ANTIOXIDANT COMPONENTS AND COLOR CHARACTERISTICS OF BLUEBERRIES DRIED BY DIFFERENT METHODS

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ABSTRACT

Blueberries have recently become the subject of focus for some scientists owing to their high antioxidant activity and potential health benefits beyond nutrition. The components likely to be responsible for this activity are the phenolic compounds and vitamin C. Dehydration can be successfully applied to this fruit to make it available all year round. While freeze-drying and air-drying are two conventional methods used for dehydration, vacuum microwave dehydration is a relatively new method of food dehydration, which has the potential of producing high quality novel dehydrated products.

This study was designed to investigate the effects of vacuum microwave, air, freeze drying and a combination of air/vacuum microwave drying on vitamin C, anthocyanin, phenolic content and antioxidant activity of two blueberry cultivars, Bluecrop and Hardy Blue. In addition the color characteristic of the berries were assessed.

Vacuum microwave dried blueberries retained higher amounts of vitamin C than the air and air/vacuum microwave dried ones. The vitamin C content of air-dried and combination treatments were too small to be detected by the method employed in this study. Total anthocyanin level of the vacuum microwave dried sample was higher ($p \leq 0.05$) than those of the air-dried and combination treatments in one of the two cultivars, and was comparable to the level of anthocyanin in the frozen berries. Vacuum microwave and freeze-dried berries had the highest concentration of phenolic compounds ($p \leq 0.05$). All blueberry extracts exhibited antioxidant activity, which was positively correlated with total anthocyanin, total phenolic ($p \leq 0.05$) and vitamin C content ($p \leq$

0.058). There was no significant difference between the antioxidant activities of various dehydrated blueberries. In Hardy Blue berries, all dehydration methods caused loss of antioxidant activity. In Bluecrop berries, some dehydration methods such as vacuum microwave drying, air/VMD, and freeze-drying were able to preserve the antioxidant activity to the level which was comparable to that of the frozen berries of that cultivar.

In terms of color characteristics the puffed structure of vacuum microwave and freeze-dried berries likely influenced the Hunter lab values. In these treatments despite higher anthocyanin content, due to the distribution of the pigment over a bigger surface, they had higher Hunter L (indicating lightness), and lower Hunter a (indicating redness) and Hunter b (indicating blue color) values than the air-dried samples. No clear trends were observed between Hunter a, b, and L values, and the extent of heat and oxygen exposure.

Therefore, vacuum microwave drying showed a better efficiency in retaining some bioactive components, when compared to air-drying. Vacuum microwave drying shortens the drying time considerably, and by doing so, it has a definite advantage over methods such as air-drying which has a prolonged drying time.

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LIST OF ABBREVIATIONS

AOAC	Association Official Analytical Chemists
BC	Bluecrop
BHT	butylated hydroxy toluene
°C	degree Celisus
cm	centimeter
DHAA	dehydroxy ascorbic acid
DNA	deoxyribonucleic acid
ε	molar absorbance
g	gram
GAE	gallic acid equivalent
h	hour
HB	Hardy Blue
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
H_2SO_4	sulfuric acid
KCl	potassium chloride
kg	kilogram
KPa	kilopascal
MDA	malonaldehyde
MFW	methanol/formic acid/water
µl	microliter

ml	milliliter
mm	millimeter
Pa	Pascal
pmol	picomole
RH	relative humidity
rpm	rotation per minute
RP-	Reverse phase
TBAR	thiobarbituric acid reagent
V	Volt
VMD	Vacuum microwave dried

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To my children; Arash and Avishan

who are teaching me the biggest lesson of all every day; the lesson of loving in face of all shortcomings

And for that I feel truly blessed!

I. INTRODUCTION AND OBJECTIVES

Positive health effects of fruits and vegetables have been the focus of many researchers, especially in the last few decades. A positive correlation between consumption of fruits and the health condition has been established (Ames, 1993). Many fruits have become the subject of the scrutiny of various fields of science such as Medicine, Biochemistry, Pharmacy, Food Science and so on. One such fruit, which has intrigued scientists, is the blueberry.

Traditionally blueberries have been part of North American food culture. This dates back to the Native American who used sun-and smoke-dried blueberries to flavor foods. Early settlers learned to rely on blueberries as a staple ingredient in cooking. In fact, the blueberry is one of the oldest known fruits, estimated to be more then 13,000 years old (Villata, 1998). Today, due to the short shelf life of the fresh fruit, a large proportion (60%) of blueberries are consumed in the frozen or dehydrated form.

Dehydration is the oldest preservation method used by mankind. Along with many advantages, dehydration imposes some disadvantages on dehydrated fruit as well. Most of the dehydration techniques involve some kind of heat treatment to evaporate the water from the fruit. This heat treatment often has an adverse effect on the heat labile components of the fruits.

Various components of blueberries have been indicated as possibly responsible for its suggested health promoting effects. The phenolic compounds, the anthocyanin content and vitamin C content of blueberries are some examples (Heinonen *et al.,* 1998; Block *et al.,* 1992; Elliot, 1994). All of these compounds are heat sensitive, and their

concentration is reduced when blueberries are exposed to heat during various dehydration procedures.

The objective of this study was to dry blueberries by some common and some novel methods, and to analyze and compare the resultant blueberries for the concentration of the above mentioned components and total antioxidant activity.

It was hypothesized that blueberries dried by the vacuum-microwave drying method would preserve these components better compared to the berries dehydrated by the conventional temperature dependent dehydration methods, such as hot air drying.

II. LITERATURE REVIEW

A. Dehydration

Fruits and vegetables are an important part of the human diet. They are valuable sources of carbohydrates, essential vitamins and minerals, besides providing crude fiber. In addition they add color, flavor, and variety to our diet. However, due to their high moisture content (above 80%) they are highly perishable. Losses estimated at 40-50% occur in many developing countries in the tropical and subtropical regions due to inadequate refrigeration and freezing facilities (Jayaraman and Das Gupta, 1992). These losses are endured in developed countries as well, although on a much smaller scale. To minimize these losses, fruit processing industries have been established in many developed and developing countries. The main goal of these industries is to convert perishable commodities to stable products, which can be stored and used out of season and at places far away from the site of production. This industry has focused on three major preservation methods for fruits and vegetables; canning, freezing, and dehydration. Among these techniques, dehydration is perhaps the most common and universally used technique.

Dehydration is the oldest preservation method used by mankind. Dehydration of fruits and vegetables is achieved by a variety of techniques. In all these techniques, dehydration involves the manipulation of temperature to vaporize water and some means of removing water vapor after it is separated from the fruit/vegetable tissue. Hence, it is simultaneously a heat and mass transfer operation for which energy must be supplied. Three basic types of drying processes are recognized: solar drying, atmospheric drying

including batch and continuous processes, and sub-atmospheric dehydration (vacuum shelf/belt/drum and freeze dryers) (Jayaraman and Das Gupta, 1992).

The desire to maintain the nutritional quality of foods following processing is an extension of man's desire to preserve his food supply. Chemical analysis of the processed food for certain nutrients provides us with insight into which are preserved by a certain method, and which are not. The following sections (sections I-V) are a review of some of the most commonly used dehydration methods, as well as some of the relatively new dehydration methods, which are not yet used on a large scale commercially.

II. A. 1. Air drying

While sun drying is one of the most primitive and inexpensive methods of drying certain fruits, it is restricted to climates with hot sun and dry atmosphere, and is not applicable to such fruits as blueberries. Air-drying, however, does not have this limitation. With this method, the food is exposed to a hot air current, and the moisture is removed from it by evaporation. Within the food, the heat is transferred by conduction, and the rate of drying is the function of many factors (the internal factors in food and the external factors around the food) among them the volume to surface area of the food (Somogyi and Luh, 1975).

The air-drying of fruits was reported by Saravacos and Charm (1962) to occur mostly in a single falling rate period. Nonetheless, this is not the general pattern with all fruits. For many fruits, such as banana slices (Sankat *et al.*, 1996) drying occurs in two falling rate periods. During the first falling rate period, moisture migration occurs mostly by capillary flow and water is depleted in the sub-surface reservoirs of the product. Consequently, the wetted area on the product surface disappears (Treybal, 1980). The

liquid surface recedes into the capillaries and goes deeper as drying progresses, accounting for the second falling rate period during which evaporation occurs below the food surface and diffusion of vapor occurs from the place of vaporization to the surface (Jayaraman and Das Gupta, 1992). In the study of convective-air drying kinetics of osmotically pre-treated blueberries, Ramaswamy and Nsonzi (1998) reported that the convective-air drying of blueberries occurred in two falling rate periods, and among the pre-treatment conditions, the temperature and sucrose concentration during osmotic dehydration significantly influenced the air-drying time.

Many of the dried fruits produced today are processed by the air-drying method. To achieve dehydrated products of high quality at a reasonable cost, dehydration must occur fairly rapidly (Jayaraman and Das Gupta, 1992). However, if the rate of drying is too fast, moisture will be removed too rapidly from the surface and case hardening will occur (Yang and Atallah, 1985). Furthermore, loss of some nutrients and volatile compounds inevitably occurs during air-drying. Since the products are exposed to high temperature for a long period, volatile compounds are vaporized and lost with water vapor. This causes a significant loss of characteristic flavor in dried products. High temperature and long drying time also degrade the product's original color, and can introduce new flavor compounds to the food. Therefore, alternative, energy efficient drying methods are necessary for the food industry in order to manufacture products of high quality (Yongsawatdigul and Gunasekaran, 1995). However, after sun drying, air-drying is the most inexpensive dehydration method while the other dehydration methods such as freeze-drying are usually restricted to high value products (Brown, 1973).

II. A. 2. Vacuum drying

Vacuum oven drying is another conventional dehydration method. In this method food is placed in a vacuum chamber and the dehydration occurs by introducing the heat into this chamber. Due to the absence of oxygen many degradative and oxidative reactions can be minimized. Vacuum drying has been successfully applied to many fruits and yields a product with low moisture content and unique quality. Because of the vacuum which lowers the boiling point of water, and the absence of oxygen during the dehydration process, it is suggested to be a good method for foods that are sensitive to heat and would deteriorate quickly at the high temperatures and from oxidation (Somogyi and Luh, 1975). Due to a decrease in drying time, when compared to the air-drying method, the overall color, texture, and flavor of dried products are improved. This method is the recommended AOAC method for estimation of the moisture content of blueberries, due to their high sugar content (AOAC, 1996). However, industry does not employ this method for dehydration purposes in a large scale, partially due to the non-continuous nature of the process. Moreover vacuum drying has high installation and operating costs (Woodroof and Luh, 1986).

II. A. 3. Freeze drying

Freeze-drying is a dehydration method, which has gained attention in recent years. In this method, the frozen products are placed in a chamber in which the temperature and the pressure are reduced to the point that causes the sublimation of the water (from ice crystals to water vapor). This method is one of the most advanced dehydration methods, which yields a dry product with porous structure and little or no shrinkage (Somogyi and Luh, 1975). Rahman (1972) applied this method to produce densely packed blueberries

for military use. In order to better control the quality of freeze dried blueberries during production and storage, data are needed on physical properties including equilibrium moisture content (EMC)/ equilibrium relative humidity (ERH) relationships, which may be expressed as moisture sorption isotherms. Loong *et al.* (1995), reported first order moisture adsorption kinetics for freeze-dried blueberries. A major drawback of freeze-drying is the economics of the process. Since the cost of freeze-drying depends on the amount of the moisture removed, the pre-treatment such as osmotic drying can reduce the processing expense. Also the syrup remaining after osmotic drying can be recycled as suggested by Bolin *et al.* (1983), to further reduce the cost. Rahman (1972) proposed a thermal conditioning to plasticize the crunchy, and sometimes fragile, freeze-dried fruits to produce a space-saving, compressed, dehydrated product; this approach was adopted by Yang *et al.* (1987), to induce chewiness to finished freeze-dried blueberries.

II. A. 4. Microwave drying

Microwave drying is a rapid and uniform drying process. In this method the food is placed, in the microwave oven, in the path of microwaves, and some of the electromagnetic energy is absorbed by the food and converted to heat. This heat is then used to vaporize the water in the food. The amount of energy converted to heat varies from one food to another, depending on the loss factor of the food. Loss factor itself is determined by the mobility, charge, and symmetry of molecules in the food. The presence of these regions is in fact a prerequisite for the absorption of microwave energy by the food material. The penetration power of microwaves is a function of factors such as frequency of the incident microwave and the nature (moisture content and dielectric constant) of the food being radiated (Gruenberg *et al.,* 1992). The overall governing

parameters in microwave heating are the mass of the material, its specific heat, dielectric properties, geometry, heat loss mechanism and coupling efficiency (Schiffmann, 1987).

The heat is transferred throughout the food by convection (if the food is liquid) as well as conduction. Microwave shortens the drying time by more than 30% when compared to conventional methods (Attiyate, 1979; Kock, 1989) and leads to a substantial improvement of the final product quality. In microwave drying it is reported that the drying time can be considerably reduced, since the least efficient portion of the conventional method such as air-drying, is near the end. During this stage, two third of the time may be spent removing the last one third of the moisture content (Decareau, 1985; Tulasidas, 1994.

A combined method of microwave and vacuum drying system has been described which produces puff dried products from fruits and vegetables, and apparently maintain the color, flavor and shape of fresh fruits (Teranishi, 1988). In this method, drying takes place in a microwave oven chamber whose pressure can be reduced. Applying microwave energy under vacuum affords the advantages of both vacuum drying and microwave drying - improved energy efficiency and product quality (Yongsawatdigul and Gunasekaran, 1995). Kiranoudis *et al.* (1992), also reported that the parameters of vacuum microwave drying (VMD) were found to be greatly affected by the microwave power level while vacuum pressure only slightly affected the process. However, a major disadvantage is the high cost of energy required to generate microwaves. This high cost of energy dictates that microwave vacuum techniques could be used only in cases where drying of final products has to meet high quality specifications or as a supplementary drying method for further product quality improvement (Drouzas and Schubert, 1996).

In an attempt to optimize the quality of the end product and also to reduce the cost of VMD, a treatment that combines VMD with another drying treatment may be advisable. For example fruits may be first treated by air-drying, to remove 50%-90% of the moisture content (depending upon the fruit), and the dehydration process may be completed by vacuum microwave drying. When most of the water has been removed by conventional heating methods, microwave heating removes the rest of moisture from the interior of the product quickly and efficiently, to reach the target moisture content without overheating the already dried material. Vacuum microwave drying of foods acts, in many cases, as an excellent complementary method to the conventional ones, to bring the moisture content of a partially air dried fruits and vegetables down to a target moisture content (Attiyate, 1979; Kock, 1989). In a study on tomatoes dried by the combination of air drying and vacuum microwave drying, the cost associated with a combination method of air drying and vacuum microwave drying were calculated. This study revealed that although the energy used in vacuum microwave drying is more expensive than that used in the conventional methods (electricity versus natural gas), due to the efficiency of the dehydration especially towards the last time segment of the drying process (which results in reduced drying time and fuel consumption), overall the combination of air drying and VMD was less expensive than either the air drying or vacuum microwave drying methods by themselves (Durance and Wang, 1999).

II. A. 5. Other dehydration methods

Many other preservation methods have been investigated to prolong shelf life of fruits such as blueberry and blueberry products. Kim and Toledo (1987) reported that high temperature fluidized-bed drying reduced dehydration time as compared to

conventional air-drying, and the dried product had lower bulk density, larger diameter and higher rehydration ratio. Sullivan *et al.* (1982), used a continuous explosion puffing process to dry rabbit eye blueberries. They found berries were very susceptible to skin rupture which caused severe 'bleeding'. It has also been reported that batch explosion puffing of high bush blueberries created rapid drying and quick rehydrating blueberries (Eisenhardt *et al.,* 1964). In testing explosion-puffed dried blueberries, it has been reported that when the dry berries were put in storage, they did not develop unfavorable textural changes. Other favorable characteristics of the explosion-puffed product were excellent flavor and color, fast rehydration, ambient temperature storage, minimal storage and transportation costs, and durability (Sullivan *et al.,* 1982).

Osmotic dehydration is another alternative to the above mentioned methods. It basically consists of removing water to the extent of 30%-50% of the weight of fruit pieces by placing them in contact with sugar or syrup, then draining to remove excess syrup and rinsing if desired. Osmotic water removal is possible because the cell membranes are semi-permeable and allow water to pass through them more readily than sugar. However if the processing time in sugar or syrup is too long there is a considerable diffusion of sugar into the fruit. Along with water, acid is removed rapidly at first and then more slowly. These factors may alter the taste and texture of the fruit, and are to be kept in mind. On the other hand, sugar is a very effective inhibitor of polyphenol oxidase, the enzyme which catalyses oxidative browning of many cut fruits, and it prevents loss of volatile flavoring constituents during dehydration (Ponting, 1973).

B. Blueberries

Berries have been part of North American food culture for a long time. This dates black to the Native American who used sun-and smoke-dried blueberries to flavor foods. Early settlers learned to rely on blueberries as a staple ingredient in cooking. High bush blueberries make up more than half of all blueberries produced in North America. The top three production regions for cultivated blueberries are Michigan, New Jersey, and British Columbia. Blueberries, available in various forms and products, are commonly used in the food and baking industries. Products include individually quick frozen (IQF) and straight-pack, puree, puree concentrate, canned, and dried blueberries (Villata, 1998).

Blueberries belong to the genus *Vaccinium* and grow throughout North America. This genus includes *V. corymbosum* and *V. ashei*, which are cultivated, and native North American wild low bush, *V. angustifolium*, which grows primarily in Maine, Nova Scotia, and Quebec (Villata and Kenyou, 1997). Each cultivar within a species possesses certain features that separate it from the other cultivars. Professionals have worked to identify and enhance the most desirable features of various cultivars of high bush blueberries through breeding in an effort to produce blueberries with optimal flavor, color, and texture (Villata, 1998).

Vaccinium breeding is a relatively recent development (Eck and Childers, 1967). High bush breeding began in the early twentieth century in New Jersey, with the first hybrid being released by Dr. Frederick Conville of the United States Department of Agriculture in 1920. The genetic variability found within *Vaccinium corymbosum* L. has made the breeding progress rapid (Gough and Korcak, 1995). Since the first high bush cultivar was released in 1920, blueberries have become a major fruit crop. Qualities such

as disease resistance, plant vigor, high production, and suitability to machine harvest have encouraged growers to process high bush blueberries (Sullivan *et al.,* 1982).

In 1996, high bush blueberries accounted for 56% of the blueberry production in North America. Cultivated blueberries accounted for 97% of the fresh blueberries, and their production has tripled during the last thirty years. Based on projections by Michigan State University, East Lansing, continued growth is expected through the year 2000 (Villata, 1998). The huge, and somewhat unanticipated increase in production of this fruit promoted the development of new products from this fruit (Yang and Atallah, 1985). Blueberries have a brief harvest season and fresh berries may not be kept for more than six weeks after harvesting. Blueberries are high in moisture content (about 80-85%). Like most of high moisture fruits they are highly perishable. Up until 1987, most of the blueberry crop in North America was frozen with only a small portion canned (Yang *et al.*, 1987). However, the frozen products entail increased storage and transportation costs. Frozen storage results in fractures at the cellular level. Leaching of cellular content and soft texture that occurs with thawing limits applications for frozen blueberries (Loong *et al.,* 1995). As well, frozen storage life is limited to 6 months or less because of textural problems of woodiness or grittiness (Sullivan *et al.*, 1982).

Dehydration as a preservation method may be easily applied to blueberries due to the relatively small size of the fruit. The removal of moisture prevents the growth and reproduction of microorganisms, which can cause deteriorative reactions otherwise. It brings about a substantial reduction in weight and volume, minimizing packing, storage and transportation costs (Jayaraman and Das Gupta, 1992). At the usual temperatures permitting microbial growth, most bacteria require a water activity in the range of 0.90-

1.00. Some yeasts and molds grow slowly at the water activity as low as 0.60. When the water activity of a food is reduced to <0.60, none of the three main microorganisms; bacteria, mold and yeast, are able to grow (Potter and Hotchkiss, 1995).

The dehydrated product may have a high nutritional content because dehydration has little effect on the mineral content, and vitamin losses may be equal to or less than other preservation methods (Yang *et al.,* 1987). The low moisture content of dried blueberries makes them suitable for baking mixes that require extensive mixing. Dried blueberries are used in bakery mixes, cookies, muffins, yeast breads, cereals, and other products in which low moisture ingredients are desired.

An intermediate moisture (IM) blueberry product containing 16-25% water may have several advantages. On a per weight basis of IM blueberry may have more than five times the mineral and stable vitamin contents of the fresh counterpart. The ready-to-eat texture, easier rehydration, and then low moisture have made the IM blueberries a promising product in the snack and convenience food industry (Yang *et al.*, 1987).

II. B. 1. Composition and nutritive value

Blueberries are high in iron and manganese (Hope, 1965) and their vitamin content equals or exceeds that of most fruits (USDA Handbook #8, 1963). In making dietary recommendations for disease prevention, dietitians encourage higher fruit, vegetable, and fiber intake, as well as lower fat intake. Incorporating a variety of fruits, such as blueberries, with potential health benefits, into a diet is easy because they can be consumed in a variety of forms and products. Fresh blueberries are an excellent healthy snack. They can significantly contribute to the flavor and color of the food. Cultivated blueberries on average contain water (80-85%), protein (1.12%), ash (0.19%), lipids

(0.02%), carbohydrates (13.51%) including fructose (5.2%), glucose (5.1%), and sucrose (0.3%), and fiber (2.86%). In addition, blueberries contain vitamin C (13 mg/100 g), and beta carotene (100 IU/100 g), both of which are antioxidants (Villata, 1998), and perhaps play an antioxidant role in blueberries.

II. B. 2. Antioxidant capacity

II. B. 2. 1. Mechanism of lipid oxidation

Lipid oxidation in a biological environment may produce harmful chemical moieties of human. These moieties can cause damage to many vital systems including the structure of DNA. Lipid oxidation occurs in three stages (Scheme 1)

Initiation	$L \rightarrow L^{*} + H^{*}$
Propagation	$L^{*} + O_2 \rightarrow LOO^{*}$
	$LOO^{*} + LH \rightarrow LOOH + L^{*}$
Termination	$LOO^{*} + H^{*} \rightarrow LOOH$
	$L^{*} + H^{*} \rightarrow LH$

* Denotes free radical

LH= unsaturated fatty acid

LOO* = peroxy radical

LOOH = lipid hydroperoxide

Scheme 1. Three stages of lipid oxidation

Although, theoretically, termination is a part of this chain reaction, in practice, due to the unfavorable kinetics of this step, it happens at a much lower rate than the other two steps (Naswar, 1996). Lipid hydroperoxides (LOOH), produced during the propagation step are the fundamental primary product of lipid oxidation. They can react again with oxygen to form secondary products. The secondary products eventually undergo decomposition. Some of the compounds produced from the decomposition of the secondary products of lipid oxidation serve as an index of the extent of lipid oxidation. Among such products is malonaldehyde (MDA) (Naswar, 1996).

Since the formation of free radicals in the initiation step is thermodynamically difficult (due to the very high activation energy), production of the first few radicals (initiation) to start the propagation reaction must be catalyzed. The presence of agents termed "prooxidants" can catalyze these reactions. Example of such compounds is metals such as iron (Fe) and copper (Cu) (Naswar, 1996). Free radicals are also generated as a byproduct of many physiological reactions. In the human body there is a delicate balance between prooxidants and antioxidants. Antioxidants are agents that disturb the process of oxidation at some point and inactivate the free radicals (Scheme 2).

$LOO^{*} + AH \rightarrow LOOH + A^{*}$ Less harmful radicals

$A^{*} + LOO^{*} \rightarrow LOOA$

Non-radical products

$A^{*} + A^{*} \rightarrow A_2$

*Denotes free radical.

AH = antioxidant

LOO* = peroxy radical

LOOH = lipid hydroperoxide

A*= a less harmful radical produced as a result of the action of antioxidant

Scheme 2. Mechanism of action of antioxidants.

An antioxidant (AH) enters at the point that a harmful free radical such as peroxy radical (LOO^{*}) has already been formed, and it either transforms it to a harmless or less potent radical, or combines two radical and produces non-radical products. As well, antioxidants can play a role through various ways, such as being a metal chelator, or an oxygen scavenger (a free radical acceptor or an electron donor) (Naswar, 1996).

The two basic categories of antioxidants are synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures and various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), carotenoids, as well as ascorbic acid (Larson, 1988; Hudson,

1990; Hall and Cupett, 1997). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used since the beginning of this century. Restrictions on the use of these compounds however are being imposed because of their potential carcinogenicity (Branen, 1975; Ito *et al.,* 1983). Thus, the interest in natural antioxidants has increased considerably (Loliger, 1991).

Berries are known to possess antioxidant activity. Many of the biological functions, such as anti-mutagenicity, anti-carcinogenicity, and anti-aging originate from this property (Cook and Samman, 1996; Huang and Frankel, 1997).
Natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects. The search for natural replacements of synthetic antioxidants has led to the evaluation of a number of plant sources.

II.B. 2. 2. Estimation of antioxidant capacity of blueberries and other fruits

The antioxidant capacity of fruits and vegetables are estimated by various methods. Conventional methods include measurements of chemiluminescence (Loeliger and Saucy, 1984) and thiobarbituric acid reactive substances (TBARs) (Tarladgis *et al.,* 1960), while novel methods include estimation of ORAC (oxygen radical absorbing capacity) (Cao *et al.*, 1993) and DNA cleavage (Wijewickreme and Kitts, 1998).

Thiobarbituric acid reactive substances (TBARs) measurement is a colorimetric assay based on the measurement of the red color at 532 nm, when malonaldehyde (MDA), a product of the decomposition of the secondary products of lipid oxidation, reacts with thiobarbituric acid reagent. Figure 1 demonstrates the scheme of the formation of MDA from the primary products of lipid oxidation.

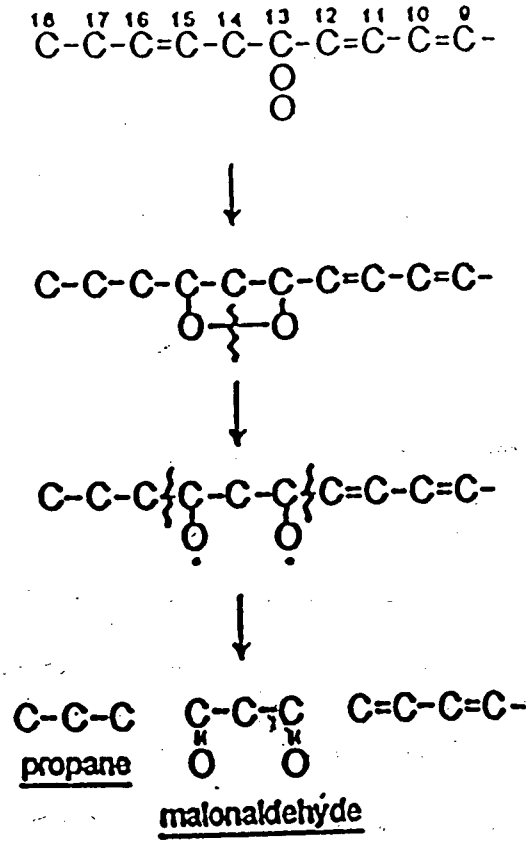

Figure 1. Speculative mechanism of malonaldehyde formation (from Pryor *et al.*, 1976)

Despite the dark color of blueberries, which could interfere in colorimetric assays, the colorimetric method of TBARs can be employed to estimate the antioxidant activity of blueberries. Since the color of the anthocyanins is very much dependent upon the pH of the medium increasing, the pH can convert them to their colorless carbinol form, and help to overcome the interference. In addition a large dilution of the analyte can be a helpful factor in minimizing this interference.

Some tissue aldehydes, such as sugars, and acetaldehyde, also react with thiobarbituric acid reagent to produce a chromophore absorbing at 532 nm (Buege and Aust, 1978). Hubert *et al.* (1975), have suggested reducing the temperature in the heating step from 100° C to 80° C to avoid interference from sucrose.

The DNA cleavage method is a relatively new method to estimate antioxidant activity. In this method all the requirements for a Fenton reaction and the generation of free radicals are set up, and then DNA is added to this setting. The free radicals generated will cleave the supercoiled DNA and the degree of the damage to the supercoiled DNA is measured by agarose gel electrophoresis. Antioxidants, with the ability to inactivate free radicals prevent this damage, and a notable reduction in the percent nicked DNA is observed. This reduction has been interpreted as an index of the antioxidant activity of that specific antioxidant or the overall antioxidant activity of certain food extract (Wijewickreme and Kitts, 1998).

Data on the antioxidant activity of berries and fruits, their juices, and berry wines vary widely, partly due to the use of different oxidation systems and methods to analyze extracts for phenolic compounds. Extracts of berries of several cultivars of blackberries, black and red currants, blueberries, and black and red raspberries showed a remarkably

high scavenging activity toward chemically generated superoxide radicals (Costantino *et al.*, 1992.

II.B. 2. 3. Antioxidants in blueberry:

The protection provided against diseases by fruits and vegetables has been attributed to the various antioxidants contained in these foods (Ames, 1993; Gey *et al.*, 1991; Steinberg *et al.*, 1989). The antioxidant activity of several plant materials has recently been reported (Oomah and Mazza, 1996; Wang *et al.*, 1996; Amarowicz *et al.*, 1996). Research has shown that fruits and vegetables contain antioxidant nutrients, in addition to the well-known vitamin C and E, or carotenoids (Wang *et al.,* 1996). Some of these antioxidants present in blueberries are anthocyanins, and other phenolic compounds. Some of the current researches on antioxidant action focus on phenolic compounds such as flavonoids (Heinonen *et al.*, 1998).

II. B. 2. 3. 1. Phenolic compounds

Berries contain a wide range of flavonoids and other phenolic compounds that possess antioxidant activity. These flavonoids have a strong antioxidant capacity (Wang *et al.*, 1997). Figure 2 shows the structure of anthocyanin as a member of the flavonoids family.

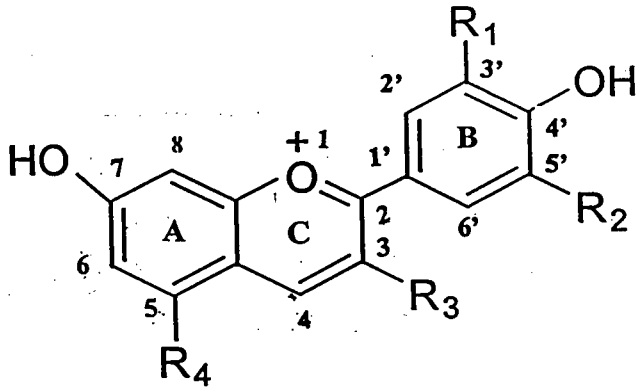

Figure 2. General structure of anthocyanin as a member of the flavonoid family, in its flavylium cation form. R_1 and R_2 are H, OH or OCH_3, R_3 is a glycosyl or H and R_4 is OH or a glycosyl.

Berries are rich in flavonoids and phenolic acids. Strongly colored berries such as blueberries, black currents, and crowberries contain up to 100 mg/kg flavan-3-ols, up to 300 mg/kg flavonols, up to 200 mg/kg hydroxycinnamates and a relatively high amounts of anthocyanins (Macheix *et al.*, 1990).

II. B. 2. 3. 2. Vitamin C

Vitamin C is another important component of blueberry with antioxidant activity. This vitamin is water-soluble with the reduced form referred to as ascorbic acid (AA), and the oxidized form referred to as dehydroxy ascorbic acid (DHAA). In humans, both forms are biologically active. The total vitamin C activity is the sum of both forms (Dodson *et al.*, 1992).

Vitamin C is one of the major antioxidant vitamins in the body and the main water-soluble antioxidant capable of regenerating vitamin E. It works both singly and synergistically to prevent or delay oxidative reactions (Block *et al.*, 1992; Block and Langseth, 1994; Elliot, 1994). Besides being an antioxidant itself, vitamin C protects and regenerates vitamin E, an even more powerful antioxidant vitamin. This role of vitamin C has a remarkable physiological significance in the human body, and is an important factor to consider when treating vitamin E deficiency or toxicity (Marks, 1985).

The quantity of vitamin C in fresh blueberries has been estimated to be in the range of 8-12 mg/100 g weight. Being a water soluble vitamin and sensitive to chemical, enzymatic, and thermal degradation, the vitamin C content is reduced by the time the berries get to the consumer as a processed blueberry. Karel and Nickerson (1964), Jensen (1967), Vojnovich and Pfeifer (1970) and Lee and Labuza (1975) have studied the

stability of reduced ascorbic acid in various low and intermediate moisture dehydrated foods and model systems as a function of moisture content, water activity and storage temperature. Results reported by these investigators showed that the rate of destruction of reduced ascorbic acid increased as the moisture content and water activity increased. Lee and Labuza (1975) have interpreted the increase in destruction rates to be the result of dilution of the aqueous phase, which results in a decreased viscosity, and thus increased mobility of reactants. Kinetic data generated by Jensen (1967), Vojnovich and Pfeifer (1970) and reported by Lee and Labuza (1975) have shown the energy of activation required for the destruction of reduced ascorbic acid to increase with moisture content in some foods while in other foods the opposite effect was observed. The reason for this discrepancy was not indicated.

Analytical methods estimating vitamin C content based on ascorbic acid (AA) or dehydroxy ascorbic acid (DHAA) quantification have limitations because, in many foods vitamin C occurs as both AA and DHAA. Unless DHAA is converted to AA or vice versa, misleading low results may be obtained (Dodson *et al.*, 1992). Vanderslice and Higgs (1989) examined a large number of food products and found that more than 90% of the products contained DHAA (the oxidized form of ascorbic acid) rather than ascorbic acid, at different levels.

II. B. 2. 3. 3. Anthocyanins

A great deal of antioxidant activity of phenolics is attributed to the presence of a well-known group of pigments called anthocyanins. Anthocyanins are an important member of the flavonoid family. Some of the other important members of the flavonoid family are flavonols, flavones, flavanones, catechins, isoflavones, dihydroflavonols, and

chalcones. Generic structure of flavonoids has been shown in Figure 2 of section B-1. The three phenolic rings are referred to as A, B, and C rings. The biochemical activities of flavonoids and their metabolites depend on the orientation of various moieties on different rings of the molecule (Cook and Samman, 1996).

Of all the berries, blueberries contain the highest amount of anthocyanin followed by cranberries, and strawberries (Kalt *et al.*, 1999). A previous work on the anthocyanin content of high bush blueberries (*V. corymbosum L.*) has reported 25-495 mg of anthocyanins/100 g of fresh blueberries, with variations being seen from one cultivar to another (Mazza and Miniati, 1993). The daily intake of anthocyanins in humans has been estimated to be as much as 180-215 mg / day in North America. Despite their relative high concentration in the diet of humans, the physiological impact of anthocyanins is not well studied. Among the possible physiological effects of anthocyanins are their antioxidant properties (Wang *et al.,* 1996).

Anthocyanins have been approved by Food and Drug Administration to be used as food colorants. In Canada according to the Food and Drug Act, anthocyanins are approved as food colorant and their limit of use in food products is upon the "good manufacturing practice" (The Food and Drugs Acts and Regulations, 1984). The composition and the distribution of anthocyanins in blueberries are affected by genetic factors and environmental conditions (Sapers *et al.*, 1984). Fifteen anthocyanins have been identified in blueberries cultivated in North America (Sapers *et al.*, 1984; Ballington *et al.*, 1988; Macheix *et al.*, 1990; Mazza and Miniati, 1993). Kader *et al.*, (1995), also reported the presence of fifteen anthocyanins in high bush blueberries after analyzing blueberry extracts by high performance liquid chromatography and thin layer

chromatography. Fifteen anthocyanins identified were the 3-monoglucoside, 3-monogalactoside, and 3-monoarabinoside of delphinidin, cyanidin, malvidin, peonidin and petunidin. No acyl anthocyanin was detected in this study, and derivatives of malvidin and delphinidin were the most abundant anthocyanins observed in high bush blueberries. Anthocyanins play a definite role in attracting animals in pollination and seed dispersal. They may also have a role in the mechanism of plant resistance to insect attack (Strack and Wray, 1989). The following section is a brief look at the chemistry of anthocyanins.

II. B. 2. 3. 3. 1. Chemistry of anthocyanins:

Anthocyanins are an important component of the phenolic composition of blueberries. They are natural colorants belonging to the flavonoid family. Anthocyanins are water-soluble pigments responsible for nearly all the red, purple, and blue colors of fruits and flowers (Mazza and Brouillard, 1987).

Anthocyanins are glycosides of anthocyanidins. The chromophoric aglycones (anthocyanidins) are red polyhydroxylated salts which, due to their instability, are seldom found in their free form in plant tissues. Differences between the individual anthocyanins are the number of hydroxyl groups in 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 sugar in the molecule. Since each anthocyanidin may be glycosylated and acylated by different sugars and acids, at different positions, the number of anthocyanins are 15-20 times greater than the number of anthocyanidins (Mazza and Brouillard, 1987).

The sugars most commonly bonded to anthocyanidins are glucose, galactose, rhamnose and arabinose. Sugars, acylated sugars, methoxyl and hydroxyl groups have a marked effect upon the color and reactivity of anthocyanins. There are over 250 naturally occurring anthocyanins (Strack and Wray, 1989), and all are O-glycosylated with different sugar substitutes (Francis, 1989). The common anthocyanins are either 3- or 3,5- glycosylated. When the number of sugar residues is higher than three, they may be attached to the basic molecule with alternating sugar and acyl linkages (Francis, 1989). Anthocyanin pigments can be described as being pH indicators- that is, their hue (shade of color) and intensity (depth of color) change with pH. At pH 1, anthocyanins exist predominantly in the highly colored oxonium or flavylium ion form, while at pH 4.5, they are mostly in the colorless carbinol form. The nature of the chemical structures which these anthocyanins can adopt upon changing the pH has been clarified (Brouillard and Dubois, 1977; Brouillard and Delaporte, 1977; Brouillard *et al.*, 1979; Brouillard, 1982). In acidic aqueous solutions, four anthocyanin species exist in equilibrium. They are: the quinoidal base A, the flavylium cation AH +, the pseudobase or carbinol B, and the chalcone C. At pH values below 2, the anthocyanin exists primarily in the form of the red (R3 = O-sugar) flavylium cation (AH+) (Figure 2). As the pH increases rapid proton loss occurs to yield a red or blue quinoidal forms (A). The relative amounts of cation (AH +), quinoidal form (A), pseudobase (B) and chalcone (C) at equilibrium vary with both pH and the structure of the anthocyanin (Mazza and Brouillard, 1987). Figure 3 exhibits anthocyanin structural transformations with pH.

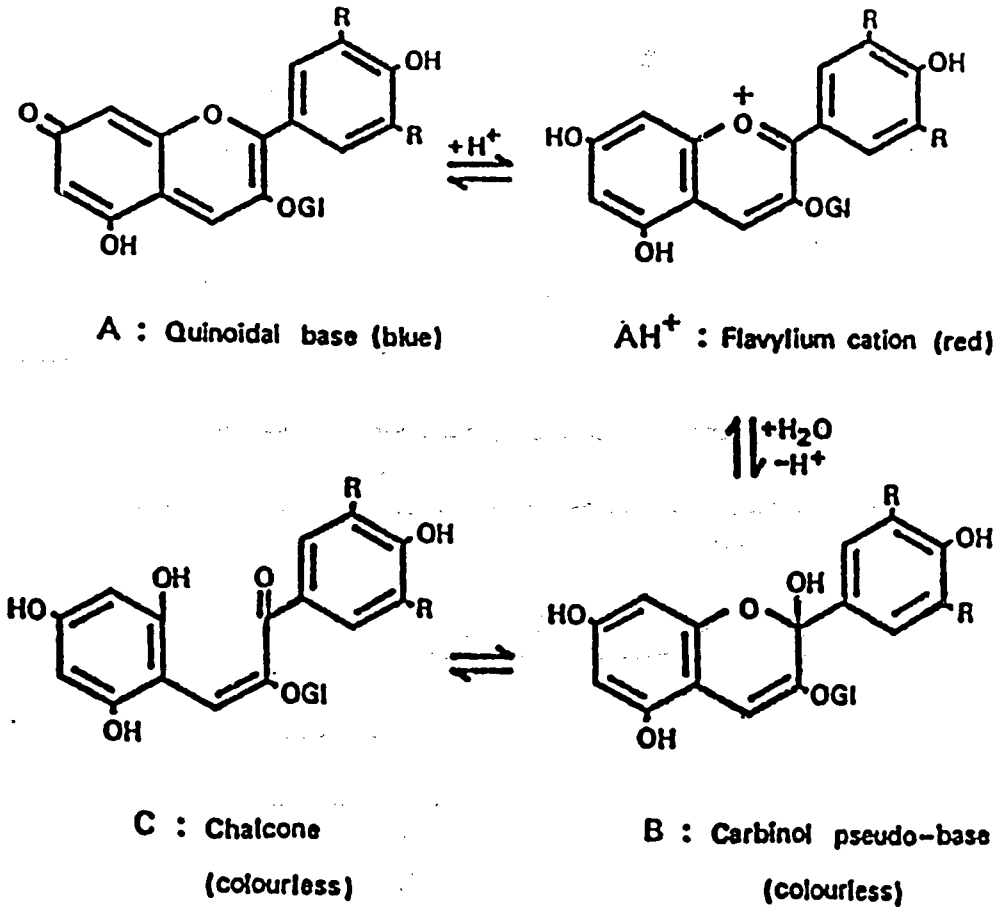

Figure 3. Anthocyanin structural transformations with pH. Malvidin-3-diglucoside (25° C, 0.2 M ionic strength) (Timberlake and Bridle, 1980).

Various spectroscopic and chromatographic methods have been used to determine the total anthocyanin content of different fruits. It is preferred to calculate anthocyanin concentration in terms of the major anthocyanin present in the fruit. While delphinidin-3-glucoside is the major pigment in blueberries, some workers use malvidin-3-glucoside to calculate the anthocyanin content of the blueberries. In reporting the results, it is important that the molar absorbance used in calculating pigment concentration be given. (Wrolstad, 1976).

II. B. 2. 4. Health effects of fruits and vegetables, including blueberries:

There is convincing epidemiological evidence showing that fruits and vegetables are beneficial to health and contribute to the prevention of degenerative processes (Ames, 1993). Other epidemiological studies showed that the consumption of fruits and vegetables is related to a reduced risk of cancer and cardiovascular disease (Steinmetz and Potter, 1991; Criqui and Ringel, 1994). At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Free radical may lie at the heart of the etiology or natural history of number of diseases, including cancer, heart, vascular, and neuro-degenerative diseases (Halliwell, 1994; Yu, 1994). Therefore antioxidants which can neutralize free radicals may be of central importance in the prevention of these disease states. The flavonoid content of foods is a major dietary factor responsible for this possible dietary effect (Hertog *et al.*, 1993, 1994, 1995; Kenkt *et al.*, 1996).

Therapeutic or physiological effects have been reported for the anthocyanins from berries, pharmaceutical preparations containing anthocyanin extracts from the fruits are used for medical treatment of various microcirculation diseases in Europe (Baj *et al.*,

1983). Anthocyanins have been shown to have some positive therapeutic effects including in the treatment of diabetic retinopathy (Scharrer and Ober, 1981), in fibrocystic disease of breast in human (Leonardi, 1993), and on vision (Timberlake and Henry, 1988). Anthocyanins may also have other potential physiological effects as anti-neoplastic agents (Kamei *et al.*, 1995), radiation protective agents (Akhmadieva *et al.*, 1993), vasotonic agents (Colantuoni *et al.*, 1991), vasoprotective and anti-inflammatory agents (Lietti *et al.*, 1976), chemoprotective agents against platinum toxicity in anti-cancer therapy (Karaivanova *et al.*, 1990), and hepatoprotective agents against carbon tetrachloride damage (Mitcheva *et al.*, 1993), and possibly other effects due to their diverse actions on various enzymes and metabolic process (Carpenter *et al.*, 1967; Ferrell *et al.*, 1979; Gibb *et al.*, 1987; Saija *et al.*, 1990; Costantino *et al*; 1995).

III. MATERIALS AND METHODS

A. Materials

Robinson Brothers Farm donated two cultivars of high bush blueberries, Hardy Blue and Bluecrop, in the month of December 1998 in the frozen state. Additional frozen Bluecrop berries of the 1998 crop were donated by Historic Collisha Farm. Berries had been individually quick-frozen, and were individually separatable from each other. The frozen berries underwent the various dehydration processes without being thawed.

The crop of the following year (1999) was purchased, in the freshly harvested state, from Berry Hill Farm in the month of August. The fresh berries were kept at -17.8° C cold room for up to three days, until they were processed. Portions of berries of both seasons were frozen at –33° C for freeze-drying.

All the chemicals used were reagent grade, unless they are specified differently.

III. B. Sample preparation

III. B. 1. Air-drying:

A total of 10.90 kg (5.45 kg of each cultivar) of berries from the crop of 1999, was placed on four mesh screens and the screens were placed together in a conventional air dryer, Vers- A- Belt, (Wal-Dor Industries. Ltd) Model 2.5-8. The berries were dried to a final moisture content and water activity of 13% and 0.537 for Bluecrop and 18.4% and 0.592 for Hardy Blue, by passing air at 70 ° C at a air flow rate of 1.335 m^3 /s for total of 12.5 hours. The water activity of all of the samples was measured (in triplicate) using an Aqua Lab instrument; model CX-2, after bringing the samples to room temperature. For

the crop of 1998, 9.1 kg of Hardy Blue spread on three net screens and 9.1 kg of Bluecrop placed on two net screens were air dried with the same instrument and the same settings. Each cultivar was air dried separately. Total time for this treatment was 8 hours that resulted in products with the final water activity of 0.561 and 0.550 for Hardy Blue and Bluecrop, respectively. Relative humidity of the air at the drier temperature was 12%.

III. B. 2. Vacuum microwave drying:

Drying was conducted in VMD2B, a vacuum microwave oven built by Enwave Corp (Port Coquitlam, B.C). The instrument has a drying chamber of 45 cm in diameter by 47 cm length. Microwave power of 1500 Watts, at 2450 MHz frequency, was available. Drying experiments were conducted at an absolute pressure of 5300 Pa, while the agitation of the drum was set at 5 rpm. A 10.90 kg sample of each cultivar from the crop of 1999 was divided into two batches and placed in the rotating basket of VMD2B at two separate episodes, and dried for sixty six, and fifty five minutes for Bluecrop and Hardy Blue cultivars, respectively with interruptions of approximately two minutes (to measure the temperature of sample and weigh the sample) at the following time periods: 30 minutes, 45 minutes, 55 minutes, 63 minutes, for Bluecrop cultivar, and 30 minutes, 40 minutes, 45 minutes, 50 minutes, 53 minutes, 54 minutes, for Hardy Blue cultivar. Blueberries were dried to 9% final moisture for Bluecrop and 11.7% final moisture for Hardy Blue, and the water activity of the final products were 0.490 and 0.450 for Hardy Blue and Bluecrop, respectively.

For the crop of 1998, 6.8 kg of Bluecrop (in one batch) and 9.1 kg of Hardy Blue (in two separate batches) underwent the same treatment with the same settings, as

mentioned for the crop of 1999, for 110 minutes for both cultivars. Final water activity of the products was 0.465 and 0.514 for Hardy Blue and Bluecrop, respectively.

III. B. 3. Freeze-drying:

A total of 10.90 kg of each cultivar of the 1999 crop were frozen at –33° C cold room for 48 hours before freeze-drying. The pressure in the drying chamber was set at 16 Pa, with temperature of the condensing chamber at –50° C. The samples taken out of the freeze drier after ten days had a moisture content of 8.2% for Hardy Blue and 14% for Bluecrop berries. Final water activity of the products was 0.418 for Hardy Blue and 0.514 for Bluecrop blueberries.

Total of 6.8 kg berries from each cultivar of the 1998 crop were freeze dried under the same conditions for one week, and had water activities of 0.447 and 0.445 for Hardy Blue and Bluecrop, respectively.

III. B. 4. Combination treatment:

The blueberries (10.90 kg) from the crop of 1999 were dehydrated by a combined air drying and vacuum microwave drying. The blueberries were first air dried at 70° C for four hours, under the same conditions as previously mentioned, until the final moisture content was 64% and 63.5% for Bluecrop and Hardy Blue, respectively. At this point they were transferred to the vacuum microwave oven and dried for an additional 48 minutes, with parameters set as previously described for the vacuum microwave dried samples. The moisture content and water activity of the final products were 15%, and 0.408 for Bluecrop cultivar, and 17.3% and 0.493 for Hardy Blue cultivar. The Bluecrop berries (4.55 kg) of the 1998 crop were subjected to air drying for 150 minutes to reduce their moisture content to 41.5%, and then underwent vacuum microwave drying for 21.5

minutes at four time segments to produce a final product with water activity of 0.460. Due to the scarcity of Hardy Blue berries in the 1998 crop, this cultivar did not have a combination treatment. Relative humidity of the air at the drier temperature was 12% for both years.

The time periods devoted for each kind of drying was based on a visual inspection of the sample. Blueberries in the air drier stuck to the net screen, and separation from the screen results in considerable loss of the fruit. This problem was more acute during the first few hours of the drying when the moisture content is high. Therefore, air-drying was terminated when the samples were separatable from the screen.

Upon completion of the drying procedures all the dehydrated samples were transferred to sealed bags and stored at -21° C until the time of analysis.

III. B. 5. Unprocessed blueberries:

About 10 kg of each cultivar, left in the bottom of two cardboard boxes and covered with plastic bags to prevent excessive dehydration, was stored at -18° C until the time of analysis (for 1-3 months depending upon the analysis), and denoted as unprocessed. Moisture content of these berries was 78.7% and 81% for Hardy Blue and Bluecrop, respectively.

Table 1 gives the summary of water activity and moisture content of the blueberries after the dehydration treatments.

Table 1. Summary of water activity and moisture content of the blueberries after the dehydration treatments

Treatment	Crop	Weight (kg)		Final aw		Moisture content	
		BC	HB	BC	HB	BC	HB
Air drying	1998	9.1	9.1	0.550	0.561	11.3	11.8
	1999	5.45	5.45	0.537	0.592	13.0	18.4
Air/VMD	1998	4.55	----	0.460	----**	16.6	----
	1999	5.45	5.45	0.408	0.493	15.0	17.3
VMD	1998	6.8	9.1	0.514	0.465	14.5	12.8
	1999	5.45	5.45	0.450	0.490	9.0	11.7
Freeze	1998	6.8	6.8	0.445	0.447	11.5	16.2
drying	1999	5.45	5.45	0.514	0.418	14.0	8.2

VMD= Vacuum microwave dried.

Aw= water activity

BC= Bluecrop

HB= Hardy Blue

The moisture contents of the 1998 crop are the final moisture contents, after equilibration.

** The Hardy Blue cultivar of the 1998 crop was not subjected to air/VMD treatment.

III. C. Chemical analysis of different treatments

III. C. 1. Estimation of total Vitamin C content

Blueberries from each treatment of the 1999 crop were analyzed for their vitamin C content, in five separate determinations. The AOAC (1996) microfluorometric method 968.22 was used to determine the total amount of vitamin C in the berries, by converting ascorbic acid to dehydroxy ascorbic acid (DHAA). Ascorbic acid is oxidized to dehydroxy ascorbic acid in presence of Norite, an oxidizing agent. The oxidized form of ascorbic acid is then reacted with 0-phenylenediamine (Sigma) to produce a fluorophor having activation max. at 350 nm and fluorescence max at 430 nm. The fluorescence of this compound is measured, and the concentration is estimated using a vitamin C standard curve. The vitamin C standard curve was prepared by reading the absorbance of various vitamin C solutions (0.5, 1, 3, 5, 7, 9, 10, 13, 18, 21, and 25 μg/ml) in triplicate, and plotting the mean $\pm$ standard deviation of these absorbances against their concentrations. It is noteworthy that the vitamin C solutions prepared for the construction of the standard curve were not passed through a C_{18} solid phase extraction cartridge, while the standard vitamin C solution (5 μg/ml, prepared and analyzed with every set of sample analysis) were. By doing so any deviation from the true vitamin C value, which might have occurred due to the use of C_{18} cartridge, can be estimated and corrected for accordingly.

Three grams of dehydrated or ten grams of frozen berries were blended in a General Electric explosion proof blender at low speed for three minutes with 15 ml of meta-phosphoric acid-acetic acid, 15 g HPO_3 pellets (ACS grade, Fisher Scientific) dissolved in 40 ml acetic acid 17N (ACS grade, Fisher Scientific), brought to 500 ml with distilled de-ionized water). The slurry was removed from the cup of the blender by the use of 10

ml additional extracting solution. The resulting slurry was passed through 5.5 cm No 1 Whatman filter paper in a Buchner funnel, wetted with a small amount of extracting solvent. The glassware was washed with some extracting solution and added to the filtrate, and the total volume of the filtrate was measured and recorded for each treatment. Two ml of this extract was passed through a Waters C_{18} solid phase extraction cartridge, in order to separate anthocyanins, which were found to interfere with the correct estimation of vitamin C. The cartridge was preconditioned by washing it with 3-5 ml of methanol (ACS grade, Fisher Scientific) and then 10-15 ml of de-ionized distilled. The clear solution eluting from the cartridge was analyzed for vitamin C content in Shimadzu Spectrofluorophotometer Model RF-540 (flow chart of the assay in Appendix). A standard solution of vitamin C was prepared and assayed in each set of five determinations of vitamin C in the blueberries. This standard, which was simply a 5 μg/ml solution of ascorbic acid in the extracting solution, was passed through the C_{18} solid phase extraction cartridge, as was done for all blueberry samples. A correction factor was estimated for each set of samples that were analyzed, by the use of this standard vitamin C solution. The deviation of this standard from the expected amount of 5 μg/ml was calculated as a percentage and defined as the correction factor for that specific set of analysis. The correction factor was used in calculations of vitamin C concentration of the samples by multiplying the estimated vitamin C content of each blueberry sample in a given set by the correction factor of that set of analysis. The correction factor calculated for the five sets of analyses was 1.12 ± 0.08.

III. C. 2. Estimation of total anthocyanin content

The pH differentiation method described by Fuleki, and Francis (1968) was adopted to estimate the total anthocyanin content of the blueberries of the 1999 crop. This method is based on the measurement of the absorption of the fruit extract at two different pH, at 510 nm. Altering the pH does not affect the absorption of the degradation products of anthocyanins, while it changes the absorption of the anthocyanins themselves to a great extent. The difference between the absorption values at two different pH values is an accurate indicator of the anthocyanins present (flow chart of the assay in the Appendix). Total anthocyanin reported for each sample was the mean ± standard deviation of five determinations. For each set of experiment two grams of each processed sample or ten grams of the unprocessed blueberries were pulped in a General Electric explosion proof blender with the addition of 20 ml of extracting solvent (95% ethanol (ACS grade, Fisher Scientific)/1.5N HCl (ACS grade, Fisher Scientific), 85/15 v/v ratio). Blending time was 3 minutes at the low speed, and an ice pack was wrapped around the cup to maintain the temperature at about 9° C. The resulting slurry was passed through 5.5 cm No 1 Whatman filter paper in a Buchner funnel, wetted with a small amount of extracting solvent. The glassware was washed with some extracting solvent and additional amounts of extracting solvent were added to bring up the final volume of the filtrate to 50 ml. The filtrates were covered with parafilm and stored overnight at 4° C. Since the straight line relationship between concentration and absorption can be expected only at low concentrations (Wrolstad, 1976), considerable dilution was necessary, and the filtrate was further diluted 25 fold and 5 fold, with 0.2N KCl-0.2N HCl buffer at pH= 1.0 and 1N sodium acetate- 1N HCl-water buffer at pH=4.5 respectively. The samples were then

equilibrated in the dark at room temperature for two hours. Absorbances was determined with a Unicam model II, UV/Vis spectrometer at 510 nm, and quantitation was carried out by using Beer's formula with an extinction coefficient of $\varepsilon = 77.5$ $(mg/ml)^{-1}cm^{-1}$ (Fuleki and Francis, 1968), taking the dilution ratios into consideration.

III. C. 3. Estimation of total phenolic compounds

Total phenolic content of the blueberries of the 1999 crop, was determined using Folin-Ciocalteu reagent (Velioglu *et al.*, 1998), and the results were reported as the mean ± standard deviation of five determinations. This assay uses the Folin Ciocalteu reagent (Sigma), to oxidize the phenolic compounds. Once oxidized, Na-bicarbonate (ACS grade, Fisher Scientific) was added and the phenolics were converted to compounds that absorb light at 725nm. The slurry of two grams of dehydrated samples or 5 grams of the frozen blueberries blended in General Electric explosion proof blender for 3 minutes at low speed, with 20ml of 80% methanol containing 1% hydrochloric acid, were extracted for 2 hours at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 4080 ×g at 9° C with Dupont centrifuge, model Sorvall RC 5B from Mandel Scientific Co. Ltd. for 15 minutes and the supernatant decanted into 50 ml vials. The pellets were extracted a second time, and the supernatants were combined and used for determination of total phenolics. One hundred µl of extract was mixed with 750µl Ciocalteu reagent (previously diluted 10 fold with distilled water) and allowed to stand at 22° C for 5 min. Then 750 µl of sodium bicarbonate (60g/L) solution was added to the mixture, and after 90 min incubation at 22° C, the absorbance was measured at 725 nm. The standard curve of gallic acid was prepared by plotting the absorbances of three

replicates of various concentrations (0, 50, 100, 150, 250, 350, 500 μg/ml) against their concentrations.

III. C. 4. Estimation of antioxidant activity

All of the dehydrated berries of the 1998 crop were analyzed for antioxidant activity in aqueous medium by the DNA breaking activity assay (Wijewickreme and Kitts, 1998).

The dehydrated and frozen blueberry treatments of the 1999 crop, were investigated for antioxidant activity using the thiobarbituric acid reaction (TBAR) assay (Buege and Aust, 1978). The results were reported as the mean ± standard deviation of three determinations. The assay was conducted in two sequential steps, as described later.

III. C. 4. 1. Estimation of antioxidant activity by DNA breaking activity of blueberry extracts

Blueberries from the 1998 crop were analyzed for antioxidant activity by this method (Wijewickreme and Kitts, 1998). Two grams of each processed blueberry sample was pulped in a General Electric explosion proof blender with the addition of 10 ml of 1N sodium acetate (ACS grade, Anachemia)- 1N HCl-water (100: 60: 90) buffer at pH= 4.5. Blending time was 3 minutes at the low speed, and an ice pack was wrapped around the cup to maintain the temperature at about 9° C. The resulting slurry was passed through 5.5 cm No 1 Whatman filter paper in a Buchner funnel, wetted with a small amount of buffer. The glassware was washed with same solution and additional amounts of buffer were added to bring up the final volume of the filtrate to 20ml. DNA strand scissions caused by Fenton reaction were measured with and without (control) the addition of blueberry extracts, using the DNA pBR322 plasmid DNA (from Escherichia coli strain RRI) (Sigma). A reaction mix containing 2 μl of 20 μM $FeCl_3$, 2 μl of 2 μM

ascorbic acid (from BDH), 2 µl of 100 mM sodium phosphate buffer pH 7.5, and 2 µl of 9 mM H_2O_2 was prepared. To this mixture 2 µl of blueberry extract or extracting solvent and 2 µl of DNA (0.5 µg/ml was added. Reactions were performed in 250 µl Eppendorf tubes. Deionized distilled water was added to give a final volume of 14 µl to all tubes, and samples were incubated for one hour in a water bath at 37° C. After incubation 2 µl loading dye (0.5% bromophenol blue/0.5% xylene cyanol / 50% glycerol) was added to each reaction mixture and 10 µl was loaded onto a 0.7% (w/v) agarose gel. Electrophoresis was conducted for 90 minutes at 50 V. Gels were scanned by a Bio-Rad model GS-670 imaging densitometer and the percentage of nicked and intact (double strand) DNA was quantitated using the software program Molecular Analyst (version 1.3).

III. C. 4. 2. Estimation of antioxidant activity by thiobarbituric acid reactive substances (TBARs)

Blueberries of the 1999 crop were analyzed for antioxidant activity by TBARs method. This assay is based on estimation of the concentration of malonaldehyde as an index of lipid oxidation. Malonaldehyde is a secondary product of lipid oxidation that can react with thiobarbituric acid reagent and produce a chromophore complex, which can be detected at 532 nm. The analysis was carried out in two steps.

III. C. 4. 2. 1. Preparation of blueberry-lipid mixtures:

Lipid oxidation model was set up according to the methods of McGookin and Augustin (1991). Blueberry extracts from 3g of the dehydrated samples or 5g of the unprocessed (frozen) ones were blended and filtered according to the procedure described in section C. III. The residue in the funnel was washed with the extracting solution until

the anthocyanin content, when read at 510 nm with the Unicam spectrophotometer, was 3.4% of the anthocyanin content of the first filtrate. The final volume of each extract, which varied from 25- 35 ml for different treatments, was recorded, and used in the calculations of total antioxidant activity of each blueberry sample. A fraction of 0.26 ml of each filtrate (or de-ionized distilled water for the control) was added to 0.26 ml of linoleic acid/Tween 20 (1:1). To this mixture 20 ml of 0.2M buffer of Na_2HPO_4/NaH_2PO_4, pH 7 was added and the total volume was brought up to 25 ml with de-ionized distilled water. The mixtures were transferred to Erlenmeyer flasks and shaken for approximately a minute manually. Then the flasks were left to stand for 30 minutes. The Erlenmeyer flasks were then incubated at 25° C. Prior to incubation their absorption was determined at 532 nm according to the procedure below, and recorded as the absorption for time 0.

III. C. 4. 2. 2. Assessment of lipid oxidation by thiobarbituric acid method:

Estimation of TBARs was adopted from Buege and Aust (1978). A fraction of 0.60 ml of the lipid emulsion of extract taken at 0. 6, 12, 18, 24, 36, 48, 54, and 62 hours of incubation, described above was transferred to a test tube and an equal amount of 1.5N HCl was added. To this mixture, 0.300 ml TBA reagent (0.5% 2-thiobarbituric acid (TBA) in 0.25 M NaOH (ACS grade, Fisher Scientific) containing 0.02% (w/v) freshly prepared BHT (Sigma); butylated hydroxytoluene) was added. The tubes were covered with a lid, placed in a boiling hot water bath for 15 min and cooled down to room temperature on the bench top. The absorbance of the reaction solutions at 532 nm was recorded using a Shimadzu-160 UV-Vis spectrophotometer (TekScience, ON). A standard curve of malonaldehyde was constructed by using 1,1,3,3-tetraethoxypropane in

1% sulfuric acid (Sigma), and plotting values of absorbances (three replicates of each one of: 0, 1, 2, 5, 7, 9, 10, 12, 15 and 20 (μmoles/ml) versus their concentrations. One mole of 1, 1, 3, 3-tetraethoxypropane yields one mole of malonaldehyde when hydrolyzed by an acid. The concentration of MDA in each sample (μmoles MDA/gram dry weight of blueberries) was calculated and converted to the % inhibition by comparing [MDA] of the sample to that of the control using the following formula;

$$\% \text{ inhibition} = \{([\text{MDA}]\text{control} - [\text{MDA}]\text{sample}) * 100\} / [\text{MDA}]\text{control}$$

III. D. Instrumental analysis

III. D. 1. Fractionation of anthocyanins: Fractionation of anthocyanins was carried out by a reverse phase high performance liquid chromatography adopted from Gao and Mazza, (1994b).

III. D. 1. 1. Extraction: An accurately weighed samples of blueberry (10 g) were blended in a General Electric explosion proof blender with the addition of 20 ml methanol (HPLC grade, EM Science)/formic acid (HPLC grade, BDH)/water (70/2/28). Blending time was 3 minutes at the low speed. An ice pack was wrapped around the cup to maintain the temperature at about 9° C. The resulting slurry was passed through 5.5-cm No.1 Whatman filter paper in a Buchner funnel, wetted with a small amount of extracting solvent. The glassware was washed with some extracting solvent and additional amounts were added to bring up the final volume of the filtrate to 100 ml. To determine the efficiency of the extraction and filtration method, the residue left on the filter paper was mixed with the extraction solution, agitated, centrifuged and its

absorbance was measured at 510 nm. Using Beer's law the concentration of anthocyanin in the first filtrate and the residual filtrate was calculated (ε= 77.5 $(mg/ml)^{-1}$ cm^{-1}) (Fuleki and Francis, 1968), and it was determined that the residual filtrate contained only 3.4% of the anthocyanin of the first filtrate, calculated from mean of three replicates. The efficiency of the extraction method was estimated at approximately 97%. One ml of the filtrate was passed through Sartorius Minisart sterile filter 0.45 μm. Three hundred microliter of this new filtrate was taken and mixed with 600 μl de-ionized distilled water, and the mixture was loaded on a conditioned C_{18} solid phase extraction cartridge. The cartridge was conditioned by washing it with 2 ml of methanol, and then passing 5 ml of distilled deionized water through it. Then 900 μl of the mixture of deionized water and blueberry extract was loaded into the cartridge, the initial clear liquid was discarded and the colored fraction containing anthocyanins was collected. Twenty-five microliter of this fraction was injected onto the HPLC column.

III. D. 1. 2. HPLC analysis of anthocyanins: High performance liquid chromatography was carried out on all of the treatments of both cultivars of 1998 crop and only air-dried and combination treatments of Hardy Blue cultivar of the 1999 crop. The HPLC system (Waters- M.45), equipped with an LC, Lambda-Max detector, Model 481, spectrophotometer, at 525 nm was operated with a C_{18} column, Phenomenex, type: Spherex 598, size: 250×4.60 mm, 5 micron particle size, as a stationary phase. The solvents and elution profiles were a modified version of the profile adapted from Gao and Mazza, (1994b): solvent A, 5% formic acid in water; solvent B, methanol; elution profile: 0-10 min, 10% B; 10-50 min 30% B; 50-55 min, 60% B; 55-65 min, 100% B; 65-75 min, 10% B. The flow rate was 1.2 ml/min.

The area of each peak was estimated using the integrating program of the HPLC system. A peak with the smallest standard deviation was selected for both cultivars, and designated as the reference peak. The ratio of each peak to the reference peak was calculated and expressed as the relative peak area of that specific peak.

III. D. 2. Hunter Lab color analysis of blueberries:

A multilayer sample of each treatment was placed on a plastic petri dish with the diameter of 8.5 cm, and depth of 1.5 cm for colorimetric measurements by using Hunter Lab Color Difference Reflectance Spectrophotometer (Hunter Lab Lab Scan) with a 5 cm aperture, and standard illuminate of D65 to simulate day light at a correlated color temperature at about 6500 ^{0}K. Each sample was placed on the dish and positioned over the viewing area, and readings were taken after the sample was rotated 90^o C, four times. A black tile was used to zero the colorimeter, and a white tile with the following parameters; X = 79.80, Y = 84.67, Z = 91.23, was used to standardize the instrument. Hunter L, a, and b scores were recorded on five replicates of each sample.

III. D. 3. Estimation of moisture content of the berries:

For estimation of the moisture content of the samples, the AOAC official method procedure number 934.06 (AOAC, 1996) was applied. Briefly: ca 5 g of each sample was weighed and spread as evenly as possible over bottom of a metal dish with about 8.5 cm in diameter, and dried 6 hr at 70 ± 1^o under pressure < 13.3 kPa. During drying, a slow current of air is admitted to the oven by passing through H_2SO_4. The dishes were cooled in the desiccator and weighed at the end of the mentioned period. Moisture content was calculated by:

% MC=[(initial weight - final weight) / initial weight] *100%

III. E. Statistical analyses

All data are expressed as means ± standard deviation. Two way analysis of variance followed by Tukey test was performed by using Minitab statistical software, version 12.0, State College of PA. The linear regression program (Minitab) was used to establish a correlation between different variables. The level of confidence required for significance in all of the performed statistical analysis was selected at $p \leq 0.05$.

IV. RESULTS AND DISCUSSION

IV. A. CHEMICAL ANALYSIS

IV. A. 1. Estimation of total Vitamin C content

Vitamin C content of blueberries was estimated by AOAC fluorometric method (AOAC, 1996). Detectable amounts of vitamin C were found to be present in blueberries after they had been frozen, vacuum microwave dried and freeze dried (Table 2). Vitamin C levels of air-dried and combination treatments were not high enough to be detectable by this method. In order to have the vitamin C content of a sample detected by this method the concentration of vitamin C should be ≥ 0.1 μg/ml in the solution read in the spectrofluorometer. This amount is approximately 0.1 mg/100 g dry weight. Considering the sensitivity of this method, it is clear that the two air-dried and combination treatments have experienced a severe vitamin C loss. Yang and Attalah (1985) reported as much as 91% loss of vitamin C (compared to the fresh berries) in low bush blueberries processed by forced air-drying method. In this work, the loss of vitamin C in both air-dried and combination treatment was estimated at more than 99% of the vitamin C present in the frozen blueberries of each cultivar. The degradation of ascorbic acid in the presence of hot air in air-dried and combination treatments is the possible explanation for these losses. The relatively long drying time associated with the air-drying method contributes to this severe loss of vitamin C (Schadle *et al.*, 1983). The results of vitamin C estimation for the frozen berries were 23 -37 mg/100 g frozen berries. These numbers available in the literature for the vitamin C content of fresh

blueberries are 40-60 mg/100 g berries. It is evident that freezing has also subjected blueberries to some degree of vitamin C loss.

There was a significant difference in vitamin C content associated with treatments and cultivars. Vitamin C content of frozen blueberries was higher than any of the dehydrated ones in Hardy Blue cultivar. In Bluecrop vitamin C content of the freeze-dried was comparable to that of the frozen berries. Among the dehydrated blueberries the freeze-dried samples had the highest vitamin C content followed by those that were vacuum microwave dried. Because of the low temperature (in the case of freeze drying), and low oxygen pressure (in both cases of freeze drying and vacuum microwave drying) during the drying processes, berries processed by these treatments did not incur as much vitamin C loss. The considerable reduction in vitamin C content of vacuum microwave dried berries (80% of the vitamin C content of the frozen Hardy Blue, and 59% of the vitamin C content of frozen Bluecrop) can be attributed to the thermal degradation of vitamin C resulting from heat. Tein *et al.* (1999), reported vitamin C loss of about 60% of the vitamin C content of fresh carrots in vacuum microwave dried carrots. Deactivation of enzymes responsible for ascorbic acid degradation (such as ascorbic acid oxidase) achieved by blanching prior to dehydration, can be the reason for the higher retention of vitamin C in the study by Tien *et al.*, 1999. In addition the periodic opening of the VMD basket to examine the sample exposed the warm blueberries to atmospheric oxygen. Degradation of vitamin C due to oxidation can perhaps be responsible for a fraction of this loss in our study.

Table 2. . Vitamin C content (mg/100 g dry weight) of frozen, air-dried, combination dried, vacuum microwave-dried, and freeze-dried blueberries of Bluecrop and Hardy Blue blueberries of the 1999 crop.

Cultivar	Frozen	Air dried	Combination	VMD	Freeze dried
Bluecrop	22.96^{ax}	ND	ND	9.36^{bx}	22.14^{ax}
	(3.40)			(0.83)	(2.23)
Hardy Blue	37.18^{ay}	ND	ND	7.48^{bx}	25.08^{cx}
	(2.83)			(1.124)	(4.89)

*Values are average of five determinations. Values in brackets represent the standard deviations.

Means within a column that do not have a common superscript letter (x and y) are significantly different ($p \leq 0.05$).

Means within a row that do not have a common superscript letter (a, b,and c) are significantly different ($p \leq 0.05$).

VMD= Vacuum microwave dried

Combination= Air/vacuum microwave dried

ND=Not detectable (concentration in the cuvette< 0.1 μg/ml)

When examining the degree of vitamin C loss in two cultivars, Table 2 reveals that the frozen berries of the two cultivars started with significantly different vitamin C content, and once they were vacuum microwave dried or freeze-dried, the vitamin C content was not significantly different between the two cultivars. This indicates that Bluecrop berries experienced a smaller loss.

To explore a possible relationship between berries' size and vitamin C content, frozen berries belonging to Bluecrop cultivar were sorted into three groups according to their size as follows: group A unsorted BC berries as they naturally occur, group B contains the small berries of BC cultivar, and group C contains the large berries of BC. To give an idea about the density of these berries, one hundred grams of Hardy Blue contained 70 ± 1 berries, while for Bluecrop (A) and its subclasses B, and C, 100 grams contained 46 ± 1, 68 ± 1, and 30 ± 1 berries, respectively. The results of the analysis of these berries for vitamin C content revealed the following trend (Figure 4).

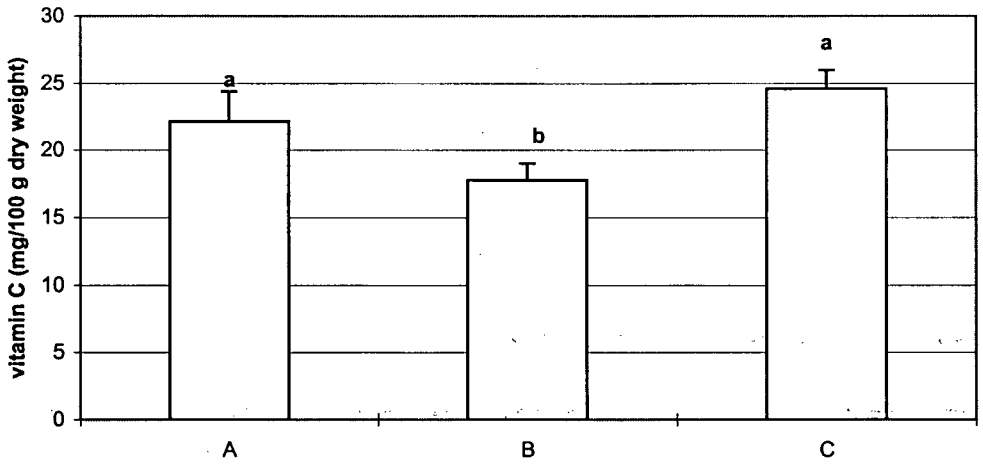

Figure 4. . Vitamin C content (mg/100 mg dry weight) in Bluecrop berries of the 1999 crop, as affected by the size of the berries.

Values are average of five determinations.

Bars having a common letter are not significantly different ($p \leq 0.05$).

A: Unsorted frozen berries (46 berries /100 grams)

B: Small berries (68 berries /100 grams)

C: Large berries (30 berries / 100 grams)

Error bars indicate standard deviations.

The data suggests that there is a direct relationship between the size of the berry and its vitamin C content within this cultivar; as the berry's size increases so does its vitamin C content. However, as it was already evident from the data of Table (1) this relationship between size and the vitamin C content does not hold true between cultivars. Hardy Blue berries, which are smaller, contain more vitamin C than the Bluecrop berries.

The data indicate that vacuum microwave drying can preserve vitamin C more efficiently than air-drying. Because vitamin C is relatively unstable to heat, oxygen, and light, the retention of this nutrient can be used as an indicator for the quality of dried fruit (Tein *et al.,* 1999). However, the quantity of vitamin C reported to be present in these products is the amount which were present within two weeks after the processing. The amount of vitamin C at the time the dehydrated berries reach to the consumer is likely to decline depending on many factors among them length of the storage time (Kmieck, 1995) and the storage temperature (Reitman, 1987). In a study carried out on the vitamin C content of hop cones, Reitman observed a nearly complete deactivation of ascorbic acid oxidase dried to the moisture content of 8-9%. He also noted that the enzyme is reactivated by different factors among them length and temperature of storage. Therefore, while the data collected from this part of analysis does support the proposed hypothesis that the vacuum microwave dried blueberries had a higher concentration of vitamin C compared to the air dried ones, further care in storage and handling is required to maintain their quality in terms of vitamin C content.

IV. A. II. Estimation of total anthocyanin content

The anthocyanin content of the berries was estimated by the pH differentiation method of Fuleki and Francis (1968). A previous report of anthocyanin content in highbush blueberries (*V. corymbosum L.*) indicated that fresh berries contained 25-495 mg/100 g fresh weight (Mazza and Miniati, 1993). Gao and Mazza (1994a) in quantifying the anthocyanin content of high bush blueberries by RP-HPLC have reported that two high bush blueberry cultivars contained about 100 mg of anthocyanin/100g fresh berries. These reports have all indicated a large variation. The results of this study indicated 46 ± 9.8 mg/100g and 113 ± 13 mg/100g frozen blueberries for Bluecrop and Hardy Blue berries, respectively. Aside from the seasonal variations, the wide variation between the anthocyanin content reported by various workers can be attributed to the different extinction coefficient values used in the calculation. While some workers use the extinction coefficient of malvidin-3-glucoside, some others use that of delphinidin-3-glucoside instead for calculations (Wrolstad, 1976). The extinction coefficient used in calculating anthocyanin concentration in this study was that of delphinidin-3-glucoside ($\varepsilon = 77.5\ (mg/ml)^{-1}\ cm^{-1}$ Fuleki and Francis, 1998) on account of delphinidin-3-glucoside being the major pigment in blueberries. The anthocyanin content of the frozen and dehydrated blueberries of the two cultivars of Bluecrop and Hardy Blue is shown in Table 3.

Table 3. . Anthocyanin content (mg/100g dry weight) of frozen, air dried, combination dried, vacuum microwave-dried, and freeze-dried Bluecrop and Hardy Blue blueberries of the 1999 crop

Cultivar	Frozen	Air-dried	Combination	VMD	Freeze dried
Bluecrop	258^{abx}	117^{ax}	129^{ax}	173^{ax}	319^{bx}
	(50)	(20)	(22)	(21)	(42)
Hardy Blue	530^{ay}	198^{bx}	218^{bx}	498^{ay}	574^{ay}
	(60)	(16)	(31)	(44)	(87)

*Values are average of four determinations. Values in brackets represent the standard deviations.

Means within a column that do not have a common superscript letter (x and y) are significantly different ($p \leq 0.05$).

Means within a row that do not have a common superscript letter (a and b) are significantly different ($p \leq 0.05$).

VMD= Vacuum microwave dried

Combination= Air/vacuum microwave dried

Table 3 reveals that in most of the treatments, anthocyanin content of Hardy Blue exceeds that of their Bluecrop counterpart ($p \leq 0.05$). It is likely that the fresh Hardy Blue berries contained more anthocyanin than the fresh Bluecrop ones. This could partially be due to the fact that Hardy Blue cultivar contains much smaller berries and as a result these have a larger amount of skin per gram basis, and anthocyanins are mostly concentrated in the skin of berries (Prior *et al.*, 1998). The concentration of anthocyanin in freeze-dried samples exceeded all other dehydrated treatments and was comparable to that of the frozen samples in both cultivars ($p \leq 0.05$). Analysis of variance also indicated that the anthocyanin content of vacuum microwave dried berries were not significantly different from that of the frozen berries in both cultivars ($p \leq 0.05$). In case of Hardy Blue the anthocyanin content of vacuum microwave dried berries was comparable to that of freeze-dried berries as well. There was not any significant difference between the anthocyanin content of air-dried and combination dried blueberries in both cultivars ($p \leq 0.05$).

Results within the Hardy Blue cultivar suggests a relationship between the increase in the length and intensity of the heat treatment and the degree of anthocyanin loss, which is in agreement with results reported by Shewfelt in 1966. Gubina and Fedotova (1977), also reported that all forms of drying led to a decline on the anthocyanin content of blueberries, the loss being 16.5-25.5% in shade-dried fruits increasing to 89-90% when dried by forced air at 100° C. Degradation of anthocyanin increases as the temperature increases (Timberlake and Bridle, 1980). Numerically, a similar trend is observed in the Bluecrop as well, but the numbers are not statistically different.

Because the anthocyanins are mostly concentrated in the skin of the blueberries, it is expected that the total anthocyanins would increase in proportion to the surface area/volume ratio of the blueberries (Prior *et al.*, 1998). In order to determine whether or not this is the case in the berries of this study, Bluecrop berries were segregated into small (B) and big (C) berries, as previously described for vitamin C analysis and analyzed for their total anthocyanin contents. The result of this analysis has been illustrated in Figure 5.

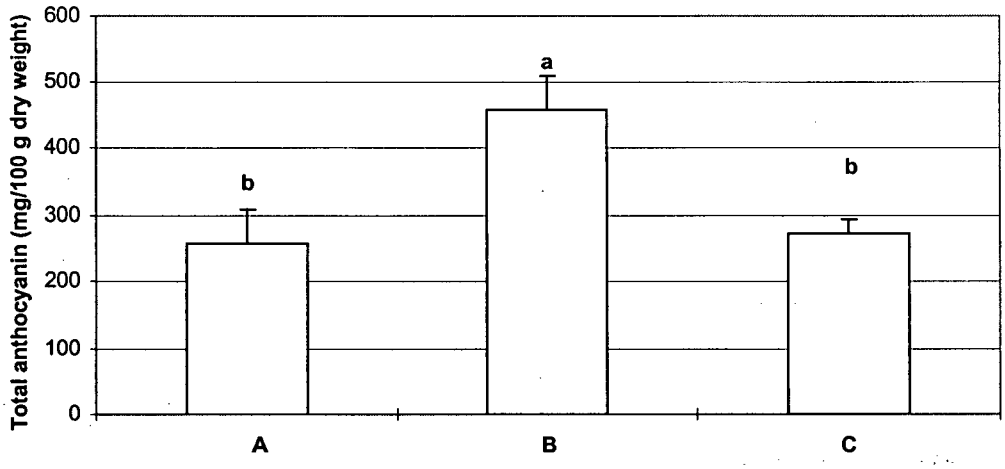

Figure 5. Anthocyanin content (mg/100 mg dry weight) in Bluecrop berries of the 1999 crop, as affected by the size of the berries.

Values are average of five determinations.

Bars having a common letter are not significantly different ($p \leq 0.05$).

A: Unsorted frozen berries (46 berries /100 grams)

B: Small berries (68 berries /100 grams)

C: Large berries (30 berries / 100 grams)

Error bars indicate standard deviations.

Data from Figure 5 reveals that the direct relationship between the surface area/volume ratio and the anthocyanin content of the berry holds true within Bluecrop cultivar; B subclass of the Bluecrop which contains smallest berries and as a result highest surface area/volume ratio has the highest anthocyanin content. It is possible that this relationship holds true across the cultivars as well, and the high anthocyanin content of the Hardy Blue cultivar (containing smallest berries) is in part due to this effect. Prior *et al.* 1998, reported that this relationship held across cultivars within *V. corymbosum* and *V. ashei Reade* species. However changes in anthocyanin content at different stages of maturity can cause this relationship to break down. Anthocyanins increase markedly with maturity, while the surface area/volume does not change (Prior *et al.*, 1998).

In view of these results, it is concluded that freeze-drying and vacuum microwave drying are two methods that preserve the highest amount of anthocyanin in blueberries. Considering the lower processing cost and shorter processing time of vacuum microwave drying, this method seems to be superior to the conventional drying method of forced air-drying.

IV. A. 3. Estimation of total phenolic compounds

The total phenolic content of the berries was estimated by the Folin-Ciocalteu method of Velioglu *et al.* (1998), with gallic acid used to construct the standard curve. The total phenolic content of berries investigated in this study varied from 424.8 ± 10.7 mg/100 g to 678.6 ± 19.8 mg/100 g of frozen berry for Bluecrop and Hardy Blue, respectively. The values reported in the literature vary widely. The method employed, the extraction solutions used and the particular phenolic compound utilized for construction of the standard curve could all influence the absolute amounts calculated. For instance, Velioglu *et al.* 1998, reported 627 mg/100 g fresh weight blueberries, estimated by Folin-Ciocalteu method for blueberries extracted by methanol/HCl, using ferulic acid to construct the standard curve. Prior *et al.* (1998), analyzed eight different cultivars of high bush blueberries and reported their total phenolic content in the range of 181.1 ± 10.4 mg/100g fresh weight to 390.5 ± 6.5 mg/100 g fresh weight. However, the extracting solution used by this group was acetonitrile/acetic acid instead of methanol/HCl used in this study. This could be one factor which explains why the amounts estimated in this work are higher than that reported by Prior *et al* (1998).

According to our results, among the dehydrated berries, vacuum microwave dried blueberries contained the highest amount of phenolic compounds ($p \leq 0.05$) (Figure 6). This again can be attributed to oxidative and thermal degradation of the phenolic compounds, which is increased as the heat intensity, and the length of heat treatment (in the case of air dried blueberries and berries dried by the combination treatment) increases.

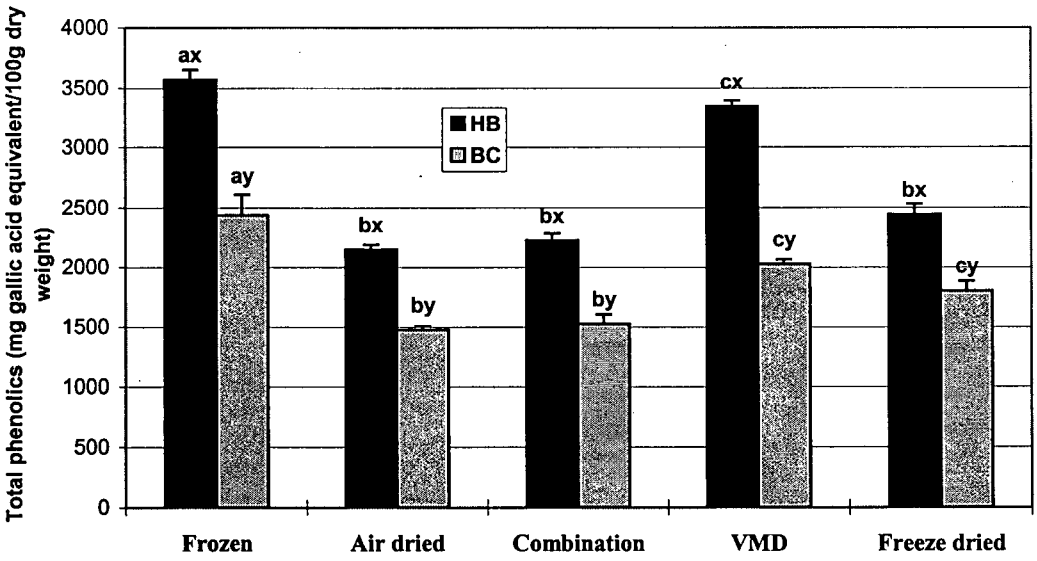

Figure 6: Total phenolic content in gallic acid equivalents of various treatments of Bluecrop and Hardy Blue blueberries, of the 1999 crop.

Values are average of five determinations.

Means adjacent to each other that do not have a common letter (x and y) are significantly different ($p \leq 0.05$).

Means within a cultivar that do not have a common letter (a, b, and c) are significantly different ($p \leq 0.05$).

VMD= Vacuum microwave dried

Combination= Air/vacuum microwave dried.

Error bars indicate standard deviations.

In case of Hardy Blue cultivar, there was a significant difference between the phenolic content of vacuum and freeze-dried blueberries ($p \leq 0.05$). A plausible explanation for this finding can be the loss of some blueberry juice and tissue during vacuum microwave drying. As a result of this juice loss the skin/tissue ratio can increase. Since phenolics are mostly concentrated in the skin of the berries (Prior *et al.*, 1998), the estimated total phenolic compounds can increase consequently. The Hardy Blue cultivar had a much larger skin/tissue ratio to begin with, and therefore was affected to a higher degree than the Bluecrop. Figure 6 also shows that, in every category, Hardy Blue berries have a higher concentration of total phenolic compounds compared to their Bluecrop counterparts ($p \leq 0.05$). Gao and Mazza (1994b) also reported variation in the total phenolic content of high bush blueberries from one cultivar to another. Prior *et al.*, 1998 observed that such a difference in the content of the phenolic compounds not only exists in the various species of blueberry; high bush and low bush, but also in various cultivars of the same species of blueberry.

Since anthocyanins are part of the total phenolic profile of blueberries an attempt was made to establish a relationship between the total phenolic content and the total anthocyanin content of the blueberries. The total phenolic content displayed a linear relationship with anthocyanin content of berries, with a positive slope and an $r^2 = 0.64$ (Figure 7). This correlation was found to be significant ($P = 0.005$, $n=10$).

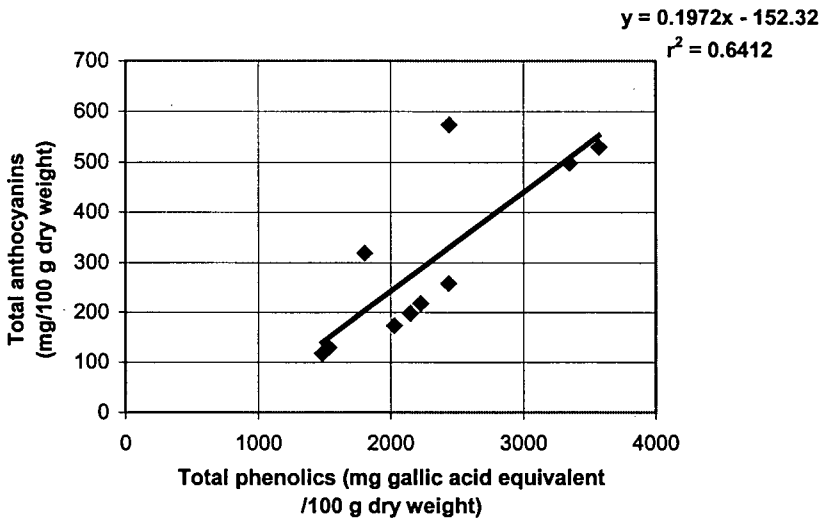

Figure 7. Relationship between total phenolics and total anthocyanin content of frozen and dehydrated Bluecrop and Hardy Blue blueberries of the 1999 crop.

Values are average of five determinations.

Correlation was significant (P= 0.005, n=10).

Anthocyanin content increased linearly with total phenolics. It is possible that higher levels of phenolics not only promote the synthesis of anthocyanins by an increased availability of precursors for synthetic purposes (Green and Mazza, 1986), but they also assist their stability while undergoing various treatments. Simrad *et al.* 1982, reported that high phenolic content was an important factor to stabilize anthocyanins in blueberry juice.

Overall vacuum microwave drying method demonstrated an excellent efficiency in preserving phenolic compounds of the blueberries, and their phenolic content did prove to be higher than the air-dried berries.

IV. A. 4. Estimation of antioxidant activity

To estimate the antioxidant activity of the frozen and dehydrated blueberries of the 1999 crop, the thiobarbituric acid reaction (TBAR) method was used (Buege, and Aust, 1978). Inhibition of linoleic oxidation was measured by a decrease in the formation of malonaldehyde (MDA), a secondary lipid oxidation product. A designated value of %inhibition was obtained by applying the following formula:

$$\% \text{ inhibition} = \{([MDA]_{control} - [MDA]_{sample}) * 100\} / [MDA]_{control}$$

As Table 4 shows, the oxidation of linoleic acid was noticeably inhibited by all dehydrated and frozen blueberry extracts. The extracts of the frozen Hardy Blue and Bluecrop berries resulted in 45.8% and 31.5% inhibition of linoleic acid. Kalt *et al.* 1999, also reported a variation in the antioxidant activity of the *Vaccinum* species from one species to another. Analysis of variance also revealed that in both cultivars there was not any significant difference in the antioxidant activity of various dehydrated blueberries ($p \leq 0.05$). Most of these dehydrated berries differed significantly from each other in terms of the concentration of their potential antioxidant components i.e. vitamin C, anthocyanin, and total phenolic compounds. Having the same degree of antioxidant activity despite the differences in the concentration of analyzed antioxidant components points at the presence of some other antioxidant components in the blueberries. In addition a possible synergistic or / and antagonistic effects of these compounds on each other is another factor to consider, while reviewing these results.

Table 4. Total antioxidant activity (lipid soluble model) of blueberries, frozen and dried by various techniques, of Bluecrop and Hardy Blue blueberries of the 1999 crop.

Cultivar	Frozen	Air dried	Combination	VMD	Freeze dried
Bluecrop	31.5^{ax}	15.6^{bx}	19^{abx}	20.4^{abx}	23.3^{abx}
	(8.0)	(4.4)	(1.5)	(3.4)	(4.1)
Hardy Blue	45.8^{ay}	19.6^{bx}	24.7^{bx}	28.2^{bx}	29.9^{bx}
	(1.0)	(3.5)	(5)	(1.3)	(4.3)

*Values are average of three determinations. Values in bracket are the standard deviations.

Means within a column that do not have a common superscript letter (x and y) are significantly different ($P< 0.05$).

Means within a row that do not have a common superscript letter (a and b) are significantly different ($P< 0.05$).

VMD= Vacuum microwave dried

Combination= Air/vacuum microwave dried

The antioxidant capacity of the 1998 crop was estimated by the DNA cleavage assay (Wijewickreme and Kitts, 1998). Blueberry extracts were added to the test tubes containing DNA and the components of the Fenton reaction. The free radicals generated from the Fenton reaction would result in the DNA cleavage in the control test tubes, and this will be evident from the % nicked DNA estimated by gel electrophoresis and subsequent densitometry of the corresponding bands on the gel. The test tubes containing blueberry extracts had a clear reduction in the % nicked DNA. This reduction is the direct indication of the antioxidant activity of the blueberry extracts; antioxidants have the capacity to deactivate free radicals, or prevent their formation thereby preventing the cleavage of DNA. Addition of all blueberry extracts resulted in a reduction of percent nicked DNA, suggesting that all of the dehydrated berries possess antioxidant activity. Freeze-dried berries exhibited the highest antioxidant activity by decreasing the percent nicked DNA to 39% and 33.6% for Hardy Blue and Bluecrop, respectively (Table 5).

Table 5. Total antioxidant activity (water soluble model) of air dried, vacuum microwave dried and freeze dried blueberry extracts of Bluecrop and Hardy Blue blueberries of the 1998 crop.

lane #	Content	% super-coiled DNA	% Nicked DNA
1	Control DNA	68.5	31.5
2	RXN mix + DNA	0.0	100.0
3	RXN mix + DNA +AB	55.7	44.3
4	RXN mix + DNA +VB	60.1	39.9
5	RXN mix + DNA + FB	66.4	33.6
6	RXN mix + DNA + AH	49.5	50.5
7	RXN mix + DNA + VH	52.3	47.7
8	RXN mix + DNA + FH	61.0	39.0

Control DNA = 2μg DNA

RXN mix = 2 μl of: 100mM sodium phosphate buffer with pH=7.4, 20μM $FeCl_3$, 2μM ascorbic acid, 9 mM H_2O_2, incubated @ 37° C for 1 hour.

AB= Air dried Bluecrop, VB= Vacuum microwave dried Bluecrop, FB= freeze dried Bluecrop

AH= Air dried Hardy Blue, VH= Vacuum microwave dried Hardy Blue, FH= Freeze dried Hardy Blue

The values are from a single experiment (no replicates).

The antioxidant activity of various treatments increased from the air-dried to vacuum microwave dried and to the freeze-dried blueberries numerically. Due to the lack of replicates the results cannot be analyzed statistically. However, in the 1998 crop the overall antioxidant activity of the Bluecrop was higher than that of the Hardy Blue. For the 1999 crop, this trend was reversed, and the Hardy Blue had a higher activity. This difference perhaps can be attributed to the seasonal differences in the composition of these cultivars in two crops of 1998 and 1999. Maturity at harvest is another factor to be considered in this comparison. Prior *et al.* 1998, reported a dramatic change in the antioxidant activity of the berries when estimated by ORAC, oxygen radical absorbing capacity, with a change in the harvest time and maturity. The differences in the two assays employed in different years and their underlying principles are also a factor that should not be overlooked. This factor can well contribute to the different trends of antioxidant activity found among cultivars from one year to another. DNA cleavage assay estimates the power of antioxidants in neutralizing any radical that can nick DNA strands. TBARs, on the other hand, measure the power of antioxidant in neutralizing the primary products (radicals) of lipid oxidation, which would have eventually been converted to MDA, a secondary product of lipid oxidation.

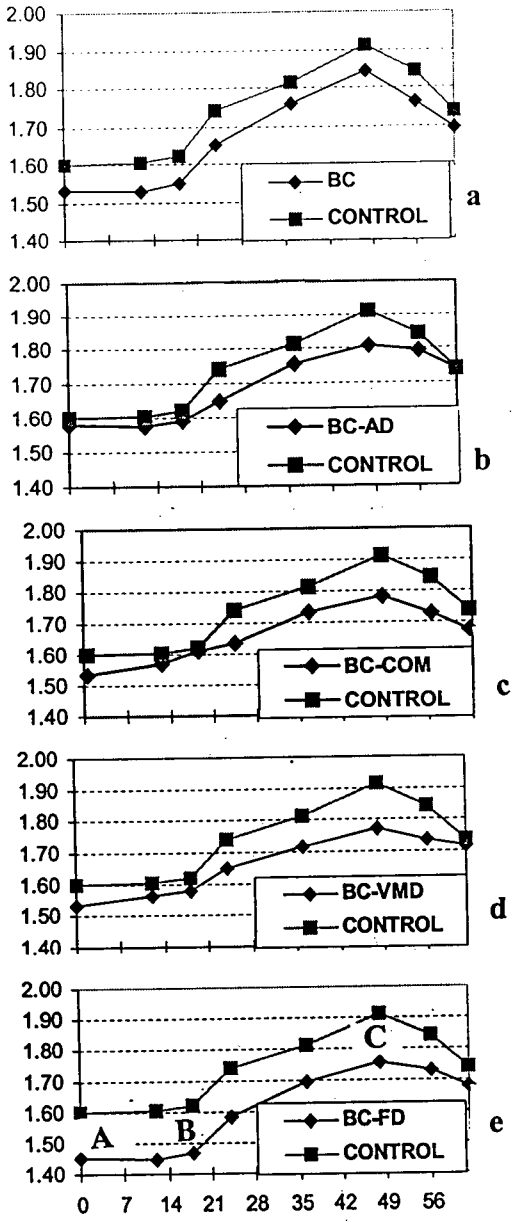

Figure 8. Progress of linoleic acid oxidation as indicated by the changes in the absorption of TBA-MDA complex, in a lipid emulsion (control) and lipid-blueberry emulsion of frozen (a), air dried (b), combination dried (c), vacuum microwave dried (d), and freeze dried blueberries (e) of Bluecrop cultivar of the 1999 crop

AB= Initiation BC= Propagation

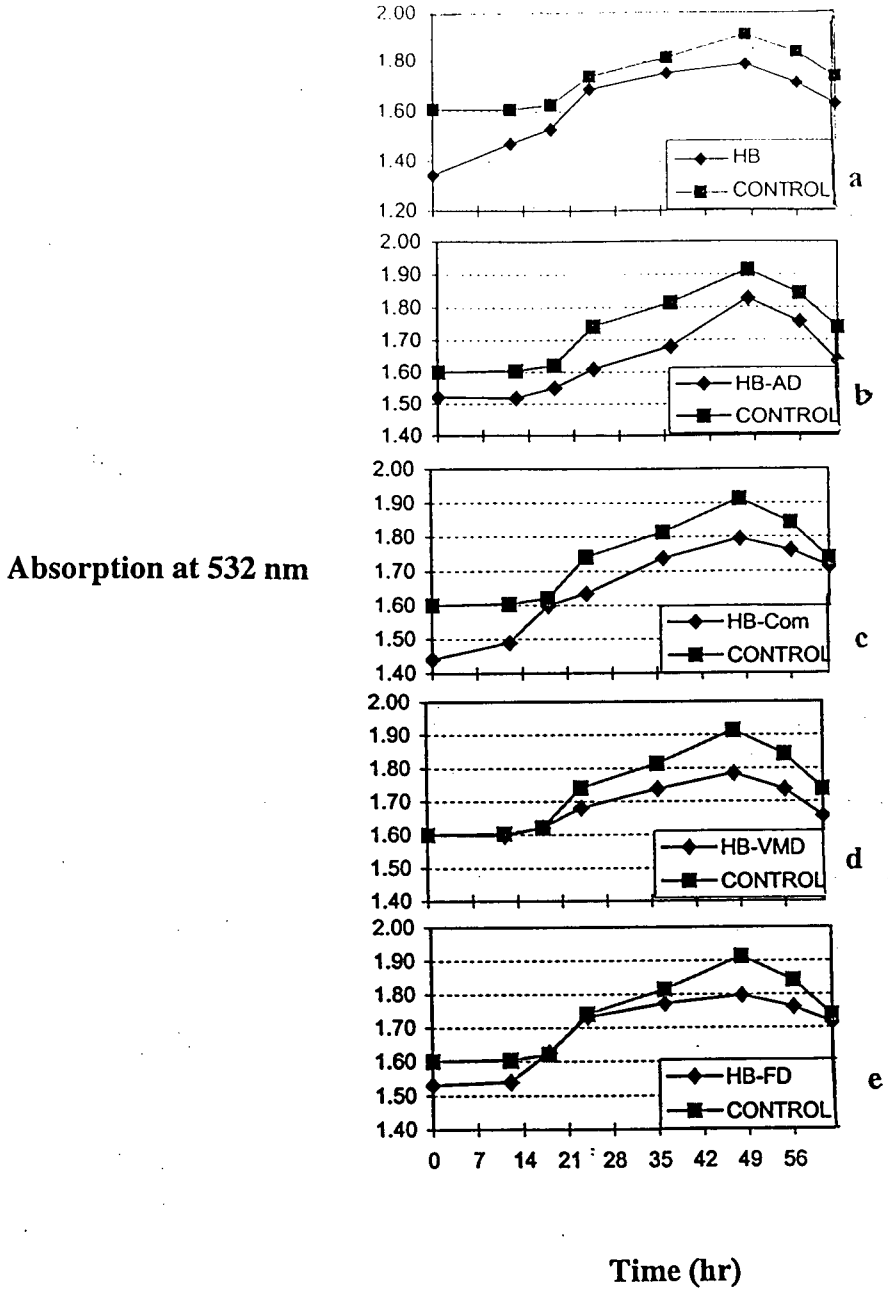

Figure 9. Progress of linoleic acid oxidation as indicated by the changes in the absorption of TBA-MDA complex, in a lipid emulsion (control) and lipid-blueberry emulsion of frozen (a), air dried (b), combination dried (c), vacuum microwave dried (d), and freeze dried blueberries (e) of Hardy Blue cultivar of the 1999 crop

Antioxidant activity and antioxidant components in blueberries:

The antioxidant activity of berry extracts in a linoleic acid- blueberry emulsion system was strongly related to the presence of anthocyanins (Figure 10). The percent of antioxidant activity of frozen and dehydrated blueberry extracts of two cultivars increased steadily as a function of their total anthocyanin content in a linear manner ($r^2 = 0.59$). Further statistical analysis revealed that this relationship is statistically significant ($p=0.009$, $n= 10$). Anthocyanins are probably the largest group of phenolic compounds in the human diet and they possess strong antioxidant activity (Wang *et al.*, 1997). In 1998, Prior *et al.* reported a linear relationship between the antioxidant activity and the anthocyanin of different varieties of fresh blueberries, with $r^2 = 0.77$. Our data suggests that the strong linear relationship between the anthocyanin content and the antioxidant activity of the fresh blueberries, previously established by many workers (Prior *et al.*, 1998; Kalt *et al.*, 1999), remains true even when they undergo various preservation treatments.

A positive relation was found to exist between the antioxidant activity and the concentration of total phenolic compounds with $r^2 = 0.73$ (Figure 11). The significance of this relationship was confirmed by estimating the correlation coefficient ($p=0.002$, $n=10$). Velioglu *et al.* 1998, also reported a statistically significant correlation between the total phenolic content and the antioxidant activity in anthocyanin- rich plants such as blueberries. The potential for prooxidant activity of some antioxidants has been previously documented. For example Mahoney and Graf in 1986, reported that some reducing agents such as α-tocopherol at high concentrations contribute to the production of highly reactive hydroxy radicals and malonaldehyde in lipid model systems. Similarly some phenolic antioxidants can produce prooxidant reactions especially at high concentrations (Wijewickreme and Kitts, 1998). However, the data collected in this study all pointed to the function of these compounds in their capacity as antioxidants in the blueberries.

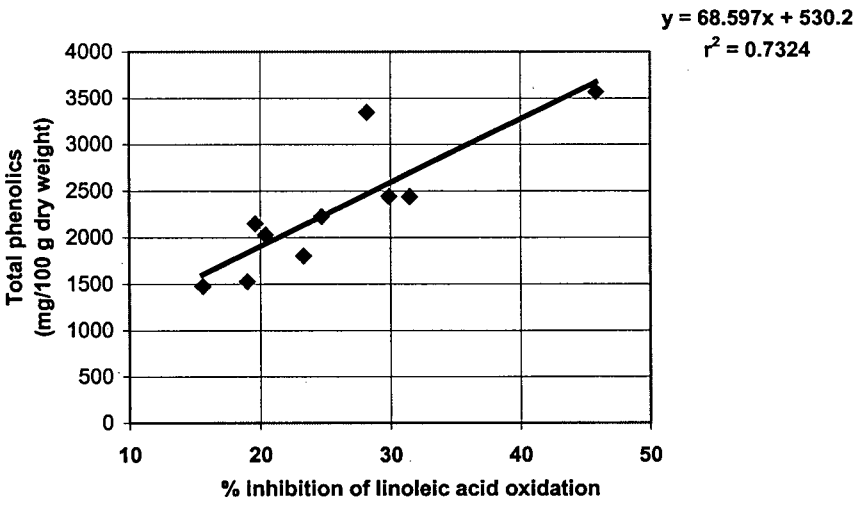

Figure 11. Relationship between antioxidant activity and phenolic compounds of frozen and dehydrated Blurcrop and Hardy Blue blueberries of the 1999 crop

Correlation was significant (P= 0.002, n=10).

The total vitamin C content of frozen and dehydrated blueberries analyzed in this study was from 7-37 mg/100 g dry blueberry. The relationship between the total vitamin C content and the antioxidant activity of the berries was found to be linear as well (Figure 12). However, the correlation observed between vitamin C content and antioxidant activity was not significant ($p= 0.058$, $n= 6$). Vitamin C is a compound that is capable of exhibiting a dual effect in an oxidation-reduction milieu. At low concentrations it has an ability to act as a pro-oxidant. Presumably this happens by an ascorbate-mediated generation of hydroxy radicals (OH^*) or other reactive species (Naswar, 1996).

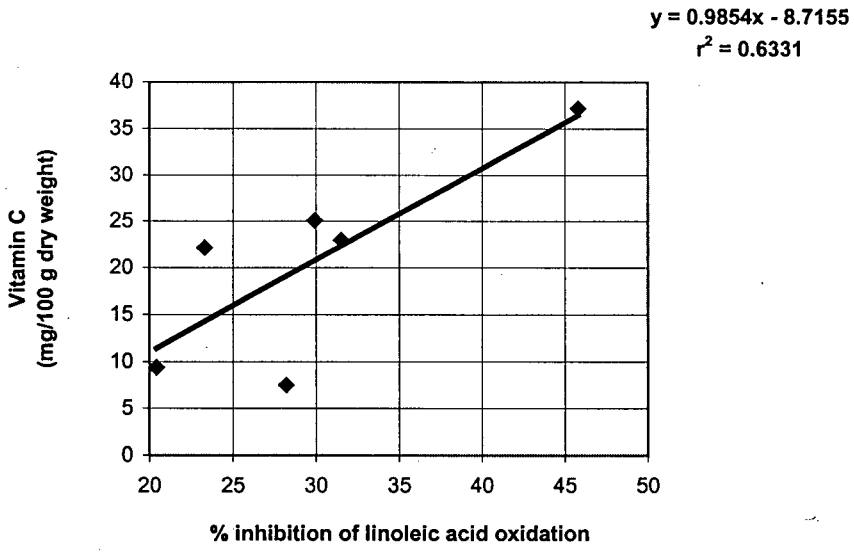

Figure 12. Relationship between antioxidant activity and vitamin C content of frozen and dehydrated Hardy Blue and Bluecrop blueberries of the 1999 crop Correlation not significant ($p= 0.58$, $n= 6$).

To relate the antioxidant activity of the berries to total phenolics, anthocyanins, and vitamin C content, multiple regression analysis was employed. The regression equation obtained was:

% Inhibition =7.32 + 0.00608 [Phenolics] + 0.364 [Vitamin C] (R^2 =0.72, n=6)

Anthocyanin content was not included in the regression equation as it exhibited a strong relationship with the phenolic content (r^2 = 0.64), and did not improve the regression equation. The regression equation indicates that the phytochemicals most likely responsible for antioxidant activity can be accounted for by the phenolics and vitamin C. However, various dehydrated berries with significantly different concentration of these phytochemicals were shown to have a comparable antioxidant activity. One possible explanation for this could be the presence of other antioxidants in blueberries. Blueberries contain about 100 IU vitamin A. The role of β-carotene, the precursor of vitamin A, in prevention of cancer is also well established (Marks, 1985). Since neutralizing the free radicals is central to some theories of the prevention of cancer (Hertog *et al.*, 1993, 1994, 1995), it is tempting to speculate that these compounds could contribute health benefits by functioning as an antioxidant. However, whether or not they function in such a capacity in blueberries, is a question that requires further investigation.

IV. B. INSTRUMENTAL ANALYSIS

IV. B. 1. Fractionation of anthocyanins by HPLC.

Fractionation of anthocyanins was accomplished by the high performance liquid chromatography (HPLC), adapted from Gao and Mazza (1994b), with some modifications to the solvent gradient and extraction method. Overall 12 peaks were observed in the chromatogram of air dried, vacuum microwave dried, and freeze dried berries of the Bluecrop cultivar extracted in acidic methanol. Cyanidin-3-glucoside (courtesy of Dr. G. Mazza, Agriculture and Agri-Food Canada, Summerland BC) was used as the standard. It co-eluted with the 4^{th} major peak in the anthocyanin profile of the blueberry samples (Figure 13).

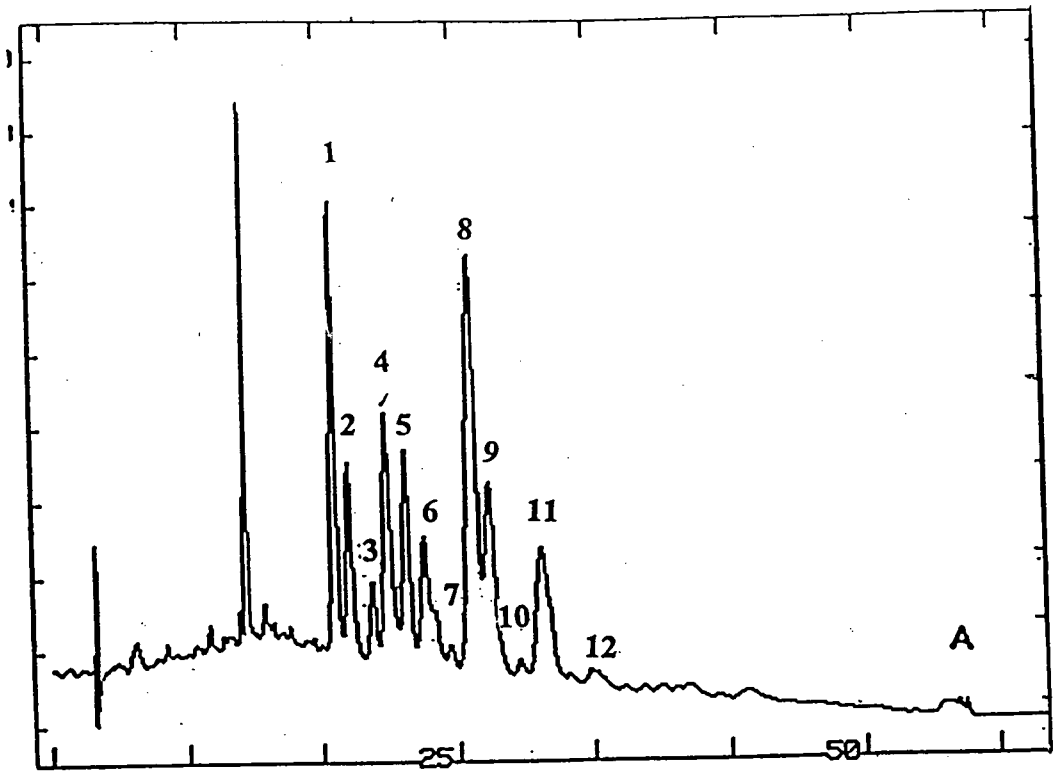

Figure 13. Reverse phase HPLC chromatogram of air-dried Hardy Blue blueberry extracts, the 1998 crop. Peaks 1-12 represent anthocyanins present in the berries. Cyanidin-3-glucoside (courtesy of Dr.G. Mazza) co-elutes with peak 4. Peak A eluted at the end may be attributed to polymeric anthocyanin pigments.

The anthocyanins in the methanolic extract of Hardy Blue blueberries were resolved into 12 peaks with the same retention time as well, (Data not shown). There is no previous work reported on the anthocyanin profile of the dehydrated blueberries. However, reports of the anthocyanins of the fresh high bush blueberries have indicated the presence of 20 (Gao and Mazza, 1994b), 25 (Gao and Mazza, 1995), 15 (Kader *et al.,* 1995), and 10 (Saper *et al.,* 1984) different peaks. The differences could be attributed to difference in ripeness (Kader *et al.,* 1995). For most blueberry cultivars, ripening is associated with the development of anthocyanins in the epidermal and sub-epidermal cells of the berry (Ballinger *et al.*, 1972). Experimental differences such as solvents and solvent gradient, the specificities of the column, and other instruments could also have a part to play in these differences. For instance in the case of our HPLC system, when washing the column with 100% MeOH, a wide peak eluted (marked as A in Figure 13), which could have been produced by polymeric anthocyanin pigments, some of which may have been formed during the extraction and storage of samples.

In order to facilitate the assessment of various peaks, the 12 resolved peaks, seven main peaks were taken and analyzed. The area of each peak was estimated using the integrating program of the HPLC system. A peak with the smallest standard deviation, peak 5 for both cultivars, was selected and designated as the reference peak. The ratio of each peak to the reference peak was calculated and expressed as the relative peak area of that peak.

There was a significant difference for four peaks with cultivar effect (Figure 14). Gao and Mazza in 1994b reported that the anthocyanin content of lowbush blueberries is variety dependent.

When the variance of the peaks was analyzed for the treatment effect, most of the fractionated anthocyanins (peaks) were not significantly different (Figures 15a and 15b). In other words neither one of these treatments and the conditions imposed by them made a significant difference in the degradation of that specific anthocyanin. For instance the anthocyanin represented by peak 1 in Hardy Blue berries had the same chance of existence in the anthocyanin profile of all blueberries treatments; whether they were air dried, vacuum microwave dried or freeze dried (Figure 15a). Anthocyanins represented by peaks 2, 6, and 11 for Hardy Blue and peaks 1, and 11 for Bluecrop (Figure 15b) did show significant difference with treatment effect. Any further explanation of this finding will require the knowledge of the structure of the anthocyanins represented by these peaks. Suffice to say that the stability of anthocyanins is in part a function of their structure and the nature of the substituents on their aromatic rings (Mazza and Brouillard, 1987). Analysis of variance also indicated that for most anthocyanin peaks cultivar*treatment effect did not make a statistically significant difference. Analysis of variance for the cultivar* treatment interaction showed that this factor made a significant difference in case of three peaks: peak 2, 4, and 6 only (complete result of ANOVA for cultivar * treatment interaction in the Appendix).

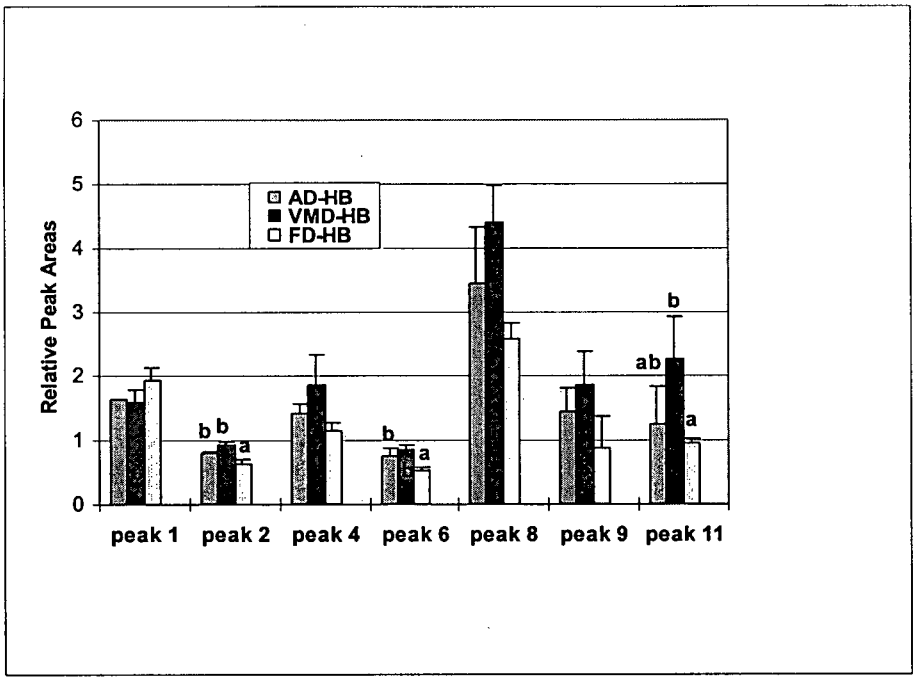

Figure 15a. . Comparison of relative peak areas of air dried, vacuum microwave dried, and freeze-dried Hardy Blue blueberries of the 1998 crop, separated by reverse phase HPLC.

Values are average of three determinations.

Treatments (within a given peak) denoted with a different letter were significantly different ($p \leq 0.05$). Peaks with no letters were not significantly different.

AD-HB= Air-dried berries of Hardy Blue cultivar.

VMD-HB= Vacuum microwave dried berries of Hardy Blue cultivar.

FD-HB= Freeze dried berries of Hardy Blue cultivar.

Error bars indicate standard deviations.

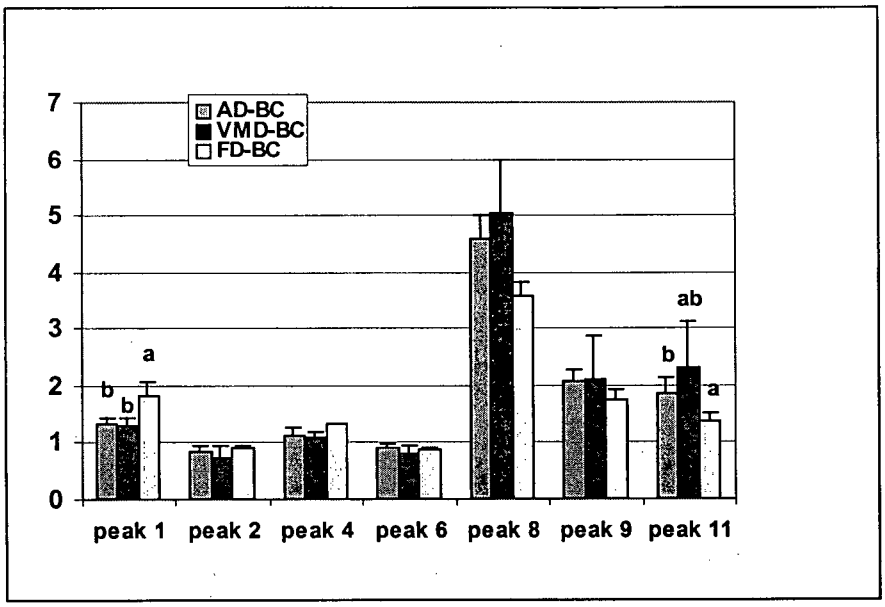

Figure 15b. Comparison of relative peak areas of air dried, vacuum microwave dried, and freeze-dried Bluecrop blueberries of the1998 crop, separated by reverse phase HPLC. Values are average of three determinations.

Treatments denoted with a different letter were significantly different ($p \leq 0.05$). Peaks with no letters were not significantly different.

AD-Bc = Air-dried berries of Bluecrop cultivar.

VMD-BC= Vacuum microwave dried berries of Bluecrop cultivar.

FD-BC= Freeze dried berries of Bluecrop cultivar.

Error bars indicate standard deviations.

From the crop of 1999 the air-dried and air/VMD treatment of Hardy Blue and the air/VMD treatment of Bluecrop were analyzed using HPLC method. When these results were compared to their counterparts from the crop of 1998, the patterns noticed were similar. The only observed difference was in the relative peak areas of some of the peaks, which was considerably higher for the crop of 1999. Figure 16-a and 16-b depicts a comparison of the relative peak areas of the air dried Hardy Blue and combination Blue crop from the two consecutive seasons.

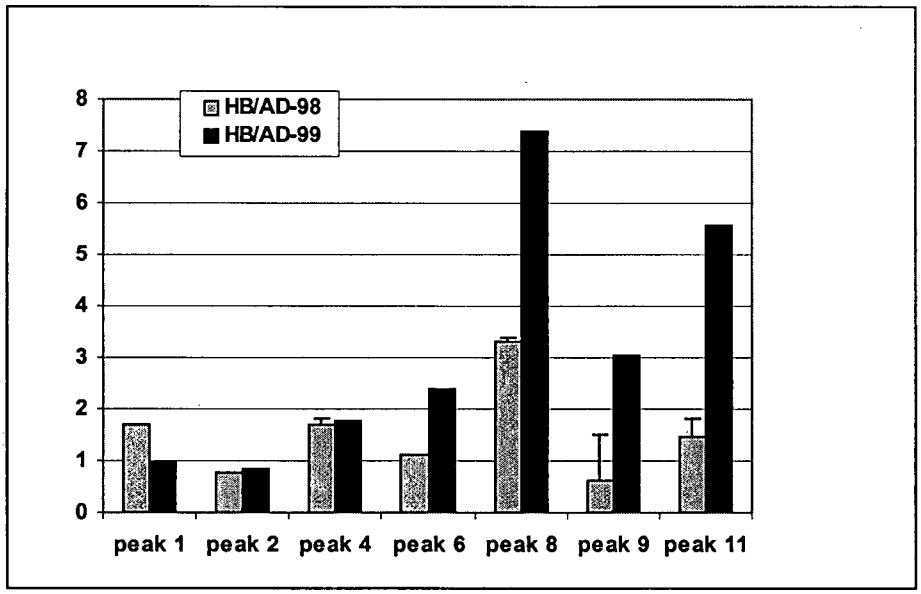

Figure 16-a. Comparison of the relative peak areas of the air-dried Hardy Blue blueberries of the 1998 and 1999 crop.

HB/AD-98 = air-dried treatment of the Hardy Blue treatment from the crop of 1998.

HB/AD-99 = air-dried treatment of the Hardy Blue treatment from the crop of 1999.

Due to lack of replicate for the air-dried treatment of Hardy Blue cultivar in 1999, the peaks do not have error bar.

Error bars indicate standard deviations.

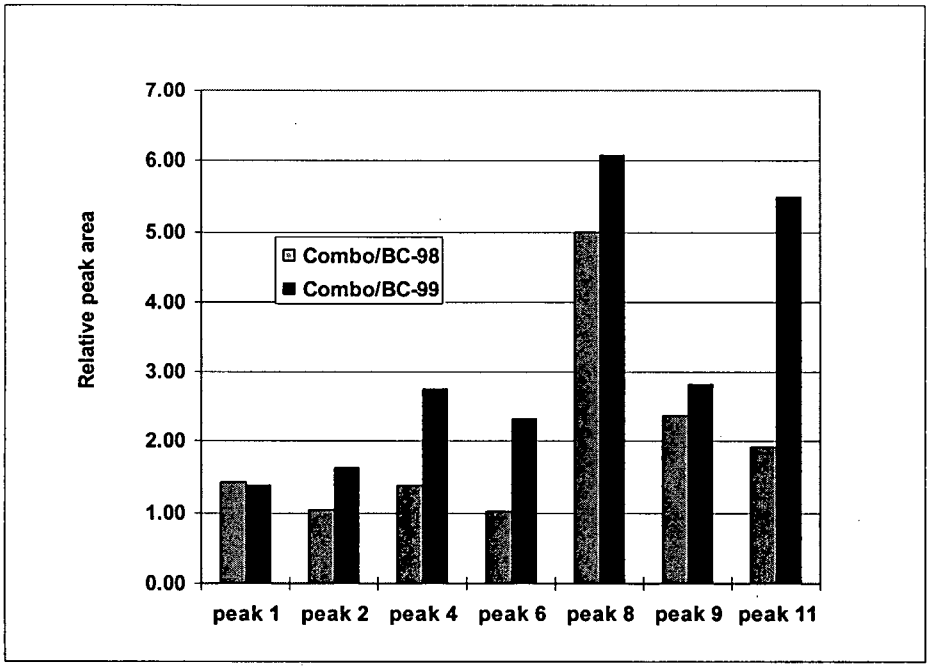

Figure 16-b. Comparison of the relative peak areas of the combination dried Bluecrop blueberries of the 1998 and 1999 crop.

Combo/BC-98 = Combination dried treatment of the Bluecrop cultivar from the crop of 1998.

Combo/BC-99 = Combination dried treatment of the Bluecrop cultivar from the crop of 1999.

Results are from a single analysis.

Overall the data collected from this part of the study indicated that within blueberries of one season the treatment, cultivar and treatment*cultivar interaction affected the presence of some anthocyanin, while some other anthocyanins were not affected by these factors. As for blueberries of two different seasons, the seasonal changes did not introduce any new peak to the anthocyanin profile of the fresh and dehydrated berries and berries from both seasons were resolved into the 12 anthocyanin peaks with some differences in their relative peak areas.

IV. B. 2. Color analysis by Hunter Lab technique:

The color of the blueberries is an important attribute of quality. The color characteristics of the dehydrated and frozen blueberries of both cultivars of the 1999 crop were analyzed for Hunter a, b, and L values by the Hunter Lab instrument.

A positive Hunter a value, indicating redness, was noticed in all blueberry treatments of both cultivars (Figure 17). Among the dehydrated blueberries, the air-dried berries exhibited the highest Hunter a. While this value was significantly different from the Hunter a value of the other dehydrated berries in the Bluecrop, it was comparable to Hunter a values of other dehydrated Hardy Blue berries. It is important to note that both freeze dried and vacuum microwave dried, and to some degree, combination dried berries have a "puffed" structure. In vacuum microwave drying a low pressure is maintained in the drying chamber, while the internal steam pressure within products are elevated. This pressure differential generates an outward force, causing the material to expand beyond its original dimensions, resulting in a "puffing" effect (Yousif *et al.*, 1999). In the case of the freeze dried berries, the vacuum created in the chamber of the freeze drier along with the low temperature provide the right condition for the moisture inside the berries to be sublimed. This phase change from solid to gas, without going through the liquid phase, contributes to the retention of the initial structure of the berries. On the other hand the air-dried berries have not retained their original structure, but have undergone an extensive folding and shrinkage, which intensifies their color. Attention should also be drawn to the size difference of the berries in two cultivars. The blueberries of the Bluecrop cultivar are almost twice the size of the berries of the Hardy Blue. The shrinking and stretching effects are both more pronounced when the berries' size is

bigger. This surface effect explains the variation in red hue intensity of the dehydrated products. Smaller brries have more surface/volume and more anthocyanin.

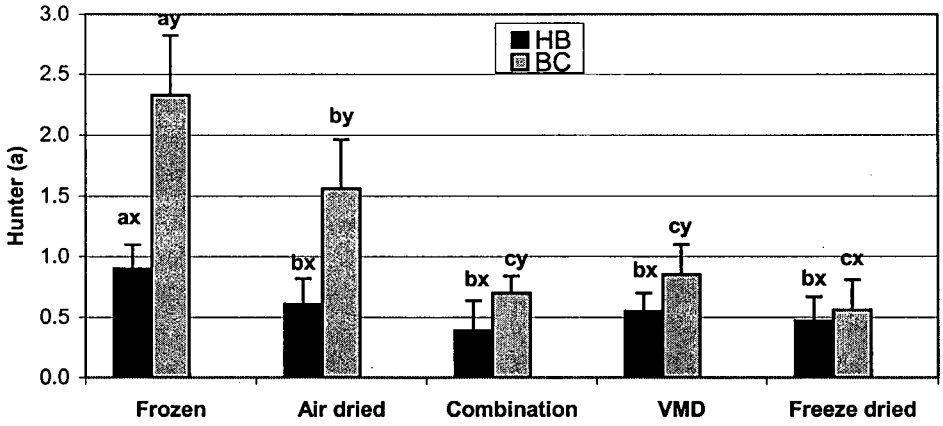

Figure 17. . Comparison of Hunter (a) values of various treatments of Bluecrop and Hardy Blue blueberries of the 1999 crop.

Values are average of five determinations.

Means adjacent to each other that do not have a common letter (x and y) are significantly different ($p \leq 0.05$).

Means within a cultivar that do not have a common letter (a, b, and c) are significantly different ($p \leq 0.05$).

Error bars indicate standard deviations

Color analysis of the blueberry samples for Hunter b (Figure 18) indicates that among the dehydrated berries the freeze-dried blueberries of both cultivars have the highest Hunter b value (absolute value). Hunter b is a value indicating the shift of color from yellowness to blueness. From the visual appearance of the dehydrated products, the freeze-dried samples are indeed the bluest ones.

Blueberries have a relatively high pigment content (Francis, 1985). Anthocyanins color can differ largely depending upon the pH of the medium. At pH values below 2, anthocyanins exist primarily in the form of the red flavylium cation, as the pH increases a rapid proton loss occurs to yield a red or blue quinoidal forms (Mazza and Brouillard, 1987). At pH value of 3.8-3.9, pH of the blueberries under study, it is expected to find them mostly in red or blue color. With this reasoning, freeze dried berries which had the highest anthocyanin content among the dehydrated berries are in fact expected to have the bluest color and the highest Hunter b values (absolute values). For this reason an attempt was made to establish relationship between the Hunter b value and anthocyanin content of the berries. Figure 19 reveals this correlation for Hardy Blue and Bluecrop cultivar, combined.

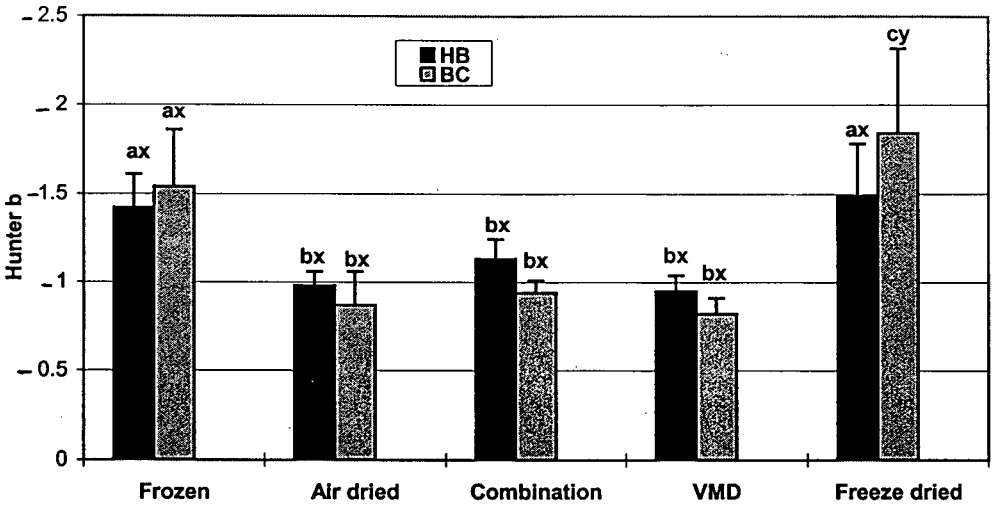

Figure 18. Comparison of Hunter (b) values of various treatments of Bluecrop and Hardy Blue blueberries of the 1999 crop.

Values are average of five determinations

Means adjacent to each other that do not have a common letter (x and y) are significantly different ($p \leq 0.05$).

Means within a cultivar that do not have a common letter (a, b, and c) are significantly different ($p \leq 0.05$).

Error bars indicate standard deviations.

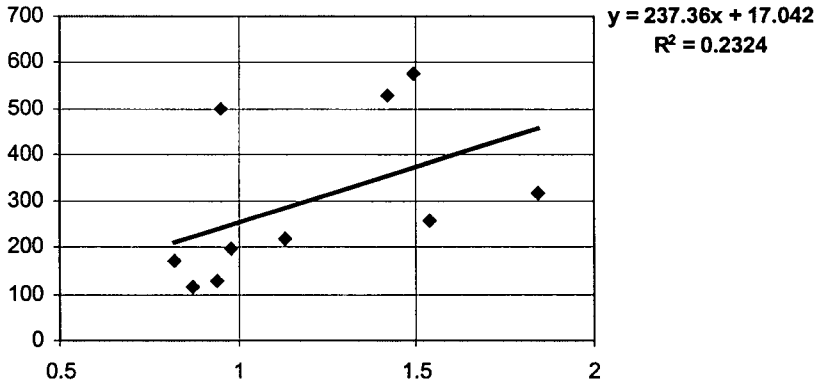

Figure 19. Relationship between Hunter b values and anthocyanins content (mg/100g dry weight) of various treatments of Hardy Blue and Bluecrop of the 1999 crop. Correlation was not significant (P= 0.158, n=10).

The results of this part of the study indicate that Hunter b value is not a good predictor of anthocyanin content of berries in all classes (r^2 =0.23), and this relationship was not significant (P=0.158, n =10). When the two cultivars were separated, there was a drastic increase in the r^2 value in case of the Bluecrop (r^2 =0.90), while the relationship was significant (P= 0.013, n = 5). However, in case of the Hardy Blue the r^2 value remained small (r^2 =0.38) and correlation was not significant (P= 0.263, n =5).

Among the dehydrated samples the freeze-dried treatments had the highest L value ($p \leq 0.05$), which indicates their degree of lightness (Figure 20). These findings were confirmed by the color of the berries when they were visually compared to each other. Freeze dried berries were the lightest color among all treatments. There is a statistically significant difference between Hunter L values of the freeze dried and other dehydrated berries ($p \leq 0.05$). The predominant condition of heat (in air drying, combination drying, and vacuum microwave drying) and the atmospheric oxygen (in air drying and combination drying) can facilitate the high enzymatic activity of polyphenol oxidase (PPO), resulting in the browning effect that characterizes many dehydrated food materials (Howard *et al.*, 1996). PPO catalyzes the oxidation of endogenous phenols into quinones that subsequently polymerized into a brown pigment (Lee, 1991). In the presence of anthocyanins, the quinones can oxidize these pigments to brown polymers (Mathew and Paripa 1971). Due to the masking effect of the color of the fruit, it is hard to detect the browning as an actual brown color on the berries. Never the less the conditions of some of the dehydration techniques favor the occurrence of this browning, and is a possibility worth being considered. Both polyphenoloxidase and peroxidase have been implicated in enzymatic browning of plant tissues (Vamos-Vigyazo, 1981).

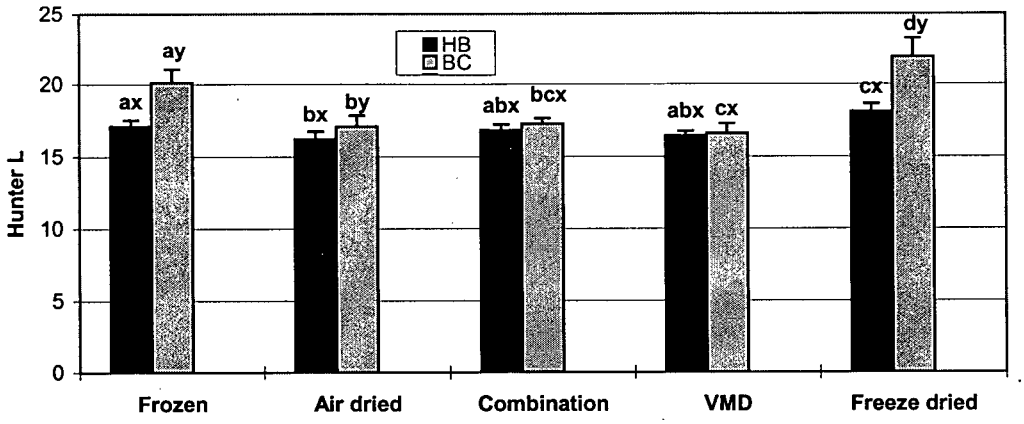

Figure 20. Comparison of Hunter (L) values of various treatments of Bluecrop and Hardy Blue blueberries of the 1999 crop.

Values are average of five determinations.

Means adjacent to each other that do not have a common letter (x and y) are significantly different ($p \leq 0.05$).

Means within a cultivar that do not have a common letter (a, b, and c) are significantly different ($p \leq 0.05$).

Error bars indicate standard deviations.

The reduced lightness (lower Hunter L value) in the berries undergoing these heat applied dehydration techniques can be the manifestation of this browning effect. In a dehydration method without high temperature and with reduced oxygen such as freeze drying, less browning should occur which would lead to lighter color and higher L values. In 1996 Kader *et al.* reported that the loss of 29% of the color in a model system containing PPO and blueberry anthocyanins indicated that PPO could act directly on these pigments. In addition, attention should be drawn once again to the wholesome and intact structure of freeze-dried berries versus the shrunk and extensively folded structures of the air-dried and combination dried berries. Distribution of pigments in a larger structure (in freeze dried berries) gives an overall lighter color to the product. Whereas when the pigments are accumulated in a smaller structure with pieces folded on each other (as in the case of the air dried and combination dried berries), the color of the product is intensified.

As a whole, the color analysis of the berries reveals that dehydration processes affect to a varying degree the quality attribute of the color. All three parameters of Hunter Lab technique show a statistically significant difference with the treatment and cultivar effect.

V. CONCLUSION AND RECOMMENDATIONS

Evaluation of data from the chemical analyses of the various frozen and dehydrated blueberry samples revealed that the vacuum microwave drying is an efficient dehydrating method in terms of preserving valuable components of blueberries that are heat and oxygen sensitive. This method preserves vitamin C content of berries much better than the air and air/vacuum microwave drying method. The anthocyanin content of vacuum microwave dried blueberries was higher than that of the air-dried ones in Hardy Blue, where in Bluecrop these values were comparable. In terms of retaining the total phenolic compounds vacuum microwave drying proved to be more efficient than all other methods for Hardy Blue berries. For the Bluecrop, VMD was as efficient as the freeze-drying, in preserving the total phenolic compounds of the berries.

Despite the efficiency of vacuum microwave drying method in preserving the potential antioxidant components of blueberries, vacuum microwave dried berries exhibited an antioxidant activity which was not significantly different from that of the air-dried blueberries. There was a significant difference in the antioxidant activity of the frozen berries and the dehydrated ones in Hardy Blue cultivar. This indicates that all these dehydration methods resulted in a loss of the antioxidant activity. In Bluecrop, however, the antioxidant activity of vacuum microwave dried berries was comparable to that of the frozen berries, while the antioxidant activity of the air-dried berries was significantly lower than that of the frozen berries.

The data collected from HPLC analysis of the blueberry disclosed 12 major peaks in the anthocyanin profile of all dehydrated berries. The overall anthocyanin profile did

not show a qualitative change from one season to another; nonetheless, some quantitative changes were noticed in the relative peak areas of some of the 12 major anthocyanins. As for treatment effect, most of these 12 anthocyanins did not show a significant difference from one treatment to another.

Due to the puffing effect, conferred on the structure of berries by the freeze drying and vacuum microwave drying methods, Hunter lab values were confounded, and data was not conclusive enough to establish a trend between the treatment and the Hunter lab values.

The combination treatment of air/vacuum microwave drying produced a product whose quality in all categories was intermediate between air drying and vacuum microwave drying method. Based on the understanding that the shorter the drying time, the more the concentration of the heat labile components in the dehydrated product, additional research is required to optimize the combination treatment (air/vacuum microwave drying) in order to improve the quality of the end products.

In consideration of these findings, it is can be concluded that the vacuum microwave drying has the potential to produce a high quality products. The greater efficiency in mass and energy transfer is inherent qualities in VMD. This method can therefore, improve the biggest draw back of air-drying i. e. prolonged drying time, and intense heat exposure. Decreasing the drying time and the heat exposure minimizes the loss of nutritive value as well as changes to the physical attributes of food, and consequently improves its quality.

Therefore, the initial hypothesis of this work i. e. the ability of vacuum microwave drying to retain sensitive bioactive components better than the conventional drying

methods of air-drying is accepted. Vacuum microwave drying is in fact an efficient dehydration method, which enhances the quality of the dehydrated products measurably.

VI. REFERENCES

Akhmadieva, A. Kh., Zaichkina, S. I., Ruzieva, R. Kh., and Ganassi, E. E., 1993. The protective action of a natural preparation of anthocyanin (pelargonidin-3, 5-diglucoside). Radiobiologiia. 33 (3): 433-435.

Amarowicz, R., Wanasundara, U. N., Karamac, M., and Shahidi, F., 1996. Antioxidant activity of ethanolic extracts of mustard seed. Nahrung. 40 (5): 261-263.

Ames, B. M., 1993. Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative diseases. Science. 221: 1265-1263.

AOAC., 1996. Official Methods of Analysis, 14th ed. Association of Official Analytical Chemists. Washington, DC.

Attiyate, Y., 1979. Microwave vacuum drying -First industrial application. Food Engineering International. 4(1): 30-31.

Baj, A., Bombardelli, E., Gabetta, B., and Martinelli, E. M., 1983. Qualitative and quantitative evaluation of *Vaccinium myrtillus* anthocyanins by high resolution gas chromatography. Journal of Chromatography. 279: 365-372.

Ballinger, W. E., Maness, E. P., Galletta, G. J., and Kushman, L. J., 1972. Anthocyanins of ripe fruit of a pink-fruited hybrid of highbush blueberries, *Vaccinium corymbosum. L.* Journal of American Society of Horticultural Sciences. 97: 381-384.

Ballington, J. R., Ballinger, W. E., Maness, E. P., and Luby, J. J., 1988. Anthocyanins, aglycon sugar content of the fruit of five species of *Vaccinium* selection *myrtillus*. Canadian Journal of Plant Science. 68: 241-246

Block, G., and Langseth, L., 1994. Antioxidant vitamins and health prevention. Food Technology. 46: 80-83.

Block, G., Patterson, B., and Subar, A.F., 1992. Fruit and vegetable and cancer prevention- A review of epidemiological evidences. Nutrition and Cancer. 18: 1-29.

Bolin, H. R., Huxsoll, C. C., Jackson, R., and Ng, K. C., 1983. Effect of osmotic agents and concentration on fruit quality. Journal of Food Science. 48: 202.

Branen, A. L., 1975. Toxicology and biochemistry of butylated hydroxy anisole and butylated hydroxy toluene. Journal of American Oil Chemists Society. 52: 59-63.

Brouillard, R., 1982. Chemical structure of anthocyanins, In: Markakis, P. (ed). Anthocyanins as Food Colors. Academic Press, New York.

Brouillard, R., and Delaporte, B., 1977. Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration and tautomeric reactions of malvidin 3-glucoside. Journal of American Society for Horticultural Sciences. 99: 84-91.

Brouillard, R., Delaporte, B., El Hage Chahine, J. M., and Dubois, J. E., 1979. Chimie des pigments anthocyaniques. 4. Spectre d'absorption UV-visible de la forme chalcone de la glucoside 3- malvidine. Journal of Chemical Physics. 76: 273-277.

Brouillard, R., and Dubois, J. E., 1977. Mechanism of the structural transformations of anthocyanins in aqueous media. Journal of American Chemists Society. 99: 1359-1364.

Brown, A. H., 1973. Drying rates and estimation of drier capacity. In: Arsdel, W. B., Copley, M. J. and Morgan, A. I. (eds.). Food Dehydration. 2nd edition. Volume 1, Avi. Pub.Co. Westport. Connecticut. pp. 347-350.

Buege, I. A., and Aust, S. D., 1978. Microsomal lipid peroxidation. Methods in Enzymology. 52: 302-310.

Cao, G., Alessio, H. M., and Culter, R. G., 1993. Oxygen radical absorbance capacity assay for antioxidants. Free Radical Biology and Medicine. 14: 303-311..

Carpenter, J. A., Wang, Y., Powers, J. J., 1967. Effects of anthocyanin pigments on certain enzymes. Proceedings of the Society of Experimental Biology and Medicine. 124 (3): 702-706.

Colantuoni, A., Bertuglia, S., Magistretti, M. J., and Donato, L., 1991. Effects of *Vaccinium myrtillus* anthocyanosides on arterial vasomotion. Arzneimittel Forschung. 41 (9): 905-909.

Constantino, L., Albasino, A., Rastelli, G., and Benvenuti, S., 1992. Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. Planta Medica. 58: 342-344.

Costantino, L., Rastelli, G., and Albasini, A., 1995. Anthocyanins inhibitors of xanthine oxidase. Pharmazie. 50: 573-574.

Cook, N. C., and Samman, S., 1996. Flavonoids chemistry, metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry. 7: 66-76

Criqui, M. H., and Ringel, B. L., 1994. Does diet or alcohol explain the French paradox? Lancet. 344: 1719-1723.

Decareau, R.V., 1985. Microwave in the Food Processing Industry. Academic Press, New York.

Dodson, Y. K., Young, E. R., and Soliman, AG. B., 1992. Determination of total vitamin C in various food matrixes by liquid chromatography or fluorescence detection. Journal of AOAC International. 75: 887-891.

Drouzas, A. E., and Schubert, H., 1996. Microwave application in vacuum drying of fruits. Journal of Food Engineering. 28: 203-209.

Durance, T. D., and Wang, J. H., 1999. Energy utilization and quality in vacuum microwave and convective air dehydration of tomatoes. In: Proceedings of Canadian Institute of Food Science and Technology, 6- 9 June 1999, Kelowna, British Columbia, Canada. Oral Presentation abstract No.11.

Eck, P., and Childers, N. F., 1967. Blueberry Culture. Rutgers University Press. New Brunswick, NJ.

Eisenhardt, N. H., Eskew, R. K., and Cording, J., 1964. Explosive-puffing applied to apples and blueberries. Food Engineering. 36: 53-57.

Elliott, J., 1994. Functional candies; scientific marketing and regulatory aspects. Manufacturing Confections. 12: 39-46.

Ferrell, J. E. Jr., Chang Sing, P. D. G., Loew, G., King, R., Mansour, J. M., and Mansour, T.E., 1979. Structure/activity studies of flavonoids as inhibitor of cyclicAMP phosphodiesterase and relationship to quantum chemical indices. Molecular Pharmacology. 16: 556-568.

Food and Drugs Acts and Regulations, 1984, Food Additives; Food Colorants. Part III-B. The Food and Drugs Acts and Regulations. Canada Communication Group Publishing. P: 67-3B.

Francis, F. J., 1985. Blueberries a colorant ingredient in food products. Journal of Food Science. 50: 754.

Francis, F. J. 1989., Food colorants: Anthocyanins. Critical Reviews in Food Science and Nutrition. 28: 273-314.

Fuleki, T., and Francis, F. J., 1968. Quantitative methods for anthocyanins. Journal of Food Science. 33: 72-77.

Gao, L., and Mazza, G., 1994-a. Rapid method for complete chemical characterization of simple and acylated anthocyanins by HPLC and capillary gas chromatography. Journal of Agricultural and Food Chemistry. 42 (1): 118-125.

Gao, L., and Mazza, G., 1994-b. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. Journal of Food Science. 59: 1057-1059.

Gao, L., and Mazza, G., 1995. Characterization of acylated anthocyanins in low bush blueberries. Journal of Liquid Chromatography. 18 (2): 245-259.

Gey, K. F., Puska, P., Jordan, P., and Moser, U. K., 1991. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. American Journal of Clinical Nutrition. 53: 3265-3345.

Gibb, C., Glover, V., and Sandler, M., 1987. In vitro inhibition of phenolsulphotransferase by food and drink constituents. Biochemical Pharmacology. 36: 2325-2330.

Gough, R.E., and Korcak, F. R., 1995. Blueberries a Century of Research. Food Products Press, Beltsville, Maryland.

Green, R.C., and Mazza, G., 1986. Relationships between anthocyanins, total phenolics, carbohydrates, acidity and the colour of Saskatoon berries. Canadian Institute of Food Science and Technology Journal. 19: 107-133.

Gruenberg, M., Schuchmann, H., and Schubert, H., 1992. Mikroweicenerwaermung Von Speisefetten und-Oelen. ZFL, 43(1/2): 1-5.

Gubina, M. D., and Fedotova, T. K., 1977. Changes in the anthocyanins during drying and storage of bilberries and blueberries. Konservnaya-I-Ovoshchesushil'naya-Promyshlennost. 1: 12-13.

Hall, C. A., and Cuppett, S. L., 1997. Structure- activities of natural antioxidants. In: Aroma, O. I., Cuppett, S. L (eds.). Antioxidant Methodology, In vivo and In vitro Concepts, AOCS press. USA. pp. 141-172.

Halliwell, B., 1994. Free radicals and antioxidants: A personal view. Nutritional Review. 52: 253-265.

Heinonen, L.M., Lethonen, P. J., and Hopia, A. I., 1998. Antioxidant activity of berry fruit wines and liquors. Journal of Agricultural and Food Chemistry. 46(1): 25-31.

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., Kvomhout, D., 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. Lancet. 342: 1007-1011.

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., and Kvomhout, D., 1994. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. Nutrition and Cancer. 22: 175-184.

Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., Fidanza, F., Giampaoli, S., Jansen, A., and Menotte, A., 1995. Flavonoid intake and long-term risk of coronary heart disease, and cancer in the seven-country study. Archives of Internal Medicine. 155: 381-386.

Hope, G. W., 1965. A review of the suitability of the lowbush blueberry for processing. Food Technology. 19(2): 115-118.

Howard, L. R., Braswell, D. D., and Aselage, J., 1996. Chemical composition and color of strained carrots as affected by processing. Journal of Food Science. 61: 327-330.

Huang, S. W., and Frankel, E. N., 1997. Antioxidant activity of tea catechins in different lipid systems. Journal of Agricultural and Food Chemistry. 45: 3033-3038.

Hubert, C. T., Edwards, H. H., and Morrison, M., 1975. The effect of lactoperoxidase-catalyzed iodination on the integrity of mitochondrial membranes. Archives of Biochemistry and Biophysics. 168: 463-472.

Hudson, B. J. F (ed)., 1990. Food Antioxidants; Elsevier Applied Science, London.

Ito, N., Fukushiman, S., Hasegawa, A., Shibata, M., and Ogiso, T., 1983. Carcinogenicity of butylated hydroxy anisole in F344 rats. Journal of the National Cancer Institute. 70: 343-347.

Jayaraman, K. S., and Das Gupta, D. K. 1992. Dehydration of fruits and vegetables-recent development in principles and techniques. Drying Technology. 10(1): 1-50.

Jensen, A., 1967. Tocopherol content of seaweed and sea meal. 3. Influence of processing and storage on the content of tocopherol, carotenoids and ascorbic acid in seaweed meal. Journal of the Science of Food and Agriculture. 20: 622-628.

Kader, F., Rovel, B., Girardin, M., and Metche, M., 1995. Fractionation and identification of the phenolic compounds of high bush blueberries. Food Chemistry. 55 (1): 35-40.

Kader, F., Rovel, B., Girardin, M., and Metch, M., 1996. Fractionation and identification of the phenolic compounds of highbush blueberries (*Vaccinium corymbosum, L.*). Food Chemistry. 55: 35-40.

Kalt, W., Howell, A., and Goldman, I. L., 1999. Antioxidant, antiadhesion and anti-platelet activity of *Vaccinium* species. In: Proceedings of Canadian Institute of Food Science and Technology, 6-9 June 1999, Kelowna, British Columbia, Canada. Poster abstract No. 12.

Kamei, H., Kojima, T., Hasegava, M., Koide, T., Umeda, T., Yukawa, T., and Terabe, K., 1995. Suppression of tumor cell growth by anthocyanin *in vitro*. Cancer Investigation. 13: 590-594.

Karaivanova, M., Drenska, D., and Ovcharov, R. A., 1990. A modification of the toxic effects of platinum complexes with anthocyanins. Eksperimentalria Meditsina I Morfologiya. 29 (2): 19-24.

Karel, M., and Nickerson J. T. R., 1964. Effects of relative humidity vacuum and browning of dehydrated orange juice. Food Technology. 18:104-108.

Kenkt, P., Jarvinen, R., Reunanen, A., and Magtela, J., 1996. Flavonoid intake and coronary mortality in Finland: a cohort study. British Medical Journal. 312: 478-481.

Kim, M. H., and Toledo, R. T., 1987. Effect of osmotic dehydration and high temperature fluidized bed drying on properties of dehydrated rabbiteye blueberries. Journal of Food Science. 52: 980-989.

Kiranoudis, C. T., Tsami, E., and Maroulis, Z. B., 1992. Microwave vacuum drying kinetics of some fruits. Drying Technology. 15: 2421-2440.

Kmieck, W., 1995. The effects of drying temperature and storage period of dried parsley leaves on vitamin C content. Bromatologia-I-Chemia-Toksykologiczna. 28: 235-239.

Kock, K., 1989. Veber den Einsatzder Mikrowellentechnik Zum Haltbarmachen Von Lebensmitteln. Lebersmitteltechn. ½: 39-43.

Larson, R. A., 1988. The antioxidants of higher plants. Phytochemistry. 27 (4): 969-978.

Lee, C. Y., 1991. Browning reaction-Enzymatic. In: Hui, Y.N. Wiley. (eds.). The Encyclopedia of Food Science and Technology. John Wiley and Sons, New York. pp. 223-230.

Lee, S. H., and Labuza, T. P., 1975. Destruction of ascorbic acid as a function of water activity. Journal of Food Science. 40: 370-373.

Leonardi, M., 1993. Treatment of fibrocystic disease of the breast with myrtillus anthocyanins. Our experience. Minerva Ginecologia. 45: 617-621.

Lietti, A., Cristoni, A., and Picci, M., 1976. Studies on *Vaccinium myrtillus* anthocyanosides. I. Vasoprotective and antiinflamatory activity. Arzneimittel Forschung. 26(5): 829-832.

Loeliger, J., and Saucy, I., 1984. Food oxidation as measured by chemiluminescence. Journal of Luminescence. 31-32: 908-910.

Loliger, J., 1991. The use of antioxidants in foods. In: Aroma, O. I., Hallwell, B. (eds.). Free Radicals and Food Additives. Tyloy and Francis, London. pp. 121-150.

Loong, T. L., Juming, T., and Jianshan, H., 1995. Moisture sorption characteristics of freeze dried blueberries. Journal of Food Science. 60(4): 810-814.

Macheix, J. J., Fleuriet, A., and Billot, J., 1990. Fruit Phenolics; CRC Press, Boca Raton, FL.

Mahoney, J. R., and Graf. E., 1986. Role of α-tocopherol, ascorbic acid, and EDTA as antioxidants in model systems. Journal of Food Science. 51: 1293-1296.

Marks, J., 1985. Vitamin C. Ch 29. The Vitamins. First edition. MTP Press Limited, Lancaster, England. pp. 185-193.

Mathew, A. G., and Parpia, H. A. B., 1971. Food browning as a polyphenol reaction. Advanced Food Research. 19: 75-145.

Mazza, G., and Brouillard, R., 1987. Recent developments in the stabilization of anthocyanins in food products. Food Chemistry. 25: 207-225.

Mazza, G., and Miniati, E. 1993. Anthocyanins in Fruits, Vegetables and Grains. CRC Press, Boca Raton.

McGookin, B. J., Augustin, M. A., 1991. Antioxidant activity of casein and Maillard reaction products from casein-sugar mixtures. Journal of Dairy Research. 58: 313-320

Mitcheva, M., Astroug, H., Drenska, D., Popov, A., and Kassarov, M., 1993. Biochemical and morphological studies on anthocyanin and vitamin E on carbon tetrachloride induced liver injury. Cellular and Molecular Biology. 39: 443-448.

Naswar, W. W., 1996. Lipids Chapter 5. In: Food Chemistry. Fennema, R. O (ed). Marcel Dekker. Inc, New York. 225-320.

Oomah, B. D., and Mazza, G., 1996. Flavonoids and antioxidative activities in buckwheat. Journal of Agricultural and Food Chemistry. 44: 1746-1750.

Ponting. J. D., 1973. Osmotic dehydration of fruits-Recent modifications and applications. Process Biochemistry. 12:18-20.

Potter, N. N., and Hotchkiss, J. H., 1995. Food deterioration and control. Ch 7 In: Food Science. Chapman and Hall, New York. pp. 113-136.

Prior, R.L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfelt, M., Kalt, W., Knewer, G., and Mainland, G. M., 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. Journal of Agricultural and Food Chemistry. 46: 2686-2693.

Pryor, W. A., Stanly, J. P., and Blair, E., 1976. Oxidation of polyunsaturated fatty acids. Lipids. 11: 370-379.

Rahman, A. R., 1972. Increased density for military foods. Technical report. No AD-750-368. U. S. Army Natick Labs, Natick, MA.

Ramaswamy, H. S., and Nsonzi, F., 1998. Connective-air drying kinetics of osmotically pre-treated blueberries. Drying Technology. 16(3-5): 770-774.

Reitman, I.J., 1987. Activity of hops oxidoreductases during post harvest treatment, processing and storage. Fermentnaya-I-Spirtovaya-Promyshlennost. 2: 33-37.

Saija, A., Princi, P., D'Amico, N., De Pasquale, R., and Costa, G., 1990. Effects of *Vaccinium myrtillus* anthocyanin on triiosothyronine transport in the rat. Pharmacological Research. 22(suppl.3): 59-60.

Sankat, K. C., Castaigne, F., and Maharaj, R., 1996. The air drying behavior of fresh and osmotically dehydrated banana slices. International Journal of Food Science and Technology. 31:123-135.

Saravacos, G. D., and Charm, S. E., 1962. A study of the mechanism of fruit and vegetable dehydration. Food Technology. 16: 78-81.

Sapers, G.M., Burgher, A. M., Phillips, J. G., Jones, S. B., and Stone, E. G., 1984. Color and comparison of highbush blueberry cultivars. Journal of American Society of Horticultural Sciences. 109: 105-111.

Schadle, E. R., Burns, E.E., and Tally, L.J., 1983. Forced air drying of partially freeze dried compressed carrot bars. Journal of Food Science. 48: 193-196.

Scharrer, A., and Ober, M., 1981. Anthocyanosides in the treatment of retinopathies. Klinische Monatsblaetter Fuer Augenheikrunde. 178: 386-389.

Schiffmann, R. F., 1987. Microwave and dielectric drying. In: Mujumdar, S. A. (ed). Hand Book of Industrial Drying. Marcel Dekker, Inc, New York. pp. 327-355

Shewfelt, A. L., 1966. The nature of the anthocyanins in pigments in South Carolina Concord grapes. Technical Bulletin of Agricultural Experiment Station. Clemson University, SC. 1025

Simard, R.E., Bourziex, M., and Heredia, N., 1982. Factors influencing color degradation in blueberry juice. Lebensmittel-Wissenschaft and Technologie. 15:177- 180.

Somogyi, L. P., and Luh, B. S., 1975. Dehydration of fruits, In: Woodroof, J. G. and Luh, B. S. (eds). Commercial Fruit Processing. AVI Publishing Co. Inc., West Port, Connecticut. pp. 374-429.

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., and Witztum, J. L., 1989. Beyond cholesterol: Modifications of low-density lipoprotein that increases its atherogenicity. New England Journal of Medicine. 320(14): 915-924.

Steinmetz, K. A., Potter, J. D., 1991. Vegetables, fruits, and cancer. I. Epidemiology. Cancer Causes and Control. 2: 325-357.

Strack, D., and Wray, V., 1989. Anthocyanins. Academic Press, New York. pp. 236-240.

Sullivan, J. F., Craig, J. C., Dekazos, E. D., and Leiby, S. M., 1982. Dehydrated blueberries by the continuous explosion-puffing process. Journal of Food Science. 47: 445-448.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., and Dugan, L., 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. Journal of American Microwave and Vacuum Drying Technologies. Up-to-Date Food Processing. 23(9): 43-46.

Tein, M.L., Durance, T.D., and Scaman, C.H., 1999. Characterization of vacuum microwave, air and freeze dried carrot slices. Food Research International. 31(2): 111-117.

Teranishi, T., 1988. Production of high quality dehydrated vegetables using microwave and vacuum drying technologies. Up-to-Date Food Processing. 23: 43-46.

Timberlake, C. F., and Bridle, P., 1980. Anthocyanins. In: Walford, J (ed). Developments in Food Colours. Applied Science Publishers. Ltd, London, England. pp. 115-142.

Timberlake, C. F., and Henry, B. S., 1988. Anthocyanins as natural food colorants. In: Harborne, J. B., Middleton, E., Cody, V., and Beretz. B. (eds). Plant Flavonoids, in Biology, and Medicine. II. Biochemical, Cellular, and Medicinal Properties. Allen. R Liss Inc, New York. pp. 107-122.

Treybal, R. E., 1980. Mass Transfer Operation. 3rd edition. McGraw Hill, New York.

Tulasidas, N. T., 1994. Combined Convective and Mcrowave Drying of Grapes. Ph.D. Thesis. McGill University, Montreal, Quebec.

USDA Hand book #8. 1963. ARS, United States Department of Agriculture, U.S. Government Printing Office, Washington, DC.

Vamos-Vigyazo, L., 1981. Polyphenol oxidase and peroxidase in fruits and vegetables. Critical Review in Food Science and Nutrition. 15: 49-127.

Vanderslice, J. T., and Higgs, D.J., 1989. Automated analysis of total vitamin C in foods. Journal of Micronutrient Analysis. 6: 109-117.

Velioglu, Y.S., Mazza, G.,Gao, L., and Oomah, B. D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry. 46: 4113-4117.

Villata, M., 1998. Cultivated blueberries- a true –blue baking ingredient. Cereal Foods World. 43: 127-130.

Villata, M., and Kenyou, N., 1997. Blueberries application in bakery foods. Technical Bulletin of American Institute of Baking. 19: 1-10.

Vojnovich, C., and Pfeifer, V. F., 1970. Stability of ascorbic acid in blends with wheat flour, CSM and infant cereals. Cereal Science Today. 19: 317-321.

Wang, H., Cao, G. H., Prior, R. L., 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry. 44: 701-705.

Wang, H., Cao, G. H., and Prior, R. L., 1997. Oxygen radical absorbing capacity of anthocyanins. Journal of Agricultural and Food Chemistry. 45: 304-309.

Wijewickreme, A. N., and Kitts, D.D., 1998. Modulation of metal-induced genotoxicity by maillard reaction products isolated from coffee. Food and Chemical Toxicology. 36: 543-553.

Woodroof, J. G., and Luh, B. S., 1986. Commercial Fruit Processing. Van Nostrand Reinhold/Avi Publishing. Co, New York.

Wrolstad, R. E. 1976. Color and pigment analysis in fruit products. Station Bulletin of Oregon Agricultural Experiment Station. Oregon State University, Corvallis, Oregon. 624

Yang, C.S.T., and Atallah, W. A., 1985. Effects of four drying methods the quality of intermediate moisture low bush blueberries. Journal of Food Science. 50: 1233-1237.

Yang, A. P. P., Wills, C., and Yang, C. S. T., 1987. Use of combination process of osmotic dehydration and freeze drying to produce a raisin type lowbush blueberry product. Journal of Science of Food and Agriculture. 74: 31-34.

Yongsawatdigul, J., and Gunasekaran, S., 1995. Microwave-vacuum drying of cranberries. Part 2. Quality evaluation. Journal of Food Processing and Preservation. 20: 145-156.

Yousif, A. N., Scaman, C. H., Durance, T. D., and Girard, B., 1999. Flavor volatiles and physical properties of vacuum-microwave and air-dried sweet basil (*Ocimum basilicum L)*. Journal of Agricultural and Food Chemistry. 47: 4777- 4781.

Yu, B. P., 1994. Cellular defenses against damage from reactive oxygen species. Physiology Review. 76: 139-162.

VI. APPENDIX

Table A. Analysis of variance of relative peak areas of anthocyanins of dehydrated Bluecrop and Hardy Blue blueberries of the 1998 crop separated using RP-HPLC. Peaks with a significant treatment*cultivar effect ($p \leq 0.05$) are shown.

Peak*	cultivar	treatment		
		Freeze dried	air dried	vacuum microwave dried
2	BC	ab	ac	a
2	HB	cde	af	bef
4	BC	ab	a	a
4	HB	a	ac	bc
6	BC	a	a	a
6	HB	b	ab	a

BC= Bluecrop HB= Hardy Blue

Vitamin C Spectrofluorometric Assay

2 ml berry extract (in metaphosphoric-acetic acid) passed through C_{18}

↓

1.5 ml clear eluent mixed with 0.03 g Norit, shaken thoroughly and centrifuged

↓ ↓

0.5 ml put in a 10 ml volumetric flask containing 0.5 ml NaOAc

↓

Diluted to volume with water

↓

2ml into three test tubes

↓

5 ml O-phenylenediamine in each

0.5 ml put in a 10 ml volumetric flask containing 0.5 ml H_3BO_3/ NaOAc

↓

Let stand for 15 minutes

↓

Dilute to volume with water

↓

2ml into three test tubes

5 ml O-phenylenediamine in each

↓

Let stand for 35 minutes in the dark at room temperature

↓

Read at excitation=340nm, emission=430nm

pH differentiation Assay for Anthocyanins

Berries extracted in 95% ethanol /1.5 N HCl
(85:15)

↓

Filtered through Whatman # 1 and stored overnight at 4° C

↓ ↓

2 ml is diluted to 10 ml with
Na-acetate-HCl buffer pH=4.5

1ml is diluted to 25 ml with
KCl-HCl buffer pH= 1

↓

Equilibrated in the dark at room temperature
for 24 hrs

↓

Absorbance is read at 510 nm