 12.5% | 2.30±0.43 [c] | 19.7% |
| | **Sum of Fractions** | **165.2±32.0** [a] | **90.6%** | **10.72±1.53** [a] | **91.9%** |

[1] Values within each column for each individual sample group followed by a different letter are statistically different at $P< 0.05$.

Total phenolic contents for both the non-fractionated and the four individual fractions from freeze-dried SBE, collected over the three harvest years are shown in Table 11. From this data, Fraction III (the butanol fraction) always contained the relatively greater amount of total phenolics; as compared to the other three fractions investigated (e.g. 3.87 and 3.5μg GAE/mg berry for the Thiessen and the Smoky variety,

respectively). Fraction I (the ether fraction) contained the least amount of phenolics across all samples (e.g. 1.5μg GAE/mg berry for both varieties). Fraction II (the acetate fraction) and Fraction IV (the remaining water fraction) were indifferent in total phenolics content (e.g. 2.8 to 3.1μg GAE/mg for the Thiessen variety and 2.3 to 2.7μgGAE/mg berry for the Smoky variety). The sum of the different phenolic contents recovered from the four fractions totaled approximately the amount originally present in the non-fractionated samples (NFS).

Interestingly, unlike the trend found with the total anthocyanins content in these fruits, there were no significant varietal differences in total phenolics content for all Fractions ($P<0.001$). When both varieties were analyzed together, Fractions I, II, III, and IV were calculated to contain total phenolic contents of 15.4, 29.4, 35.0, and 23.0 μg GAE/mg berry respectively.

**Antioxidant Activity**

**DPPH Radical Scavenging**

The free radical scavenging capacity of the individual SBE fractions of DPPH radical are shown in Table 12, along with the associated scavenging activity for the non-fractionated samples. The sum of the four individual fractions for each sample group was calculated and was less than the activity of the original, non-fractionated samples, (e.g. recovery was about ~80% for the overall average). Fraction I provided the least scavenging activity for the DPPH radical across all samples investigated. Fraction I provided scavenging activity at a low of 10.1μmol TE and 7.0μmol TE/mg berry for the Thiessen and Smoky varieties respectively. Fraction II had DPPH radical scavenging

activity in the range of 23.8 and 18.1μmol TE/mg berry for the two varieties. For Fraction III, the Thiessen variety provided DPPH scavenging activity of 27.0μmol TE/mg berry, while Smoky provided 25.9 μmol TE/mg berry. Fraction III was slightly higher in scavenging activity towards the free radical compared to the other fractions; however, a significant difference ($p<0.05$) was noticed only for the Smoky variety. In the general case of the Thiessen berries, and the overall average for all berries, the scavenging activity towards the DPPH radical for Fraction III was not significantly different than the Fraction II scavenging activity. Fraction IV provided activity in the range measured at 17.8 and 12.8 μmol TE/mg berry for the Thiessen and Smoky varieties respectively; this fraction had significantly lower activity than Fractions II and III, but higher in activity when compared to Fraction I. When the DPPH radical scavenging activities for the two varieties were combined, no significant differences were found between Fraction II and III. However, the activities found in these two fractions were still significantly higher than either Fraction I or IV.

**Table 12.** DPPH radical and ABTS radical scavenging activity, and reducing activity of fractionated freeze dried Saskatoon berry extracts (FD-SBE). Calculated as pooled measurements over three harvesting years (2000, 1999, and 1998) for each variety of Thiessen and Smoky berries. NFS=non-fractionated samples for the individual sample group.[1]

| Sample Group | | DPPH Radical Scavenging Activity (μmol TE/mg) | ABTS Radical Scavenging Activity (μmol TE/mg) | Reducing Activity (μg AAE/mg) |
|---|---|---|---|---|
| Thiessen | **NFS** | **92.8±3.6** [a] | **85.9±7.5** [a] | **32.6±6.7** [a] |
| | I | 10.1±0.4 (10.9) [b] | 7.6±1.1 (18.6) [b] | 3.1±0.9 (9.5) [b] |
| | II | 23.8±2.7 (25.6) [c] | 20.4±1.7 (23.7) [c] | 7.7±1.3 (23.6) [c] |
| | III | 27.0±1.5 (29.1) [c] | 27.2±1.9 (31.7) [d] | 8.7±0.9 (26.7) [d] |
| | IV | 17.8±1.8 (19.2) [d] | 15.5±2.4 (18.0) [c] | 6.9±0.7 (21.2) [c] |
| | **Sum of Fractions** | **77.0±6.4 (83.0)** [f] | **70.7±7.1 (82.3)** [f] | **26.4±3.8 (81.0)** [a] |
| Smoky | **NFS** | **84.1±3.0** [a] | **72.2±1.9** [a] | **28.3±0.6** [a] |
| | I | 7.0±1.3 (8.3) [b] | 7.8±0.7 (10.8) [b] | 3.3±0.9 (11.6) [b] |
| | II | 18.1±3.8 (21.5) [c] | 17.7±0.9 (23.7) [c] | 6.3±0.9 (21.8) [c] |
| | III | 25.9±1.3 (30.8) [d] | 23.5±0.3 (32.5) [d] | 7.9±0.9 (27.9) [d] |
| | IV | 12.8±2.7 (15.2) [e] | 12.0±1.6 (16.6) [c] | 6.2±1.0 (21.9) [c] |
| | **Sum of Fractions** | **63.8±9.0 (75.9)** [f] | **60.9±3.4 (84.3)** [f] | **23.6±3.6 (83.4)** [a] |
| Average | **NFS** | **88.4±8.4** [a] | **79.1±18.7** [a] | **30.4±2.6** [a] |
| Of Both | I | 8.5±1.9 (9.6) [b] | 7.7±0.8 (9.7) [b] | 3.2±0.8 (10.5) [b] |
| Varieties | II | 21.0±4.3 (23.8) [c] | 19.1±1.9 (24.1) [c] | 7.0±1.3 (23.0) [cd] |
| | III | 25.6±1.3 (29.0) [c] | 25.4±2.3 (32.1) [d] | 8.3±0.9 (27.3) [c] |
| | IV | 15.3±3.4(17.3) [d] | 13.7±2.7 (17.3) [e] | 6.6±0.9 (82.2) [d] |
| | **Sum of Fractions** | **79.4±10.9 (79.6)** [a] | **65.8±7.7 (83.2)** [a] | **25.0±3.8 (82.2)** [a] |

[s]Values within each column for each individual variety followed by a different letter are statistically different at $P< 0.05$.
Numbers in parenthesis represent retention percentages as compared to the unfractionated sample.

**ABTS Radical Scavenging**

The ABTS radical scavenging capabilities for the different fractions of FD SBE was also measured and are presented Table 12, along with the non-fractionated samples

(NFS). Similar to the DPPH assay, the highest scavenging activity of the ABTS radicals was found in Fraction III for each sample. Values ranged from 29.8 μmol TE/mg berry for the Thiessen to 25.1μmol TE/mg berry for the Smoky berries. Fraction I provided the least scavenging activity against the ABTS radical, across all samples investigated, with only 8.5 and 7.9μmol TE/mg berry for the Thiessen and Smoky varieties respectively. Fraction II had higher scavenging activity, which was double that of Fraction I, at around 21.8 and 18.8 μmol TE/mg berry. The last fraction, Fraction IV also contained scavenging activities that were lower than Fractions II and III, at around 16.5 and 12.7 μmol TE/mg berry only for the two varieties, Thiessen and Smoky. The calculated sum of the four Fractions for each sample group was found to total closely to the original, unfractionated sample. When comparing the ABTS radical scavenging activities for the two varieties investigated, it was found that the Thiessen berries had a higher ABTS scavenging activity in all fractions measures. The only exception was Fraction I, which had relatively low activities regardless. The sum of the four individual fractions for each sample group was significantly less ($p<0.05$) than the activity of the original, unfractionated samples for both the Thiessen and Smoky sample groups. Similar trends in ABTS scavenging activity distribution among the four fractions were observed when the two varieties were combined.

**Reducing Power**

The reducing powers for the individual fractions obtained from FD SBE extracts are tabulated in Table 12. Calculations of reducing power were based on a standard curve of ascorbic acid, measured as ascorbic acid equivalents (AAE). For each sample,

Fraction I consistently had the lowest reducing power as compared the other three fractions, at about 3.1 and 3.3 μg AAE/mg berry for the Thiessen and Smoky varieties respectively. For each sample group, Fraction II and IV, were equal in reducing potential. These two fractions had reducing powers between 6.0 to 7.0 μg AAE/mg berry. The highest reducing activities were seen with Fraction III, at 8.7 and 7.9μg AAE/mg berry for the Thiessen and Smoky varieties respectively. The reducing power approached the value found in the non-fractionated samples when expressed as the sum of the all four fractions for each sample. Non-fractionated samples contained 27.8 to 33.1 μg AAE/mg berry. When the reducing activities for the two varieties were combined, no significant difference ($p<0.05$) was found between Fraction II and III. Also, no difference was found between Fraction II and IV. However, Fraction III was still higher in reducing activity than Fraction IV.

**Correlation Coefficients Among Measured Parameters**

Correlation coefficients among all the parameters measured for the SBE fractions are shown in Table 13. Correlation data was obtained from analyzing all the fractions. A highly significant correlation was observed for the total anthocyanin content and the total phenolic content in the berry samples ($r=0.906$, $p<0.001$). Also, highly significant correlations were observed among total anthocyanin and antioxidant activities (as measured with both the DPPH ($r=0.884$, $p<0.001$) and ABTS ($r=0.834$, $p<0.001$) radical scavenging activity). Total anthocyanin content was correlated with the reducing power of the fractions ($r=0.772$, $p<0.001$). A highly significant correlation was observed with the total phenolic content and the scavenging for the DPPH radical ($r=0.975$, $p<0.001$)

and the ABTS radical (r=0.890, p<0.001). The reducing power of the fractions and the total phenolic content also was highly correlated (r=0.849, p<0.001). The two different radical scavenging assays (e.g. DPPH and ABTS) were highly correlated (r=0.930, p<0.001). The reducing power of the extracts was also correlated with the DPPH and ABTS radical scavenging activity (r=0.876 and r=0.778, respectively, p<0.001). Thus, all assays measured in this experiment were highly correlated to each other, among all fractions investigated. Both the fruit characteristics (total anthocyanins and total phenolics content) in the fractions correlated highly with the both radical scavenging assays and also with the reducing power assay.

**Table 13.** Correlation coefficients among the antioxidant activities (DPPH radical and ABTS radical scavenging), reducing activity, total anthocyanin, total phenolic concentrations as calculated for all the fractions of Saskatoon berry extracts. (n=24)

| | **Total Anthocyanin (c3g/100 g dry fruit)** | **Total Phenolics (GAE/g dry fruit)** | **Antioxidant Activity: DPPH scavenging (μmol TE/mg dry fruit)** | **Antioxidant Activity: ABTS scavenging (μmol TE/mg dry fruit)** |
|---|---|---|---|---|
| **Total Phenol** | 0.906*** | | | |
| **DPPH** | 0.884*** | 0.975*** | | |
| **ABTS** | 0.834*** | 0.890*** | 0.930*** | |
| **Reducing** | 0.772*** | 0.849*** | 0.876*** | 0.778*** |

*** designate significance at P<0.001.

## DISCUSSION

The analysis of anthocyanins in fruits and vegetables is complicated because of instability associated for with structural transformations and complexation reactions. Moreover, anthocyanins are difficult to measure independently to other flavonoids because they have similar structural and reactivity characteristics. In addition, pure standard compounds are not easily available (Mazza and Miniati, 1993). Thus the

method of choice for extracting anthocyanins depends on the purpose of the extraction and also on the characteristics of the anthocyanins (Timberlake and Bridle, 1980).

The ultimate use for this study will be to use knowledge concerning antioxidant capacity for potential incorporation into commercial products such as beverages and food colorants. Thus, greater focus was placed on the hydrophilic constituents of these extracts.

Liquid-liquid extractions have been used in the assessment of antioxidant activities of particular phytochemicals from fractionated samples of extracts from herbs and plants (Hung and Yen, 2002; Lim et al., 2002). The use of a liquid-liquid extraction method in this study allowed for the separation of compounds with similar polarity present in the Saskatoon berry samples. For example, the liquid-liquid solvent system extracted out the polar, water-soluble anthocyanins using a procedure whereby solvents were chosen based on differences of increasing polarity. Ethyl ether or the least polar solvent (Fraction I) would extract very minimal amounts of the anthocyanins, compared to ethyl acetate, which would remove greater amounts of the total anthocyanins (Fraction II). The more polar solvent used herein (e.g. butanol fraction) theoretically would remove the highest amounts of the polar anthocyanins, along with phenolics (Fraction III). The remaining compounds present in the SBE following these extraction procedures would remain behind in Fraction IV.

From the total monomeric anthocyanins measurements conducted with the individual fractions, Fraction I (the ether fraction) contained very minimal amounts (e.g. only 5.1% of the anthocyanins) of measurable total anthocyanins. Fraction II had higher recovery of total anthocyanins than that of Fraction I, removing 24.8% of the

anthocyanins. The greatest proportion of anthocyanins successfully extracted occurred in Fraction III (the butanol fraction) for all samples investigated. This third fraction contained almost half of the total SBE anthocyanin content or around 48.2%. The distribution of anthocyanins in the medium-to-high polar fractions suggests that anthocyanins in Saskatoon berries are more likely to be the glycoside forms, as indicated by Mazza (1986) who reported Saskatoon berries to contain 61% cyanidin 3-galactoside and 21% cyanidin 3-glucoside.

The total phenolic content for the individual fractions was also investigated. From the data, it was noticed that the Fraction I contained very low amounts of phenolics, and that the differences between phenolic content in Fractions II, III, and IV were not as great as that seen for the total anthocyanins measurement. Even though Fraction III contained the highest phenolic content, only 30% of the total phenols accounted for occurred in Fraction III, as compared to the original extract, when assessing all samples investigated. For Fraction II, and IV, total phenolic content varied, accounting for about 25% and 19%, respectively of the total phenolic present in the original extract. From the total phenolic distribution data, it can be noted that phenol distribution among Fractions II, III, and IV was as widely dispersed in these three fractions. Even though a higher percentage of total phenolics were extracted into the third fraction, the phenolics present in the sample were probably a collective of a wide variety of phenolic compounds, which had great variances in polarity characteristics. Thus, the results in this study suggest that the liquid-liquid extraction method used herein was more effective at separating out anthocyanins in the berry extract according to polarity differences. For the determination

of the type and variety of phenolics present in these fractions, an HPLC profile or NMR spectroscopy would be beneficial, which was not performed in this study.

Antioxidant activities for the different fractions were investigated by measuring the scavenging activities towards both DPPH and ABTS free radicals. All fractions were found to contain varying degrees of scavenging activities towards these specific free radicals. Similar retention in activities was found for both scavenging assays. Very low scavenging capacity was found in Fraction I, which also contained minimal amounts of total anthocyanins and total phenolic compounds. In Fractions II, III, and IV, the free radical scavenging activity was found to be higher than that of Fraction I. Fraction III was shown across all data to contain the highest radical scavenging activity, but in some cases not significantly different ($p<0.05$) than Fraction II. Likewise, Fraction IV was found to contain the least radical scavenging activity across all data but in some cases not significantly different ($p<0.05$) than Fraction II. Nonetheless, the free radical scavenging activities was significantly correlated to the amount of anthocyanins and phenolics in the individual fractions.

Other studies have reported the link between the anthocyanin and phenolic content of fruits with antioxidant activities. In a study performed by Oki and colleagues (2002), 5 purple-fleshed sweet potato cultivars were separated into two fractions based on an ethyl acetate liquid-liquid extraction, which represented anthocyanin- and phenolic-rich fractions, respectively. The associations of extracted anthocyanin and phenolic content and free radical scavenging activity of DPPH radical was also made. Cultivar differences were found to account for the differences noted in the total radical-scavenging activity of the sweet potatoes. Moreover, for some cultivars, the determining factor for

the free radical scavenging activity was the anthocyanin content. For other cultivars, a similar correlation was made with the presence of phenolic compounds. These researchers also provided evidence to show that both the anthocyanins and polyphenols present in the purple-fleshed sweet potatoes were responsible for the radical-scavenging activity. This was especially true when the total radical scavenging activity was assessed by summing the effect of the radical scavenging activity of the individual phytochemical components.

In another study performed with polyphenolic fractions collected from Italian red wine, researchers reported that the anthocyanin-rich fraction was the most effective fraction for scavenging reactive oxygen species and inhibiting lipoprotein oxidation and platelet aggregation, when compared to the procyanidin or the phenolic acids fraction (Ghiselli et al., 1998). They reported that the anthocyanin fraction accounted for approximately 70% (w/w) of the total phenol content in the red wine. Furthermore, decreases in hydroxyl and peroxyl radical scavenging activity by polyphenolic fractions of Italian red wine was reported relative to the original unfractionated red wine.

When looking at the relative free radical scavenging activities of the individual SBE fractions in this study, it is interesting to note that the total phenolic content of the different fractions was proportionally similar. Thus the total phenolics in SBE were more likely to be the component that was responsible for the antioxidant activities shown in the Saskatoon berries. However, since correlation statistics also implicated the anthocyanins for antioxidant activities found in these berries, it may be concluded that the overall antioxidant capacity in Saskatoon berries is most likely a combination effect attributed to the phenolics including anthocyanins in these berries.

In relative terms, recovery of antioxidant activity in different fractions was much lower as compared to the recovery of total anthocyanins and phenolics data. One possible reason for the apparent discrepancy may be explained by the loss of possible synergistic effects of the two components in the sample due to the fractionation process. Since different components in the SBE sample were isolated and collected in different fractions, possible synergistic effects that were observed with the unfractionated samples may have been lost. Possible synergism may be due to the mixture of phenol groups (anthocyanins and phenolics) in the berry extracts, being more effective to act as hydrogen donors in scavenging the ABTS and DPPH radicals (Figure 4), as compared to just their individual components. There is similar evidence supporting the idea of synergistic activity between of different components in fruits that contribute to overall antioxidant activity (Kayano et al., 2002; Stahl et al., 1998).

In a study by Kayano et al., (2002), antioxidant activity from prune (*Prunus domestica)* extract constituents was compared from different fractions. It was concluded that the overall antioxidant capacity of prunes, as measured by the ORAC method, was not dependent on only a single phenolic compound such as caffeoylquinic acid isomers, but rather a number of unknown antioxidant compounds with possible synergistic activity. However, characterization of these unknown antioxidant compounds were not reported.

Synergistic effects of phytochemicals producing antioxidant activity has also been reported with lycopene and lutein (Stahl, et al., 1998), where mixtures of carotenoids were more effective than a single carotenoid for antioxidant activity expressed in a

multilamellar liposomes assay where inhibition of thiobarbituric acid-reactive substance formation was reported.

However, the loss of antioxidant activity in the fractions may have been brought about by loss in bioactivity of the berry constituents in these fractions and not due to the lack of synergism between berry components. Hence, further studies are required to identify the specific berry extract constituents responsible for the antioxidant activity and to assess the individual and possible synergistic activities.

The results for the reducing power assay, in the present study also indicated that Fraction III possessed the highest reducing activity. The reducing activities for Fraction II were similar to that measured in Fraction IV and Fraction I had the least reducing power. These data support the trends seen with the ABTS radical scavenging assay. Moreover, the reducing activities reported in the various fractions were correlated to both ABTS and DPPH radical scavenging activity and the total anthocyanins and total phenolics content. These suggest that the majority of the reducing power measured in SBE was due to the phenolic compound content, more so than the anthocyanins contents. Reducing power exemplifies an affinity to donate electron and hydrogen atoms and thus potentially provide an antioxidant characteristic. Thus, since high reducing activities were found in fractions with high anthocyanins and phenolics content, it is apparent that the antioxidant activity of the Saskatoon berry samples was attributed to the affinity of both anthocyanins and phenolic compounds to act as a hydrogen- or electron donors.

## CONCLUSION

A solvent fractionation study was performed with Saskatoon berry extracts, based on polarity differences, with the aim to determine which constituents in SBE were responsible for antioxidant activity. Antioxidant activity, measured by the affinity to scavenge free DPPH and ABTS radicals, correlated highly to both the anthocyanin and phenolic content present in the sample. Fractions with higher total anthocyanin and phenolic content related to higher free radicals scavenging activities. When expressing the observed trends as recovery percentage for each fraction, it was observed that the antioxidant capacity of SBE constituents related more so to the phenolic compounds present in these fractions, but anthocyanins content also played a major role as seen by the strong correlations. Possible synergistic effects of the different components in each fraction were also observed as a result of a significant loss in antioxidant activity when expressed as a sum of the individual fractions. Reducing power of the individual fractions was also related to phenolic content of SBE fractions, and also the anthocyanins. The reducing activity of the Saskatoon berries suggested that the reported antioxidant capacity of the SBE berries was related to the ability of both anthocyanins and phenolic compounds to act as hydrogen- or electron donating antioxidants.

## GENERAL CONCLUSION

The Saskatoon berry is a well known fruit commodity to many prairie provinces, especially Saskatoon and Manitoba. As a result, the present market for Saskatoon berries tends to be in the prairie provinces. The long-term market opportunity for Saskatoon berries lies in reaching consumers in other locations. To increase the market for Saskatoon berries means that consumers in other regions must become familiar with the berry. To become familiar with the berry, studies pertaining to the potential benefits in health and disease prevention will be an asset.

Saskatoon berry extracts showed antioxidant properties as measured by the DPPH and ABTS free radical scavenging assays. The degree of antioxidant capacity was found to be comparable to well studied soft fruits such as blueberries. The antioxidant activity was found to be correlated to the total anthocyanin and total phenol content in the berries. Moreover, the reducing power assays conducted suggests that the Saskatoon berry extracts are good sources of electron and hydrogen donors and therefore could terminate the free radical chain reactions by converting free radicals to more stable products.

Results also show that there was a definite varietal difference between the Thiessen and Smoky cultivars; in which, the Thiessen berries were found to be consistently higher in measured fruit chemical characteristics (total anthocyanin and total phenol content) and antioxidant activity (as measured via scavenging assays towards the DPPH and ABTS radicals) as compared to the Smoky vareity. This varietal difference held up throughout the three consecutive harvest years tested (1998, 1999, 2000). Harvest year, however, has been determined to not be a factor for prediction of the fruit characteristics and associated antioxidant activity.

Dehydration processing was found to result in lower retentions of antioxidant activity associated with the anthocyanin pigments and phenol contents. Extracts from freeze-dried Saskatoon berries were found to retain the highest amounts of these measured parameters. The vacuum-microwave dried berries retained the second highest of the measured parameters. The poorest retention of anthocyanins and phenols were found with extracts from air-dried Saskatoon berries. The combination processing method, combining air drying and vacuum microwave drying, showed retentions in measured parameters (total anthocyanin and phenol contents) that were in between the two individual dehydration methods.

The solvent fractionation study proved that fractions containing higher anthocyanin and phenol content exhibited higher antioxidant activities. Possible synergistic effects of the different components in each fraction were suggested as a result of the significant loss in antioxidant activity when expressed as a sum of the individual fractions. However, the issue of loss in bioactivity in the individual fractions due to the fractionation procedure can not be ruled out.

From these studies, it can be concluded that Saskatoon berries contain notable antioxidant activities due to high anthocyanin and phenolic contents. Future research might entail a more detailed analysis of the specific anthocyanin and phenolic components in the Saskatoon berries responsible for the established antioxidant activities. Moreover, chemical trials are required to determine if consumption of fresh or dried Saskatoon berries will influence the antioxidant status of the consumer.

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## Appendix A

Retention percentage (%) of supercoiled DNA strand with the addition of SBE for the 2000 harvest year, in peroxyl radical induced supercoiled DNA strand nicking.

| | Berry Extract | 2000T | | 2000S | |
|---|---|---|---|---|---|
| **Lane** | **Concentration** | **50 ug/ml** | **500 µg/ml** | **50 µg/ml** | **500 µg/ml** |
| **3** | **AD** | 0 | 71.1±2.4 $^{ax}$ | 0 | 71.9±1.2 $^{ax}$ |
| **4** | **FD** | 0 | 88.9±2.2 $^{bx}$ | 0 | 86.2±2.9 $^{bx}$ |
| **5** | **FF** | 0 | 52.0±2.2 $^{cx}$ | 0 | 49.1±3.9 $^{cx}$ |
| **6** | **VMD** | 0 | 83.5±1.8 $^{dx}$ | 0 | 82.0±0.5 $^{dx}$ |
| **7** | **CD** | 0 | 78.1±0.6 $^{ex}$ | 0 | 76.0±1.9 $^{ex}$ |

Values represent data from two replicates. Control (+) designates a positive control with only DNA. Control (-) designates a negative control with DNA + AAPH. All other lanes represent sDNA + AAPH + SBE. AD=air dried; FD= freeze dried; FF=fresh frozen (fresh weight); VMD=vacuum microwave dried; CD=combination dried (AD+VMD).
Values within each column for each concentration measured followed by a different letter are statistically different at $P< 0.05$.
Values within each row for each individual dehydration method followed by a different letter are statistically different at $P<0.05$.

## Appendix B

Retention percentage (%) of supercoiled DNA strand with the addition of SBE or the 1999 harvest year, in peroxyl radical induced supercoiled DNA strand nicking.

| | Berry Extract | 1999T | | 1999S | |
|---|---|---|---|---|---|
| **Lane** | **Concentration** | **50 µg/ml** | **500 µg/ml** | **50 µg/ml** | **500 µg/ml** |
| **3** | **AD** | 0 | 69.4±1.2 $^{ax}$ | 0 | 67.1±2.1 $^{ax}$ |
| **4** | **FD** | 0 | 86.8±2.5 $^{bx}$ | 0 | 85.3±3.1 $^{bx}$ |
| **5** | **FF** | 0 | 54.1±3.1 $^{cx}$ | 0 | 55.3±1.2 $^{cx}$ |
| **6** | **VMD** | 0 | 81.3±1.7 $^{dx}$ | 0 | 78.6±2.6 $^{dx}$ |
| **7** | **CD** | 0 | 74.0±2.1 $^{ex}$ | 0 | 73.1±1.5 $^{ex}$ |

Values represent data from two replicates. Control (+) designates a positive control with only DNA. Control (-) designates a negative control with DNA + AAPH. All other lanes represent DNA + AAPH + SBE. AD=air dried; FD= freeze dried; FF=fresh frozen (fresh weight); VMD=vacuum microwave dried; CD=combination dried (AD+VMD).
Values within each column for each concentration measured followed by a different letter are statistically different at $P< 0.05$.
Values within each row for each individual dehydration method followed by a different letter are statistically different at $P<0.05$.

## Appendix C

Retention percentage (%) of supercoiled DNA strand with the addition of SBE for the 1998 harvest year, in peroxyl radical induced supercoiled DNA strand nicking.

| | Berry Extract | 1998T | | 1998S | |
|---|---|---|---|---|---|
| **Lane** | **Concentration** | **50 µg/ml** | **500 µg/ml** | **50 µg/ml** | **500 µg/ml** |
| **3** | **AD** | 0 | 69.1±2.2 $^{ax}$ | 0 | 59.7±4.2 $^{ay}$ |
| **4** | **FD** | 0 | 89.4±3.5 $^{bx}$ | 0 | 82.8±2.1 $^{bx}$ |
| **5** | **FF** | 0 | 49.2±1.7 $^{cx}$ | 0 | 44.6±1.6 $^{cx}$ |
| **6** | **VMD** | 0 | 80.5±1.9 $^{dx}$ | 0 | 76.4±1.8 $^{dx}$ |
| **7** | **CD** | 0 | 73.2±2.3 $^{ex}$ | 0 | 67.3±3.3 $^{ex}$ |

Values represent data from two replicates. Control (+) designates a positive control with only DNA. Control (-) designates a negative control with DNA + AAPH. All other lanes represent DNA + AAPH + SBE. AD=air dried; FD= freeze dried; FF=fresh frozen (fresh weight); VMD=vacuum microwave dried; CD=combination dried (AD+VMD).
Values within each column for each concentration measured followed by a different letter are statistically different at $P< 0.05$.
Values within each row for each individual dehydration method followed by a different letter are statistically different at $P<0.05$.