

**Fruit Yield and Composition of Native Fruits, Partridgeberry  
(*Vaccinium vitis-idaea* L.) and Bakeapple (*Rubus chamaemorus* L.)**

by

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Submitted in partial fulfilment of the requirements  
for the degree of Master of Science

at

Dalhousie University  
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DALHOUSIE UNIVERSITY  
FACULTY OF AGRICULTURE

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## **ABSTRACT**

Plant growth, fruit yield and composition of nutritional constituents of bakeapple and partridgeberry were monitored and analyzed over 2011 and 2012 growing seasons. Data were collected from three locations on different latitudes at Lanse'au Clair, Red Bay and Cartwright in southern Labrador. Soluble solids content, titratable acidity, total phenolics, total anthocyanins, chlorogenic acid, caffeic acid, quercetin-3-glucoside, cyanidin-3-glucoside and ellagic acid (bakeapple only) were determined by using standard laboratory procedures. The fruit yields were higher than in Finland, Norway and USA. Temperature, precipitation and other environmental factors can affect growth and composition of harvested fruits. Soluble solids content, titratable acidity, anthocyanin, chlorogenic acid and cyanidin in partridgeberry and phenolic compounds and anthocyanins of bakeapple were significantly ( $P < 0.05$ ) affected by the combination of growing season and location. To our knowledge this is the first comprehensive study of plant growth, yield, and fruit composition of partridgeberry and bakeapple conducted in southern Labrador.

## **LIST OF ABBREVIATIONS AND SYMBOLS USED**

A	Absorbance
AAFC	Agriculture and Agri-Food Canada
DF	Dilution Factor
FRAP	Ferric Reducing Antioxidant Power
HPLC	High Performance Liquid Chromatography
NL	Newfoundland and Labrador
NMR	Nuclear Magnetic Resonance
NRCS	Natural Resources Conservation Service
Mv-3-G	Malvidin-3-Glucoside
ORAC	Oxygen Radical Absorbance Capacity
SSC	Soluble Solids Content
TA	Titrateable Acidity

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## CHAPTER 1 INTRODUCTION

It is a long tradition for people living in Atlantic Canada to use wild edible plants in their diet. Even now they grow wild berry plants for fruit in the country side (Opal Consulting Inc. 2010). For many people, berry-picking is a way to enjoy and commune with nature. Locally harvested fruits are usually served fresh. The global market for both wild berries and cultivated small fruits has greatly improved over the past two decades and keeps increasing due to the health benefits associated with their consumption. Berries are rich in minerals, vitamins, anthocyanins and polyphenols (Opal Consulting Inc., 2010). Phenolic compounds, flavonoids and anthocyanins are secondary plant metabolites deposited in fruits (Opal Consulting Inc. 2010). Environmental factors such as light, temperature and radiation influence their biosynthetic pathways and thus content (Macheix et al. 1990). To southern Labrador two types of berries are of special economic and cultural important, the partridgeberry (*Vaccinium vitis-idaea* L.) and the bakeapple (*Rubus chamaemorus* L.).

Partridgeberry, also called lingonberry, is a short woody, evergreen shrub, which spreads by underground stems (Figure 1.1). The plant is native to Arctic tundra and grows in most northern regions ranging from Eurasia to North America. The stems are round and grow about 10 to 20 cm in height. Leaves are alternate and oval, approximately 5 to 10 mm long with a smooth margin. The flowers



**Figure 1.1 Partridgeberry in southern Labrador in 2011.**

are white to pink, 3 to 8 mm long, produced in the early summer. The fruit is a dark red to purple berry with acidic taste, ripening in late summer to fall. Native partridgeberries are found in more than 24 countries.

The bakeapple is a rhizomatous herb which commonly grows in peat bogs (Figure 1.2). The bakeapple, also called a cloudberry, is native to parts of northern Europe and North America. The plants produce a large, amber-colored, edible aggregate fruit consisting of 5 to 25 drupelets, similar to raspberry. Unlike



**Figure 1.2 Bakeapple in southern Labrador in 2011.**

other *Rubus* species, bakeapple is dioecious and a female plant requires pollination from a male plant in order to produce fruit (Nilsen 2005). Bakeapple grows 10 to 25 cm high, with 2 to 7 alternate leaves. After pollination, an aggregate fruit is formed in a manner similar to raspberry (Nilsen 2005). The fruit is pale red in its unripe stage, to amber color when ripening in the middle of August in Labrador. Only one fruit per plant is produced on the top of the short stem (Figure 1.2). Once the bakeapple fruits ripen, the leaves start turning dark and dry out (Greene 2009).

Partridgeberry and bakeapple are important wild berries in Atlantic Canada, next to the wild blueberry which is produced on much larger areas and has much greater economic value (Opal Consulting Inc. 2010). Labradorians say the productivity of bakeapple has shown significant differences among different habitats and varies from year to year. Newfoundland and Labrador is the largest producer of partridgeberry and

bakeapple in North America with an average partridgeberry yield of 212,750 pounds per year (Heidenreich 2010). Revenue from the wild berries varies from location to location and year to year, and overall fruit quality and supply/demand. The price of partridgeberries purchased from a harvester is between \$1.2 and \$1.5 per pound in Labrador, competitive wholesale price in China can be less than \$1 per pound (Opal Consulting Inc. 2010). The processors in Newfoundland pay a harvester about \$6 to \$8 per pound for bakeapple fruits, but sometimes the price can reach as high as \$10 per pound (Opal Consulting Inc. 2010). On the other hand, the price of bakeapple has not appreciably changed over the past two decades, farm gate prices can reach \$80 per gallon (Karst and Turner 2011).

Both bakeapple and partridgeberry contain an abundance of antioxidants, such as phenolic compounds, vitamins, anthocyanins phenolic acids and their esters. These compounds are attributed to the prevention of high blood pressure, improvement of blood circulation, and protection from cancer in humans (Koukel 2007). Phenolic compounds include flavonoids, phenolic acids and polyphenols. The anthocyanins are among the most important flavonoids as they protect plants from ultraviolet radiation and determine fruit color (Koukel 2007). Bakeapple is orange or red due to high concentration of carotenoids, which contain higher antioxidant bioactivity (Lashmanova, et al. 2012). The wild berries are also rich in potassium, zinc and magnesium (Koukel 2007).



## **CHAPTER 2 LITERATURE REVIEW**

### **2.1 LABRADOR**

The area of Labrador covers 154,000 square kilometers, twice that of Newfoundland Island. Labrador starts from Blanc Sablon (northern Quebec), the fifty-first parallel of latitude in the north, to 62° latitude north and west to the Romaine River. Labrador is a part of the Canadian Shield and the oldest rock in the world formed about 800 million years ago by forces deep within the earth and shorn and torn by wind, rain and ice for millions of years (Rompkey 2003). Ice and glaciers carved its surface into majestic mountains and rocks, resulting in the land of endless variety. In 1944 Tanner described the geography of Labrador as “endless wooded solitudes of the central part of the peninsula,” also “a great plain, strewn with a strange multitude of lakes and a veritable labyrinth of ponds, alternating with very extensive swamps and bogs.” There are a myriad of bays, fiords and islands along the Labrador coast, most of them are bare rock and windswept barrens. In the central of Labrador grow some of the best black spruce (Rompkey 2003).

In the fall (approximately mid-August to late October), berries are ripe everywhere with wonderful colors, including wild blueberries, redberries (partridgeberries) and bakeapples. Local people, both the young and the old are riding on their all-terrain vehicles (ATV) to fields for picking berries with their pans and baskets. They used the berries for pies, puddings and jams during the long winter (Rompkey 2003).

### **2.2 NORTHERN FRUITS IN GENERAL**

Although the growing season in Labrador is very short and only a few fruit crops in this part of Canada can be grown, surprisingly many wild berries are thriving in southern

Labrador. The most popular wild berries include wild blueberry (*Vaccinium angustifolium*), crowberry (*Empetrum nigrum*), partridgeberry, wild red raspberry (*Rubus idaeus* L.), and bakeapple. The demand for wild berries has doubled over the past ten years and continues to increase. In 2009, Canadians consumed about 318 million kg of berries (Opal Consulting Inc. 2010). In Newfoundland, about 300 to 400 thousand pounds of berries are harvested, with a total value of \$1.5 to \$2.0 million, and the total value of fresh berries is higher (Opal Consulting Inc. 2010).

### **2.2.1 WILD BLUEBERRY, CROWBERRY AND RASPBERRY**

Wild blueberry is an economically important small fruit in Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland and Labrador. Agriculture and Agri-Food Canada (AAFC) indicated that the lowbush blueberry market increase in Canada from 56,921 tons in 2006 to 92,517 tons in 2010. Nova Scotia and Quebec have the most production of wild blueberry. However, the market value of lowbush blueberry decreased from \$129,105,000 in 2006 to \$56,140,000 in 2010 (AAFC 2010). Wild blueberries predominately spread by rhizomes. The rhizomes differentiate into roots and stems. Wild blueberries are usually harvested in August and September in the Atlantic Provinces of Canada (Aalders et al. 1972). Their fruits are known for the abundance of antioxidants, linked to the protection of human from cancer, heart diseases and premature aging (AAFC 2008). Most wild blueberries are being frozen after harvest using “Individually Quick Freezing (IQF)” technology. Both fresh and frozen blueberries are used for processing into products such as ice cream, yogurt, jams and syrups. Most of these products are exported to over 30 countries. Wild blueberries, next to apples and strawberries, are the major fruits used for processing in Eastern Canada (AAFC 2008).

Crowberry is a common short evergreen shrub with needle-like leaves, bearing edible fruits, black in color, and grows widely in Atlantic and Northern Canada. This native plant grows in various environmental conditions such as spruce stands, heath land and rocky mountain slopes (Robert 2009). Crowberries are picked in the fall. The berries also contain impressive amounts of antioxidant compounds including organic acids, vitamins, tannins, and anthocyanins (Robert 2009).

Raspberries have been used as fresh fruits in Canada for a long time. Raspberries are also popular for commercial production in North America. Fruit yields in commercial farms in excess of 2,500 to 3,000 pounds of fruits per acre have been reported (AAFC 2007). Commercial production is limited by cultivars, red and purple cultivars being the most popular because of higher profit (Smith et al. 2007). Although there are many different colors of raspberry species, including red, black, yellow and purple, the red raspberries (*Rubus idaeus*) are the major type for commercial production. Raspberries are produced all over Canada, but 83% of production comes are from British Columbia (AAFC 2007). There are two types of raspberries which determine the commercial production system, a primocane-fruiting (fall-bearing) and a florican-fruiting (summer-bearing) (Pritts 2009). The raspberries in southern Labrador are of the florican-fruiting type and are gathered from wild stands. There are numerous problems with growing raspberries mainly due to diseases and pests. Fungal, bacterial and viral diseases, insect pests, mites, and pathogenic nematodes' caused disease can result in significant decline of production (AAFC 2007). Infested fruits are also of the lower quality and create problems for processors (AAFC 2007). It is hard to remove insects and dirt from

aggregate fruit during washing, similar applies to bakeapple. In addition, aggregate fruits are also difficult to process.

### **2.2.2 PARTRIDGEBERRY**

Partridgeberry (*Vaccinium vitis-idaea* L.) belongs to the Ericaceae family and is native to Scandinavia, Alaska, Northeastern Canada and New England. The plant has numerous names. It is also called lingonberry, foxberry, or northern mountain cranberry. The name has its roots coming from Swedish immigrants. Partridgeberry has been called a mountain cranberry in New England. In Nova Scotia where wildlife often feeds on partridgeberries, it got a nickname, a foxberry. People in Labrador almost always call it redberry, dry ground cranberry, or rock cranberry. Partridgeberries are small, red, edible berries. The plant is a perennial, evergreen short shrub, spreading by underground rhizomes. The dormant buds on the rhizome can continue spreading horizontally as new growth rhizome or upright to produce shoots (Hjalmarsson and Ortiz 1998). The fruit structure is close to cranberry, which is bright red, broadly oval berry (false berry), with numerous tiny seeds inside.

There are two types of partridgeberries in the world, one is the Northern American type, *Vaccinium vitis-idaea*, which is short and blooms only once in the season. The others are European/Asian types, *Vaccinium vitis-idaea* variety *majorus*, which is taller than the North American, with larger leaves, and blooms twice every year. Most people select the European/Asian type for commercial production because the height (20-30 cm) that makes harvest easier (Greene 2009). The wild partridgeberry flowers are 3-8 mm long, bell shaped, with white to light pink color, usually produced in the early summer. They are very sensitive to frost (Greene 2009). A degree of self-pollination exists in

partridgeberries, but predominantly flowers are cross-pollinated by insects. Although wind pollination can also be significant on the open tundra and coastal areas (NRCS 2007).

The fruit of partridgeberries could be damaged by a fruit worm, the larva of the moth *Grapholita libertina*. The white, 3 to 4 mm long worms feed inside berries from late August/early September, prior to harvest (Scott 2010). Before picking, people usually check the berries for worm presence.

Once the berries are harvested, they need to be refrigerated as soon as possible, or frozen for further processing to slow down the respiration and extend their storage life (Greene 2009). Frost will enhance berries' flavor, and make them very juicy (Scott 2010). Partridgeberries contain an abundance of antioxidants, such as phenolics, flavonols, anthocyanidins, catechins and proanthocyanidins (Ek et al. 2006). Although the amount of antioxidant would be lower when partridgeberries are frozen and processed, they still have higher antioxidant levels than many other small fruits (Koukel 2007). The sugar concentration of partridgeberry increases for a period of time during storage, but organic acids and anthocyanins decreases (Saario 2000).

### **2.2.3 BAKEAPPLE**

Bakeapple (*R. chamaemorus* L.), also called cloudberry, or maltberry is a small (5 to 10 cm high) dioecious perennial plant belonging to the Rosaceae family. It is found in circumpolar areas of North America and Northern Europe (Karst and Turner 2011). It is a very popular fruit in certain areas including parts of Newfoundland and Labrador. The plants mainly grow in bogs and spread *via* underground rhizomes. The flowers and leaves are produced from rhizomes every year. Rhizomes also differentiate to roots and the

number of roots usually depends on the environmental conditions. There is no visible difference between male and female vegetative parts unless the plants are flowering (Nilsen 2005).

Both bakeapple male and female flowers usually contain four to ten white petals. The size of flowers is affected by both genotype and environment. The sex ratio relies on many factors, including environment, soil fertility and genetic makeup, but usually male flowers are in larger numbers than female flowers (Nilsen 2005). The flowers require insects, butterflies and/or spiders for pollination. Frost in late spring can damage flowers and reduce yield of berries (Pelletier et al. 2001). More fruits are found in the coastal areas and on the island, because weather conditions are moderated by water and reproductive organs escape late frosts (Scott 2010).

The bakeapple fruit is an aggregate, raspberry-like structure composed of five to twenty-five drupelets, the only harvested part in this species. The pith stays attached to the plant upon detachment of the fruit. In each drupelet, there is a smooth and hard stone. The size of the berries and number of drupelets per fruit is based on the quality of pollination. Large number of female flowers may result in high fruit yield, but the yield also depends on plant growth conditions. Between 20 and 50% of flowers and young fruits could die due to the climate and/or carbohydrate limitation during the growing season (Nilsen 2005). At present there is no mechanical harvest for the bakeapple since it is very soft. The locals hand pick the fruits. Pickers use buckets for picking the berries as there is always plenty of juice inside at the bottom of the buckets.

Fresh bakeapple is rich in vitamin C, benzoic acid and a variety of micro- and macronutrients (Thiem 2003). It also contains large amount of flavonoids and phenolic

acids, which are a large groups of phenolic in secondary metabolites (Thiem 2003). Ellagic acid is the major phenolic compounds in bakeapple, however, there are also small amounts of other phenolics such as flavonol aglycons (quercetin, kaempferol, myricetin), hydroxycinnamic acid (p-coumaric, caffeic, ferulic) and p-hydroxybenzoic acids (Thiem 2003).

## **2.3 NUTRITIONAL COMPOUNDS IN FRUITS**

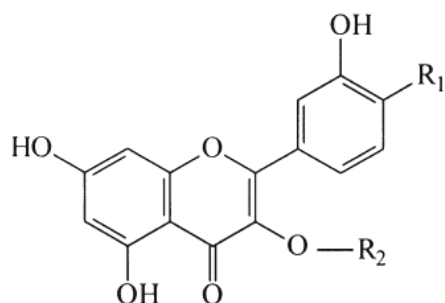
Fruits are sources of most vitamins, minerals, fibre and antioxidants in the human diet. Large amount of polyphenols found in small fruits include, phenolic acids and flavonoids (anthocyanins, proanthocyanidins and flavones) (Bakowska-Barczak, et al. 2007). These compounds are beneficial for human health and can protect humans from diseases such as cancer, high blood pressure and high cholesterol levels (Paredes-López, et al. 2010).

### **2.3.1 POLYPHENOLS**

Polyphenols are a large class of chemical compounds found in fruits, vegetables and other plants. There are more than 8000 phenolic structures and over 4,000 flavonoids that have currently been identified (Tsao 2010). Due to the wide distribution and diversity of polyphenols, they are usually divided into groups according to their source, biological function and chemical structure. Based on their different structures, polyphenols are classified into phenolic acid, flavonoids, stilbenes, tannins and diferuloylmethanes (Tsao 2010). Polyphenols are composed of multiples phenols units. Polyphenols are produced in the secondary metabolic pathways in plants. Plant secondary metabolism produce compounds that are useful for plant development, but not necessary for survive. They play a major role in coloring of leaves and fruits. Study indicated that the antioxidant

activity of polyphenols in human diet can, to some extent, protect humans from chronic and degenerative diseases (Nichenametla et al. 2006).

Phenolic acids are non-flavonoid polyphenolics, which belongs to antioxidants. They can be divided into groups based on their carbon skeleton: either benzoic acid or cinnamic acid. Phenolic acids are present in fruits and vegetables in limited forms (Tsao 2010). The general backbone structure of flavonoids is the C6-C3-C6. Since the hydroxylation pattern and variations in the C3 ring, there are many sub-groups in flavonoids including anthocyanins, flavan-3-ols, flavones, flavanones and flavonols. Although there are many kinds of structures of flavonoids, they exist in plants in glycosides forms, and their antioxidant activity depends on both structure and glycosylation patterns (Tsao 2010).



Flavonol	R1	R2
Rutin	OH	Rutinose
Quercitrin	OH	Rhamnose
Quercetin	OH	H
Kaempferol	H	H
Isorhamnetin	OCH <sub>3</sub>	H

**Figure 2.1 Structure of the five common flavonol found in plants.**

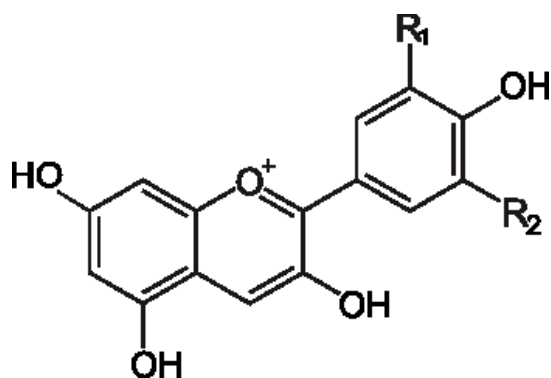
Flavonols are 3-hydroxyflavone skeleton compounds, synthesized in the flavonoid biosynthetic pathway. They are present in a wide variety of plants playing important role in UV protection and as co-pigments of flowers and fruits (Downey et al. 2003). Light is the most important factor that affects the synthesis of flavonols and flavone. The highest concentration of these compounds is in the leaves, with very little in the underground parts (Herrmann 1976). As a group of plant secondary metabolite



products, the total amount of flavonols in plants is limited, due to environmental factors, growth of fruits and the size of fruits (Herrmann 1976). For example, weather conditions such as temperature significantly affect phenolic compounds concentrations in blackcurrant berries (Zheng, et al. 2012). Higher temperature and radiation during growing season resulted in increasing accumulation of glycoside phenolic compounds (Zheng et al. 2012)

### 2.3.2 ANTHOCYANINS

As flavonoids, anthocyanins are secondary plant metabolites. They are flavonoid phenolic compounds, widely distributed among fruits, berries and flowers. One function of anthocyanins is to provide reddish-blue color to plant organs that attracts pollinators. Anthocyanidin, the aglycon, is the base structure of anthocyanins (Pascual-Teresa and Sanchez-Ballesta 2008). The major property of anthocyanins is their antioxidant activity, which plays an important role to prevention of neuronal and cardiovascular illnesses, cancer and diabetes (Castaneda-Ovando et al. 2009). There are six major anthocyanidins that exist in nature (Figure 2.2). This variety of anthocyanins is synthesized by different patterns of glycosylation (Coultate 2002).



Anthocyanidin	R1	R2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Petunidin	OH	OCH <sub>3</sub>
Peonidin	OCH <sub>3</sub>	H
Malvidin	OCH <sub>3</sub>	OCH <sub>3</sub>

Figure 2.2 Structure of the six common anthocyanidins found in plants.

Anthocyanins are water soluble and are located in the cell sap. They are usually found in vacuoles, so that they are not affected by the enzymes and other substances (Coulter 2002). The appearance of the anthocyanin colors depends on pH of the solution. The flavylium cation is red in color at low pH, but at high pH, the flavylium ions form into other anthocyanins to appear somewhat blue in color (Pascual-Teresa et al. 2008).

### **2.3.3 NUTRIENT ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)**

Chromatography is widely used in the separation and analysis of nutritional compounds. HPLC is the most popular technique (Hamilton and Sewell 1982). The separation of the components of the mixture is determined by equilibrium distribution ( $K$ ) of the components between the mobile phase and the stationary phase. If  $C$  is the concentrations of different phases, then  $K=C_s/C_m$  ( $C_s$  is concentration of stationary phase,  $C_m$  is concentration of mobile phase) (Hamilton and Sewell 1982).

The HPLC equipment usually contains a solvent reservoir, an analysis column and collection tubes. In order to increase the speed of analysis, a pump to produce high pressure to the mobile phase is used. (Hamilton and Sewell 1982).

The solvent quality of mobile phase is important to HPLC, it requires high level of purification and stability so that no small molecule or air in the pump or column (Hamilton and Sewell 1982). HPLC systems can contain more than 20 types of detectors. The selection of a detector for analysis in liquid chromatography is of great importance (Nollet 1992). The common detectors include photometric, fluorescence, refractive index (RI), electrochemical (EC), liquid chromatography–mass spectrometry (LC-MS),

conductivity, chemical reaction and flame ionization (Nollet 1992). LC-MS can be used for both qualitative and quantitative analysis and provide the best result (Nollet 1992).

#### **2.3.4 OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) & FERRIC REDUCING ANTIOXIDANT POWER (FRAP)**

Oxygen radical absorbance capacity is widely used for measuring antioxidant capacities of vegetable, fruits, berries samples. Both inhibition time and the extent of inhibition oxidation are measured by ORAC assay (Haytowitz and Bhagwat 2010). ORAC values can be divided into hydrophilic-ORAC (H-ORAC), lipophilic-ORAC (L-ORAC), total-ORAC, and total phenolics (TP). Only TP is reported in mg of gallic acid equivalents per 100 grams (mgGAE/100g), the other three are all reported in  $\mu\text{mol}$  of Trolox Equivalents per 100 grams ( $\mu\text{molTE}/100\text{ g}$ ). Trolox, an antioxidant, is a water-soluble derivative of vitamin E. Trolox is used as the standard to measure the antioxidant capacity of foods in ORAC and FRAP (Nutrient Data Laboratory 2007).

Ferric reducing antioxidant power (FRAP) is another simple, automated test that measures antioxidant capacities. The theory of this method is to compare the change of the absorbance at 593 nm under known ferrous ions concentration condition (Benzie and Strain 1996). However, some literature has indicated the FRAP method is referring to the measurement of the ferric reducing ability of plasma (Pohanka et al. 2009).

#### **2.3.5 SOLUBLE SOLIDS CONTENT**

Soluble solids content (SSC), such as soluble carbohydrates, is one of the most important factors of fruit quality (Tian et al. 2007). Sugar in fruits plays an important role in the character and quality of the polyphenols as they are utilized in their synthesis. SSC also affects the taste of the fruits because most of the soluble solids content is composed

of sugars (Jordan and Seelye 2009). Studies indicated that SSC in fruits were various in different growth stage and fruit weight (Zsofi et al. 2011). In partridgeberry, SSC increased during the first week of storage after harvest, but decreased after that. Blueberry is considered related to partridgeberry, which is classified as a climacteric fruit, producing low amounts of ethylene during ripening period (Saario 2000). SSC is also different with varying fruit weight. In some grapes cultivar, the fruit weight around 1.5g contains the highest SSC concentration (Zsofi et al. 2011).

Although soluble solid content is an indicator of fruit quality, there is only limited information available on the composition and concentration of SSC in Nordic fruits (Viljakainen et al. 2002). Viljakainen et al.(2002) indicated that the major types of sugar in partridgeberry and bakeapple were glucose and fructose, approximately 86 g/L for partridgeberry juice and 52 g/L for bakeapple juice. However, sugar concentration can vary in fruits from different locations, from different harvest time and under different conditions of storage (Viljakainen et al. 2002).

### **2.3.6 TITRATABLE ACIDITY**

The acidity of nutrient solutions is one of the factors of fruit taste and quality. Acidity also greatly affects concentration of nutritional compounds in fruits (Kuwahara et al. 1996). A combination of pH and titratable acidity (TA) provides more useful and accurate information on the quality of fruit juice (Kuwahara et al. 1996). To determine TA in fruit juice, 0.1 mol/L NaOH is commonly used for titration of fruit juice samples (Xie et al. 2011). The TA is measured on a mg basis of a specific organic acid such as malic, tartaric or citric acid. Recently, newer technologies and equipment have been used to determine TA in a fast and accurate way. These include ultrasound, microwaves

absorption, nuclear magnetic resonance (NMR), and infrared spectroscopy (Xie et al. 2011). Ultrasound treatment would reduce decay of fruits and keep significantly higher level of SSC, TA and vitamin C (Cao et al. 2010). Microwave absorption method is using microwaves to inactivate enzymes in fruit juices by heat, which can stabilize fruit juice and prevent any change in viscosity, color, pH and nutrient content (Barrett et al. 2007).

Fruits from northern regions are rich in organic acids and this causes low pH (pH 2.7 – 3.5) in fruit juice (Viljakainen et al. 2002). The major organic acids of partridgeberry and bakeapple are citric and malic acid (Viljakainen et al. 2002). Benzoic acid is also found in some northern small fruits including partridgeberry, bakeapple, crowberry and cranberry. On average, the total organic acid concentration in partridgeberry and bakeapple fruits in Finland have been reported to be 16 to 19 g/L and 14.5 g/L, respectively. However, organic acid concentration in fruits can varies due to different growing conditions (Viljakainen et al. 2002).

## **CHAPTER 3 HYPOTHESIS AND OBJECTIVES**

### **HYPOTHESIS**

The levels of nutritional compounds in bakeapple and partridgeberry will depend on the location from which the fruit is collected and on the growing season

### **OBJECTIVES**

1. To measure and identify bioactive compounds in fruits at maturity, including soluble solids content, titratable acidity, total phenolics and total anthocyanins,
2. To determine the phenology of bakeapple and partridgeberry in southern Labrador including spring dormancy break, growth, blooming, fruit set, fruit ripening and harvest,
3. Identification of pollinators and the timing of their occurrence in bogs.

## **CHAPTER 4 MATERIALS AND METHODS**

A good understanding of the bakeapple and the partridgeberry physiology in addition the surrounding environment will help to develop these two species to a possible commercial level. Identification of nutritional compounds of these two fruits could expand the potential market for these northern berries.

### **4.1 LOCATION OF THE FIELDS, GENERAL COLLECTION OF SAMPLES AND WEATHER DATA**

Three fields in southern Labrador were selected for this study based on local recommendation: Lanse'au Clair (N 51°41', W 57°08'), Red Bay (51°43', W 56°26') and Cartwright (N 53°42', W 57°0'). From each location, three samples of partridgeberry and bakeapple (approximately 300 g of fruit per sample) were collected making total of nine samples per fruit type per growing season. Following collection samples were refrigerated at -5 °C for 5 hours, and then frozen at -40 °C in a freezer. Samples were shipped to Dalhousie Agricultural Campus for nutritional analysis in the autumn. Fruit were collected in both 2011 and 2012 growing seasons.

Weather data were recorded using a data logger (U23-002, Onset Computer Corp., Bourne, MA, USA) every 15 minutes, and mean day temperatures and maximum difference in temperature were calculated. The data loggers were set up at each location at the beginning of the growing season to the end of October. These weather data were downloaded from the data loggers to a laptop computer once per month during the growing season.

## **4.2 BELT TRANSECT**

There are a number of other species growing in the area surrounding bakeapples and partridgeberries in the fields. In the belt transect, the other species were identified and recorded. A square 10 x 1 m was set in all three field sites and divided into ten 1 x 1 m squares. In the 1 m x 1 m squares, the growth stage of all plants was recorded once a week (from July 3<sup>rd</sup> until August 5<sup>th</sup>, 2011 and from June 18<sup>th</sup> until August 8<sup>th</sup>, 2012). The growth stage was displayed by percentage rating for each species. The ratings were: 0-10%, 11-25%, 25-50%, 50-75%, 75-100%, with 75-100% being peak bloom. 'Pre' and/or 'post' peak were also recorded bloom (for example it is 'pre' when the plants are in bud stage, and it becomes 'post' once they are in anthesis). The belt transects were set in the fields until there were no longer flowers in the 10 x 1 m squares.

## **4.3 POLLINATOR COLLECTION**

In the summer of 2011, a 10 x 30 m square was set in the fields at all three locations. A person was standing and/or slowly walking across the square observing any flying insects in this area. When possible, the person was catching the insects using a net. This process was performed at each site for a period of 40 minutes once a week. The observation time usually started around 1:00 pm in Lanse'au Clair and Red Bay on different day in each week, approximately one hour later at Cartwright due to long distance (approximately 500km away from Lanse'au Clair). Observation day may have changed if the weather was not allowing to observe in the fields. Normal schedule was Monday for Lanse'au Clair, Tuesday for Red Bay, Wednesday for Cartwright each week. Instead of sweeping on the bogs, net was only used for catching once the pollinators were found. The reason to do so was the plants in the bog were limited (especially bakeapple),



and bakeapple flowers were easily damaged because they were higher than other plants on the ground. Pollinators were present in the field, but populations were limited. There were less than twenty pollinators in pans during bakeapple flowers in bloom, but during partridgeberry flowers in bloom, there were one or two bees found in each pans. After the pollinators were caught, they were frozen in -40°C freezer then shipped to Dalhousie Agricultural Campus for identification.

In summer of 2012, pan-traps were used to collect pollinators . Twenty-one uniform pan traps were randomly set in a 10 x 30 m square. Pans came in three colors: blue, white and yellow, seven pans of each color (Figure 4.1). Each pan



was filled  $\frac{1}{2}$  to  $\frac{3}{4}$  full with water to 5 drops of dishwasher detergent had been added. The pollinators were collected once a week, a total of 10 collections throughout the 2012 season. The pollinators were frozen in -40°C once they were taken out of the pans, and shipped to Dalhousie Agricultural Campus for identification by using insect identification field guide (Milne and Milne, 1980).

**Figure 4.1 Pan-traps set up in Red Bay**

#### **4.4 GROWTH STAGES RECORDS AND FRUIT SET**

The growth season is short in Labrador. Pictures of plants were taken in one week intervals to record the growth stage of the plants. Pictures of plants were taken at Lanse'au Clair on Monday; at Red Bay on Tuesday and at Cartwright on Wednesday,

these days were altered if the weather was adverse. One picture was selected from each week to present the growth of plants. The selected pictures were organized following progressing time in the season. Pictures were placed together to show how plants have grown week by week during growth seasons.

Fruit set of partridgeberry was determined in this project. At each field (Lanse'au Clair, Red Bay and Cartwright), five partridgeberry plants were selected and labelled with tape. The number of flowers on each plant was recorded when plants were in bloom and the number of fruits was recorded when the berries started bearing. Fruit set was calculated as the number of flowers divided by the number of fruits.

#### **4.5 YIELDS**

Total yields of bakeapple and partridgeberry in Labrador are very difficult to determine due to lack of information on the number of plants growing in these areas.

In 2011, a 20 x 20 m plot was set in each field site for harvesting. In this square, the three best yielding 1 x 1 m spots were picked by hand. The yield was estimated based on the observation.

In 2012, seven 1 x 1 m squares were randomly selected in a 20 x 20 m field site. All the berries in the selected square were harvested, frozen and the weight of frozen berries was recorded by using an electronic scale. Some of the 1 x 1 m squares were without fruits. In this situation, the empty spot was skipped, and the next randomly selected spot which had fruits was picked.

#### **4.6 COMPOSITIONAL ANALYSES**

Both of these two fruits contain a large amount of nutrients. The data and nutrients from the collected fruit samples were analyzed. Analyses included: total soluble solids,

titratable acidity, total phenolics and total anthocyanins. The amount of total soluble solids, titratable acidity, total phenolics and total anthocyanins were determined and compared with results between the three locations and the two seasons, 2011 and 2012.

#### **4.6.1 SOLUBLE SOLIDS CONTENT**

About 50 g of samples were weighed and homogenized to make juice. Fruits were divided into 10 groups (5 g each). Each 5 g sample was pressed to juice using a garlic press. The juice from samples was placed directly onto a refractometer (Atago Co., PAL-RI, Tokyo, Japan).

#### **4.6.2 TITRATABLE ACIDITY**

About 5 g of samples were weighed and pressed to juice using a garlic press. The same operation was repeated ten times. Five mL of juice was added to 45 mL of pH balanced distilled water, distilled water pH was measure by pH meter and balanced by NaOH (0.1 mol/L) and HCl (0.1 mol/L). Three 5 mL samples of the dilution were placed into Erlenmeyer flasks and a few drops of phenolphthalein indicator were added, and swirled to mix by shaking the flask generally. Titration with 0.1 NaOH to an endpoint of pH 8.2 (between 8.0-8.5 is acceptable) was performed until color change from pink to green. The number of milliliters of 0.1 N NaOH used to reach the end point was recorded. Usually titratable acidity are presented in gram L<sup>-1</sup>, the general formula for determining the titratable acidity is:

$$\text{Titratable acidity} = 64 * \text{Molarity of NaOH} * \text{Titre value (mL)}/\text{Volume of sample (mL)}$$

### **4.6.3 TOTAL PHENOLIC COMPOUNDS**

Phenolic compounds are of interest due to their antioxidant abilities. The total phenolics of bakeapple and partridgeberry were measured by using the Folin-Ciocalteu method in this project. All the preparations were carried out under dark conditions due to gallic acid being light sensitive.

#### **4.6.3.1 SAMPLES PREPARATION**

Approximately 50 g of samples were weighed and pressed to juice by garlic press. The dilution (with distilled water) factor for bakeapple was 1:10 and partridgeberry was 1:40.

#### **4.6.3.2 DETERMINATION OF TOTAL PHENOLICS**

Total phenolic contents of fruits were measured according to the Folin-Ciocalteu method using gallic acid as the standard (Folin and Ciocalteu 1927). Gallic acid standards (20, 40, 60, 80, 150 mg/L) were prepared fresh for each batch of samples was used for developing the calibration curve. For each sample, 1 mL of solution was mixed with 5 mL of Folin-Ciocalteu reagent. The mixture was incubated for 5 min, followed by the addition of 4 mL of 7.5% sodium carbonate. The mixture was incubated for 2 hours at room temperature, and then the absorbance of the solution was measured at 760 nm. The total phenolic content was expressed in mg/mL based on gallic acid equivalents (GAE) calibration curve.

## **4.6.4 TOTAL ANTHOCYANIN**

### **4.6.4.1 SAMPLE PREPARATION**

The extraction method of anthocyanin was performed according to method provided by Fuleki and Francis (1968) with some modification. Ten grams of frozen samples was weighted out with 10 mL of extraction solvent, and homogenized into a slurry. The samples were rinsed using less than 15 mL of solvent from a squeeze bottle. The beaker was wrapped in tinfoil and overnight at 4°C. This step is crucial for the pigments to equilibrate throughout the slurry. The sample was filtered through Whatman #4 filter paper (Fisher Sci. Canada) attached to a filter flask covered with aluminum foil and under vacuum. The extract in the flask was transfer to a 50 mL volumetric flask and made up to volume with extraction solvent. The flask was wrapped with aluminum foil and overnight at room temperature.

### **4.6.4.2 DETERMINATION OF TOTAL ANTHOCYANINS**

The anthocyanin extract was separated into two sets. One set was dissolved in potassium chloride buffer (pH 1.0), and the other with sodium acetate buffer (pH 4.5) with a dilution factor 1:1.5 for bakeapple and 1:20 for partridgeberry). The absorbance of the solution was measured at 520 nm and 700 nm (Fuleki and Francis 1968).

The measurement of absorbance at 700 nm was subtracted from the absorbance at 520 nm at each pH. The difference in absorbance (A) at 520 nm was determined between pH 1.0 and pH 4.5. Calculation of pigment concentrations as Mv-3-G (Malvidin-3-Glucoside) using the following formula:

Anthocyanin conc. ( $\text{mg L}^{-1}$ ) =  $((A) / (\beta * L)) * (MW * DF * 1000)$ , where,  $\beta$  (molar absorptivity) = 28000; MW (molecular weight) = 529, DF is the dilution factor and L=1.0.

## **4.7 DETERMINATION OF COMPOUNDS BY HPLC**

### **4.7.1 STANDARDS**

Chlorogenic acid, caffeic acid, quercetin 3-O- $\beta$ -D-glucoside and ellagic acid were purchased from Sigma-Aldrich Canada Co. (Oakville, Ontario, Canada). Cyanidin-3-O- $\beta$ -glucoside was obtained from Polyphenols Laboratories AS (Sandnes, Norway). All the standards were prepared as stock solution at 1 mg mL<sup>-1</sup> in methanol, except for ellagic acid which was in a 1:1 (v/v) dimethyl sulfoxide/methanol solution. The concentration range of chlorogenic acid, caffeic acid and quercetin-3-O- $\beta$ -D-glucoside standard was from 5  $\mu$ g mL<sup>-1</sup> to 100  $\mu$ g mL<sup>-1</sup>; concentrations range of ellagic acid standard was from 30  $\mu$ g mL<sup>-1</sup> to 300  $\mu$ g mL<sup>-1</sup> and 5  $\mu$ g mL<sup>-1</sup> to 500  $\mu$ g mL<sup>-1</sup> for cyanidin-3-O- $\beta$ -glucoside standard. The retention time of chlorogenic acid, caffeic acid, quercetin, cyanidin and ellagic acid were 20.1, 23.2, 28.0, 23.1 and 23.4 minutes respectively (Appendix E).

### **4.7.2 EXTRACTION**

#### **4.7.2.1 EXTRACTION OF CHLOROGENIC ACID, CAFFEIC ACID, QUERCETIN 3-O- $\beta$ -D-GLUCOSIDE AND CYANIDIN-3-O- $\beta$ -GLUCOSIDE**

The extraction method was based on the method of Mattila and Kumpulainen (2002). Two grams of sample were weighed into a 50 mL graduated plastic test tube and homogenized in 7 mL methanol by using a ploytron. The sample extracts were sonicated (Branson 5200, Branson Ultrasonics Co. Danbury, USA) for 30 min and made up to 10 mL with distilled water, and 3 mL were filtered through a 0.45 $\mu$ m, 25 mm membrane filter for the HPLC analysis.

#### **4.7.2.2 EXTRACTION OF ELLAGIC ACID**

The extraction method of ellagic acid was based on Häkkinen et al. (2000). Five grams of bakeapple fruits were weighed into a 100 mL rotavapor flask and 5 mL of water, 10 mL of methanol and 5 mL of concentrated HCl were added. The extracts were refluxed for 20 hours at 80°C. The extracts were made up to 50 mL with methanol and 3 mL were filtered with a membrane filter for the HPLC analysis.

#### **4.7.3 COMPOUNDS ANALYSIS**

A 20 µL injection of samples was separated on a Zorbax ODS 250 x 4.6 mm i.d., 5 µm LC column (Brockville, Ontario, Canada) using a Water 1525 HPLC with a binary HPLC pump and a photodiode array detector .

The analysis of chlorogenic acid, caffeic acid, quercetin 3-O-β-D-glucoside and cyanidin-3-O-β-glucoside was performed with a mobile phase consisting of acetonitrile (A) and 5% formic acid (B) as follow: isocratic elution 5% A/95% B, 0 – 5 min; linear gradient from 5% A/95% B to 10% A/90% B, 5 – 10 min; linear gradient from 10% A/90% B to 20% A/80% B, 10 – 20 min; linear gradient from 20% A/80% B to 70% A/30% B, 20 – 30 min; linear gradient from 70% A/30% B to 5% A/95% B, 30 – 35 min; post-time 5 min before next injection. The flow rate was 0.5 mL/min. Ellagic acid was quantified separately using 50 mM H<sub>3</sub>PO<sub>4</sub>, pH 2.5 (A) and acetonitrile (B) and program was the same as Mattila and Kumpulainen (2002): isocratic elution 95% A/5% B, 0 – 5 min; linear gradient from 95% A/5% B to 50% A/50% B, 5 – 35 min; isocratic elution 50% A/50% B, 35 – 40 min; linear gradient from 50% A/50% B to 95% A/5% B, 40 – 43 min; post-time 5 min before next injection. The flow rate was 0.7 mL/min.

## 4.8 STATISTICAL ANALYSIS

Minitab 16 statistical software (Minitab Inc., State College, PA, USA) was used to analyze the data. For partridgeberry and bakeapple, in total 17 responses (measurements) were recorded. They consisted of total soluble solids, titratable acidity, total phenolic compounds, total anthocyanins, chlorogenic acid, caffeic acid, quercetin-3-glucoside, cyanidin-3-O-glucoside and ellagic acid (bakeapple only). They were made in year 2011 and 2012 with three different locations: Lanse'au Clair, Red Bay and Cartwright. A two-way analysis of variance with location and year as two factors were performed. The main effects were investigated only when the interaction between the two factors is not significant (we use  $\alpha=0.05$  as criterion for significant test). For most responses, the two factors were not interactive so that the effect of one factor shows the same pattern for each level of the second factor, thus the mean value plots of one factor are parallel. But there were several cases there seem to have strong evidence of interaction. Titratable acidity of partridgeberry, for example, seemed to be dependent on both location and year. Year or location alone did not explain the variation of the response. A complete table of the statistical analysis of the data is shown in the table of P-values together with the interaction plot (matrix plots).



## CHAPTER 5 RESULTS

The objectives of this study were to determine the phenology and selected nutritional compounds' levels of both the bakeapple and the partridgeberry in southern Labrador. The experimental results can be broken into two parts: results from field sites and results from laboratory work. The field results include: belt transect, yield measurements, pollinator collection and weekly observation records. Nutrient analysis from the laboratory work includes: total soluble solids content, titratable acidity, total phenolics, total anthocyanins, caffeic acid, chlorogenic acid, quercetin, ellagic acid (bakeapple only) and cyanidin. There was no control treatment since the plants grow in wild conditions.

### 5.1 BELT TRANSECT

There was a large diversity of species/organisms in all three field locations, including fungi, various low growing plants, insects, and animals. The plants recorded in belt transects belonged to six families, Rosaceae, Ericaceae, Pottiaceae, Juncaeeae, Equisetaceae and Sphagnaceae (Table 5.1). The most species-rich family was Ericaceae, with *Vaccinium vitis-idaea*, *Arctostaphylos alpina*, *Empetrum nigrum* and *Vaccinium angustifolium*.

In 2011, partridgeberry started bloom approximately on 205 Julian date day, which reach 100% in Figure 5.1. The partridgeberry remained in bloom for 7 to 10 days. In 2012, partridgeberry started blooming 15 days earlier than 2011. In addition, wild blueberry started blooming approximately on 197 Julian date day in 2011, and 182 Julian date day in 2012 (reach 100%), which were similar with partridgeberry. The flower bloom date for Labrador tea in the field was approximately 210 Julian date in 2011 and

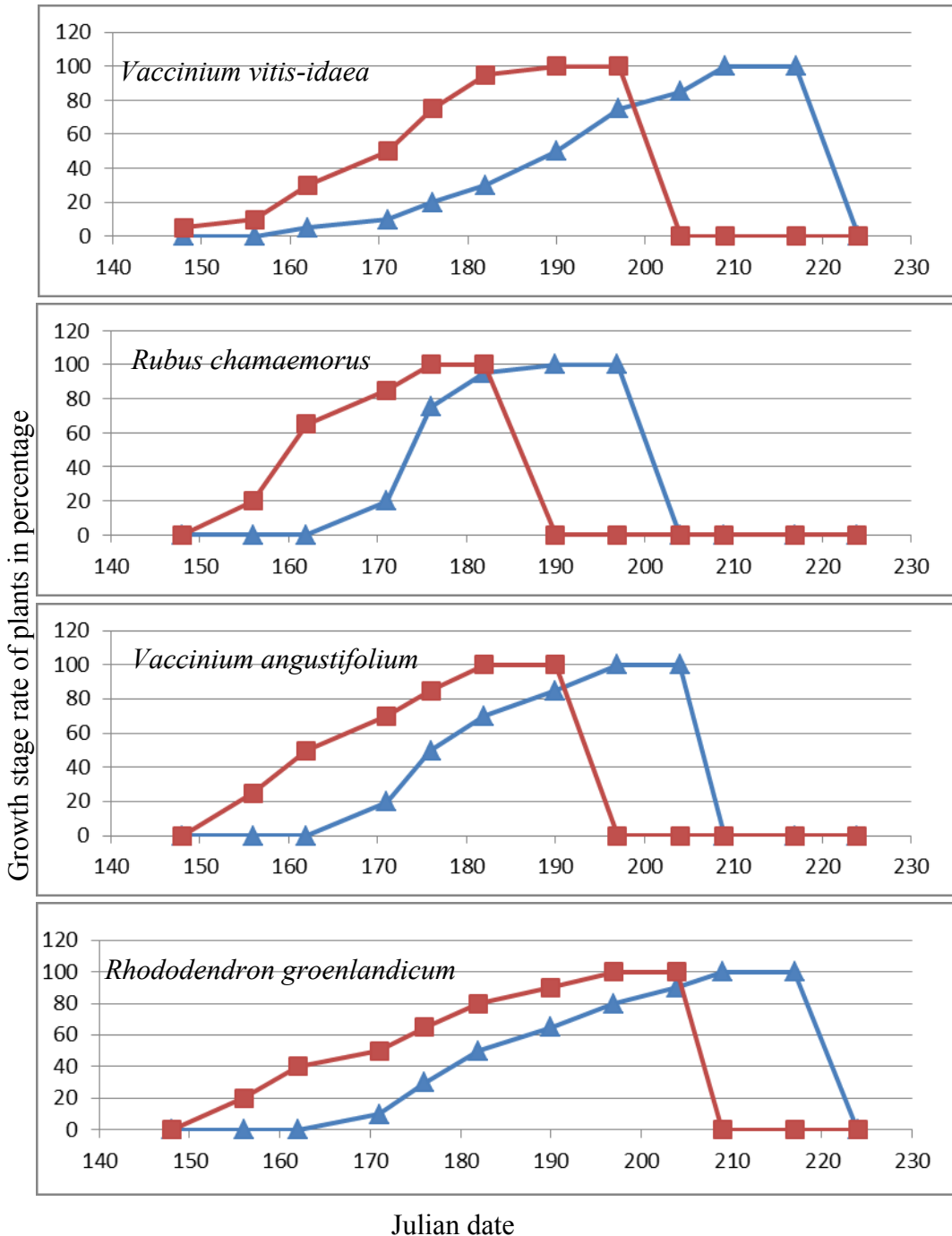
198 Julian date in 2012, which overlap with wild blueberry and partridgeberry. However, bakeapple started in the beginning of summer, which was approximately 180 Julian date in 2011 and 170 Julian date in 2012. In Figure 5.1, it is obviously visible that bakeapple reach 100% much earlier than partridgeberry.

**Table 5.1 Plant species in belt transects**

Family	Scientific name
Equisetaceae	<i>Equisetum arvense</i>
Ericaceae	<i>Arctostaphylos alpine</i>
	<i>Empetrum nigrum</i>
	<i>Rhododendron groenlandicum</i>
	<i>Vaccinium angustifolium</i>
	<i>Vaccinium vitis-idaea</i>
Juncaeeae	<i>Luzula comosa</i>
Pottiaceae	<i>Tortula ruralis</i>
Rosaceae	<i>Rubus chamaemorus</i>
Sphagnaceae	<i>Sphagnum</i> ssp.

In both 2011 and 2012, the bakeapple was the first to bloom in the fields, followed by wild blueberry, partridgeberry, and Labrador tea. The observations have shown all the plants in the belt transect bloomed approximately 7 to 10 days earlier in 2012 than in 2011. In the belt transect, the flowering period of partridgeberry overlapped with wild blueberry and Labrador tea. However, blooming of no other species overlapped with bakeapple in both years. Individual bakeapple flower lasted only for two or three days, whereas individual partridgeberry, wild blueberry and Labrador tea flowers last for approximately one week. In this study, however, bakeapple flowers were present in the field for one week as well (Figure 5.1). It was not uncommon to observe the bakeapple

plants both in bloom (Figure 5.5, stage 3) and with already formed fruits in the same field at the same time.



**Figure 5.1 Percentage rating of all plants in the belt transects in 2011 (triangles) and 2012 (squares)**

## 5.2 POLLINATOR OBSERVATION AND COLLECTION

In general, pollinators were scarce during the period of observation in both years at each location most of the time during bakeapple bloom. However, pollinators were abundant and lasted for approximately 7 - 10 days when partridgeberries were in bloom. Results have shown that pollinators were affected by weather



**Figure 5.2 A pan trap with insects at Red Bay location**

conditions. During a rainy or windy day, pollinators were not found during the observation time. Syrphidae family was the most frequently present in the field at the beginning of the growing season. Although the *Bombus* spp. was present in bogs as well, its population did not increase until August.

**Table 5.2 Orders and families of insects found in pan traps**

Order	Family	Scientific name
Hymenoptera	Apidae	<i>Bombus ternarius</i>
		<i>Bombus sylvicola</i> Kirby
	Halictidae	<i>Halictus</i> sp.
Diptera	Muscidae	<i>Helina</i> sp.
	Syrphidae	<i>Eristalis tenax</i>
		<i>Toxomerus marginatus</i>
		<i>Melanostoma mellinum</i>
Lepidoptera	Pieridae	<i>Pieris oleracea</i>

In 2012, large numbers of pollinators were caught by pan traps (Figure 5.2). Most of the specimens were from three orders: Hymenoptera, Diptera and Lepidoptera. There were five families in these three orders (Table 5.2). Among the pollinators, insects in Diptera order present the highest percentage. However, Apidae family (Figure 5.3),

which was considered most important for pollinating the fruits, occurred only in small percentage among all the pollinators. In the beginning of 2012 growing season, there were only three bumble bees caught in pans, with a large number of insects from Syrphidae family. With time progressing, more *Bombus* spp. was found in pan traps, and the population of Syrphidae family was still at the same level.



**Figure 5.3 *Bombus sylvicola* Kirby on partridgeberry flower at Cartwright location**

### **5.3 GROWTH STAGES RECORDS AND FRUIT SET**

The pictures of growth stages of partridgeberry (Figure 5.3) and bakeapple (Figure 5.4) were taken by the camera (Canon's PowerShot A2400) as of June 9<sup>th</sup> in 2011 (Julian date: 160) to October 10<sup>th</sup> (Julian date: day 283) and June 18<sup>th</sup> in 2012 (Julian date: day 169) to September 18<sup>th</sup> (Julian date: day 261). Julian date calendar refers to a continuous count of days for a whole year, with January 1<sup>st</sup> is day number 1.

Partridgeberry started to produce flower buds beginning of June (approximately day 160 Julian date). The flowers were in bloom around middle of July (approximately day 200 Julian date) and lasted for about 10 to 15 days. Partridgeberry started to form fruits by the end of July (approximately day 215 Julian date) and were ready for harvest late September (approximately day 270 Julian date) (Figure 5.4). Similar to bakeapple, the harvest date can easily change with the local climatic conditions.



**Figure 5.4 Growth stages of partridgeberry in southern Labrador during 2011 and 2012 (pictures taken by the author)**



**Figure 5.5 Growth stages of bakeapple in southern Labrador during 2011 and 2012 (pictures taken by the author)**

The partridgeberry flowers were in full bloom on approximately 200 Julian date and the number of flowers on selected plants were recorded. The berries set on approximately 215 Julian date and the number of fruits were recorded. The fruit set of partridgeberry was calculated and is presented (Table 5.3). The fruit set at all three locations were similar, approximately 72% to 75%. In addition, there was not much difference of partridgeberry fruit set between growing seasons.

	Lanse'au Clair	Red Bay	Cartwright
2011	73.43%	72.14%	73.21%
2012	72.45%	73.71%	74.19%

**Table 5.3 Fruit set of partridgeberry at three locations in 2011 and 2012**

Bakeapple plants started to produce buds underground around beginning of June (approximately day 160 Julian date). One week after, bakeapple began sprouting and developing above ground parts; leaf shoots and flowers. Flowers were in bloom around the beginning of July (approximately day 180 Julian date). Fruit started to appear around the middle July (approximately day 200 Julian date). Fruits were ready for harvest around middle of August (approximately day 230 Julian date). The harvest date, however, is dependent upon the local climatic conditions (Appendix D). The leaves started to turn brown and dried out two weeks after harvest.

## **5.4 FRUIT YIELDS**

The yields were only measured in Lanse'au Clair and Red Bay. The data from Cartwright were missing because of severe weather for both years (Table 5.4). In both seasons, it was very difficult to get to Cartwright from Lanse'au Clair during the harvest season. The distance of close to 500 km one way was extremely difficult and dangerous

to drive in heavy rain and/or fog, on unpaved road. However, for nutritional analysis, the fruit was obtained from the locals.

**Table 5.4 Total yield measurements in 20m x 20m square**

Fruits	Locations	2011 (g m <sup>-2</sup> )	2012 (g m <sup>-2</sup> )
Partridgeberry	Lanse'au Clair	195.33	235.89
	Red bay	254.15	313.72
Bakeapple	Lanse'au Clair	2.429	2.586
	Red bay	2.460	2.815

There were always more partridgeberries than bakeapples in the bog. Yields of both bakeapple and partridgeberry were higher in Red Bay than Lanse'au Clair in both seasons. Approximately 2.5 g m<sup>-2</sup> of bakeapple fruit was observed at each location and 250 g m<sup>-2</sup> of partridgeberries at Red Bay compared with 195 g m<sup>-2</sup> at Lanse'au Clair in 2011. The yields of both fruit crops were higher in 2012 than 2011 (Table 5.4).

## 5.5 WEATHER DATA

The weather data were recorded with a data logger (U23-002, Onset Computer Corp., Bourne, MA, USA) in both 2011 and 2012 summers (Appendix D). Cartwright was the warmest location in both years; the highest day mean temperature was up to 20°C in 2011 and 25 °C in 2012. The highest outdoor temperature recorded was 38°C. The daily average temperature of Red Bay (12.6°C) and Lanse'au Clair (13.2°C) were very close. The lowest temperature in Red Bay was 3°C in 2011 and 7°C in 2012. The largest day/night temperature differential (diurnal temperature) at Lanse'au Clair in 2011 was 22.9°C while it was 20.1°C in 2012. At Red Bay, the diurnal temperature in 2011 was 21.6°C and in 2012 was 16.6°C. At Cartwright, in 2011, the largest difference in



temperature was 23.7°C, and only 16.0°C in 2012. Some data are missing (2011 at Lanse'au Clair and 2012 at Red Bay) because the data loggers were plug out or destroyed most likely by bears or other wild animals.

## **5.6 NUTRIENT ANALYSIS**

As expected, the results of nutrient analysis were different for each compound in each of the fruit crops. The amount of nutrients was significantly ( $P<0.05$ ) affected by the year (growing season) and the location (Tables 5.5 and 5.6)

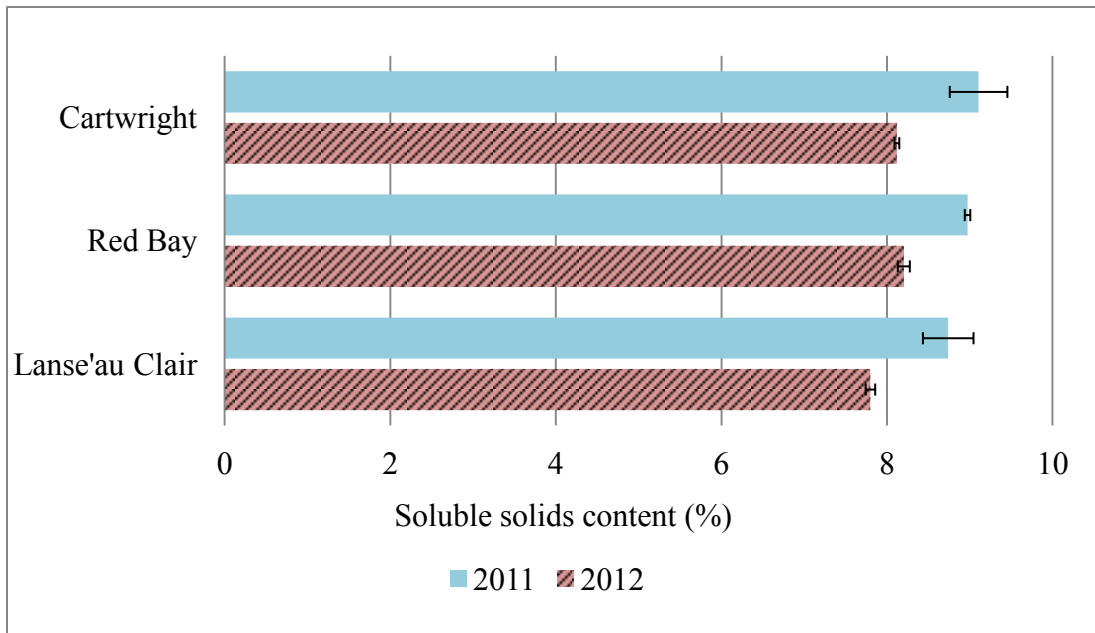
### **5.6.1 SOLUBLE SOLIDS CONTENT**

Partridgeberry fruits from Cartwright contained the highest (~13%) amount of total soluble solids contents (Figure 5.5). The concentrations in bakeapple were similar (8% to 9%) at all three locations during 2011 and 2012 (Figure 5.6). Average concentrations of soluble solids contents of partridgeberry from Red Bay and Lanse'au Clair were similar (~ 10%) and but were lower than in fruits from Cartwright by approximately 2% in 2011 (Figure 5.5). In 2012 fruits from all three locations had approximately 10% soluble solids contents. Soluble solids content in partridgeberries was affected by both the locations and the growing season. The interaction between location and growing season was significant ( $P<0.05$ ) (Table 5.5).

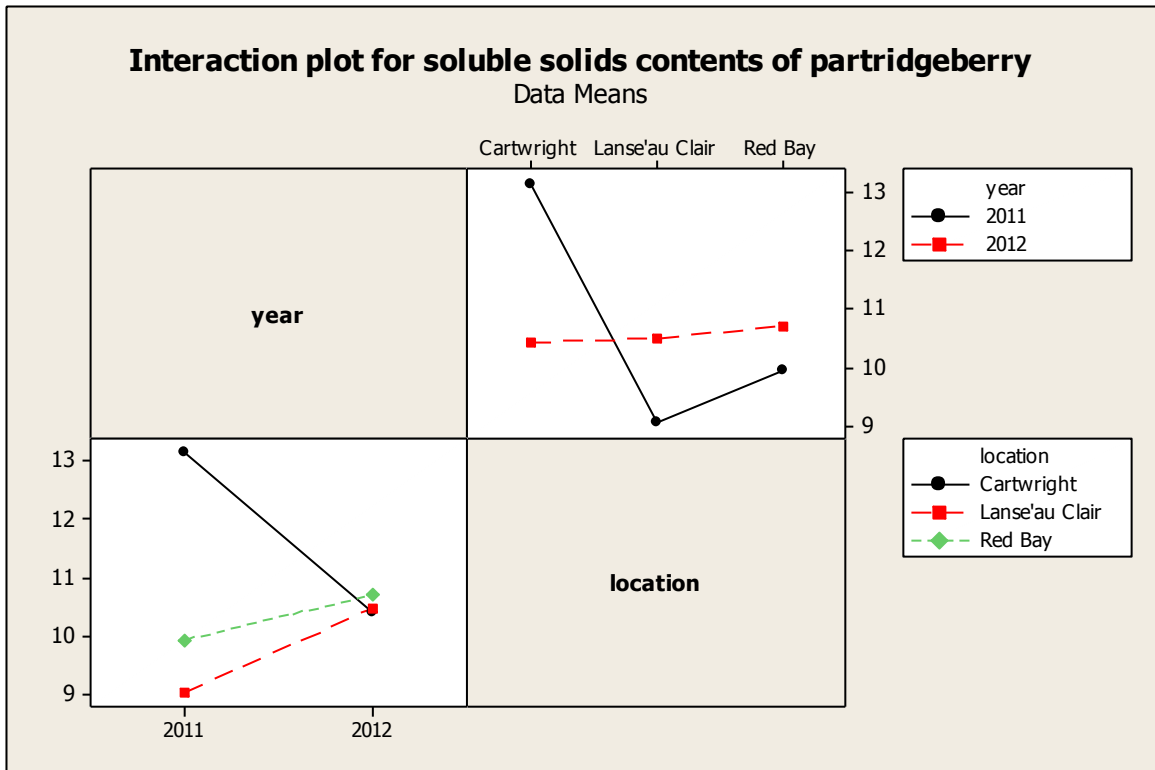
In terms of soluble solids contents of bakeapple, only the single effect of growing season was significant. The concentrations of soluble solids contents of bakeapple at all three locations were significantly higher ( $P<0.05$ ) in 2011 than 2012, 9% and 8%, respectively. The interaction between growing seasons and locations was not significant (Table 5.6).



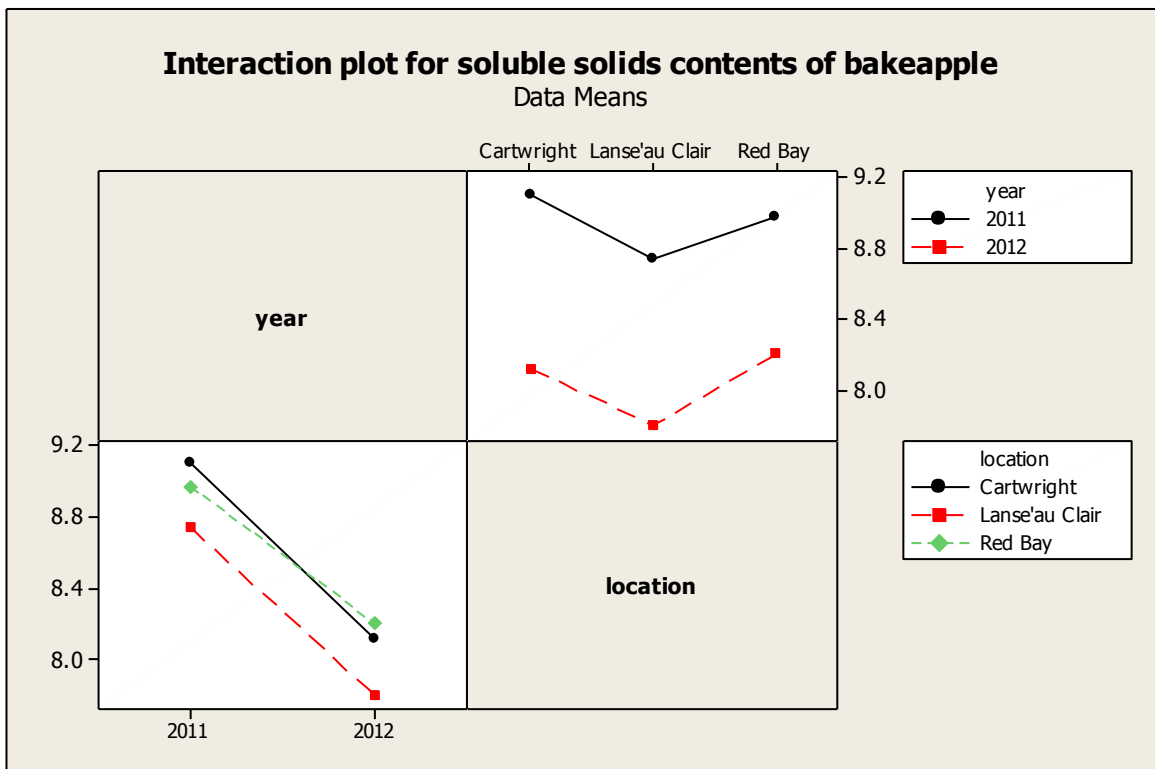
**Figure 5.5 Soluble solids content (%) in partridgeberry juice (Bars represent standard error)**



**Figure 5.6 Soluble solids content (%) in bakeapple juice (Bars represent standard error)**



**Figure 5.7** Interaction plot for soluble solids content in partridgeberry juice



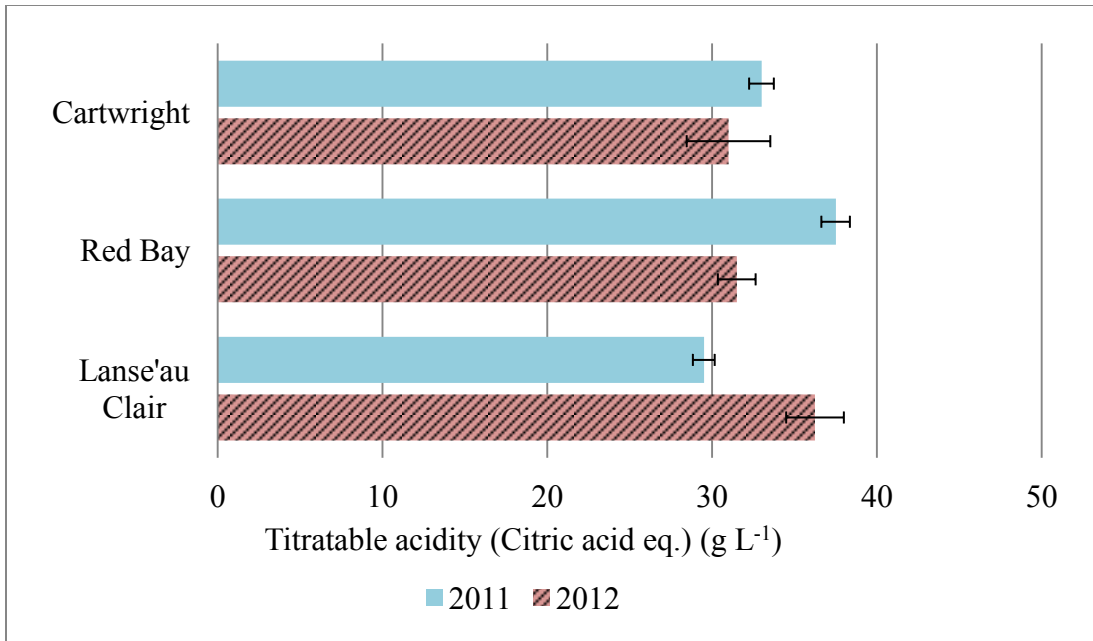
**Figure 5.8** Interaction plot for soluble solids content in bakeapple juice

### 5.6.2 TITRATABLE ACIDITY

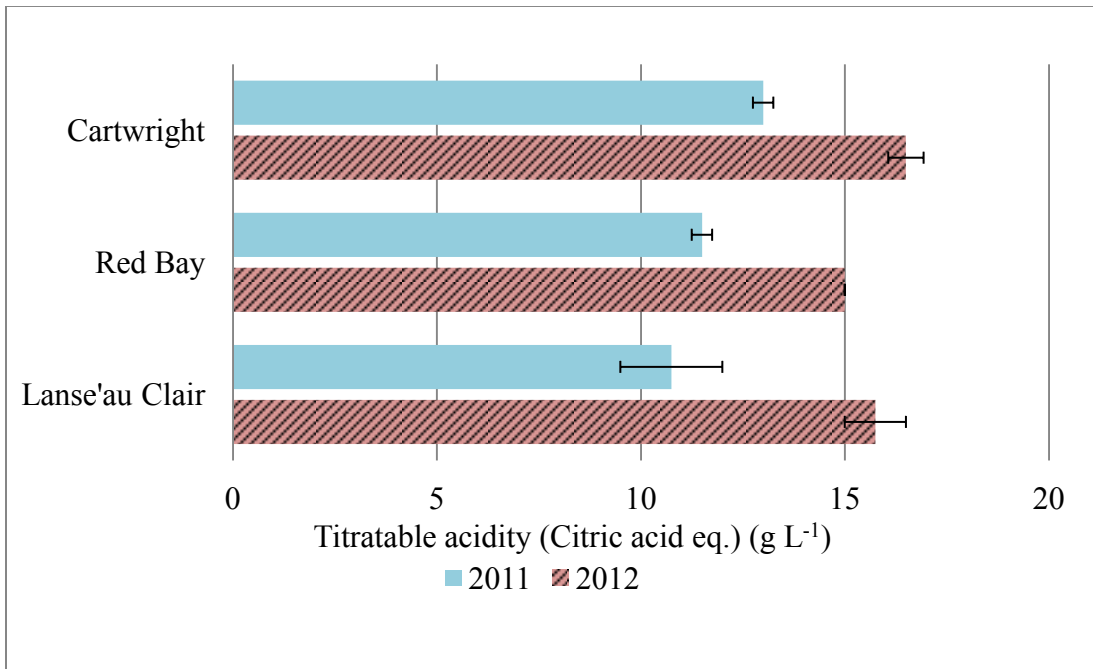
The titratable acidity in partridgeberry was 30 to 35 g L<sup>-1</sup> (Citric acid equivalents) depending on year and location (Figure 5.9). In bakeapple, the highest titratable acidity was observed in fruits from Cartwright (~15 g L<sup>-1</sup>), as compared to fruits collected at Red Bay and Lanse'au Clair (~12.5 g L<sup>-1</sup>) (Figure 5.10).

Although there were no significant single effects of location and the growing season on titratable acidity in partridgeberry, the interaction effect was significant (Table 5.5). Fruits collected in Red Bay in 2011 were observed the highest (P<0.05) for titratable acidity whereas in 2012 fruits from Lanse'au Clair were the highest (Figure 5.9).

There was no interaction effect observed for titratable acidity in bakeapple, but both single effects of growing season and location were significant (Table 5.6; Figure 5.10). Titratable acidity of bakeapple in 2012 was higher than in 2011 (~8 g L<sup>-1</sup> higher), and fruits picked from Cartwright had the highest titratable acidity of 15 g L<sup>-1</sup> compared to other two locations in both years (Table 5.6 and Figure 5.10).



**Figure 5.9 Titratable acidity (g L<sup>-1</sup>) in partridgeberry juice (Bars represent standard error)**



**Figure 5.10 Titratable acidity (g L<sup>-1</sup>) in bakeapple juice (Bars represent standard error)**

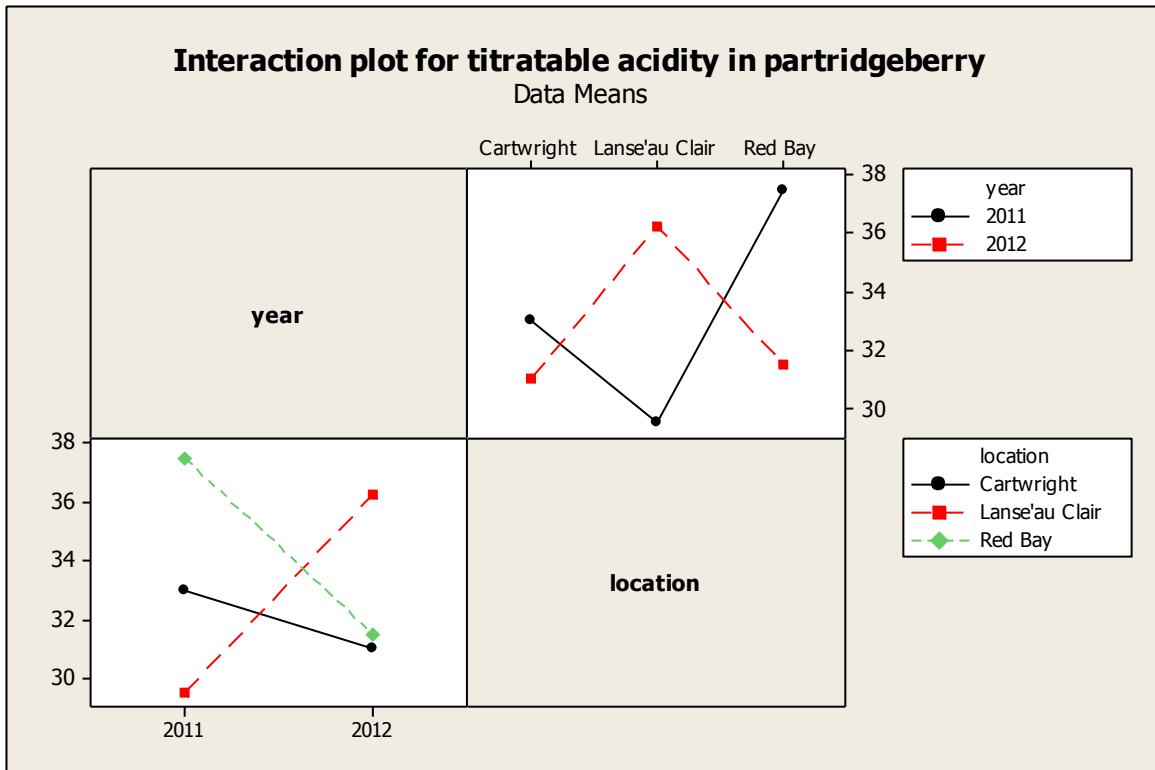


Figure 5.11 Interaction plot for titratable acidity in partridgeberry juice

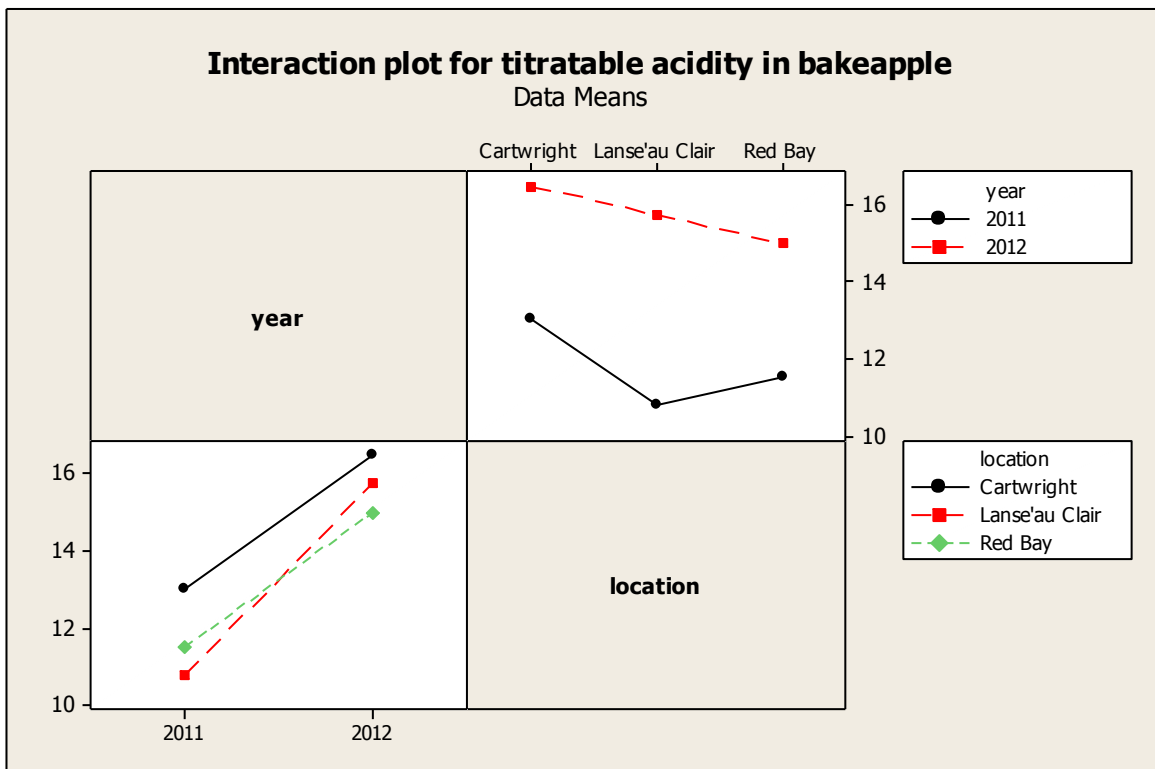


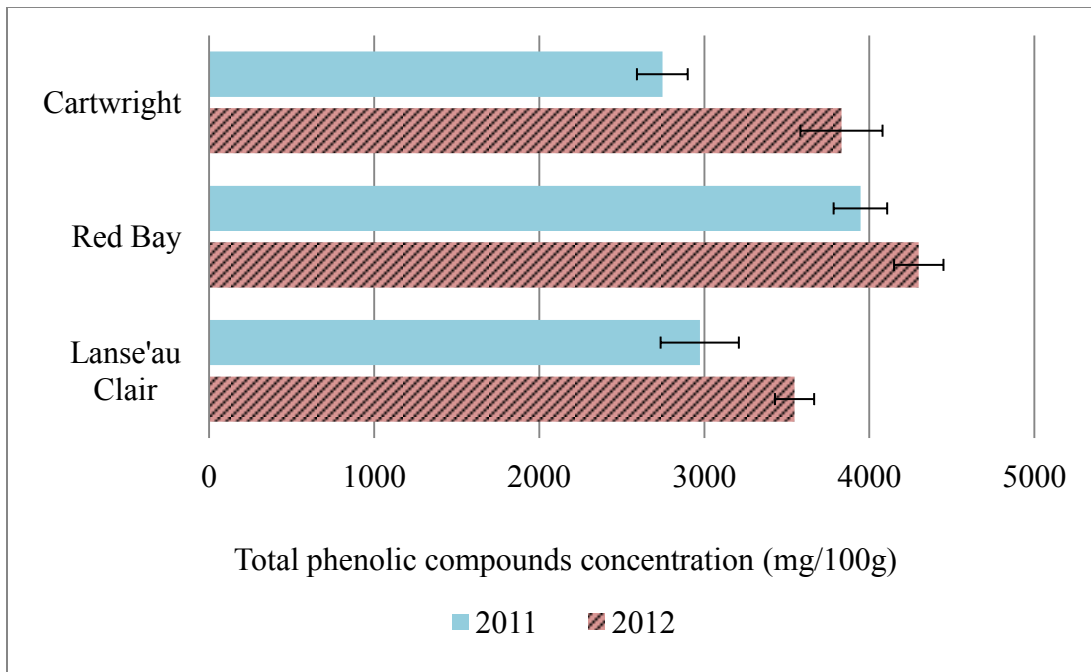
Figure 5.12 Interaction plot for titratable acidity in bakeapple juice

### 5.6.3 TOTAL PHENOLIC COMPOUNDS

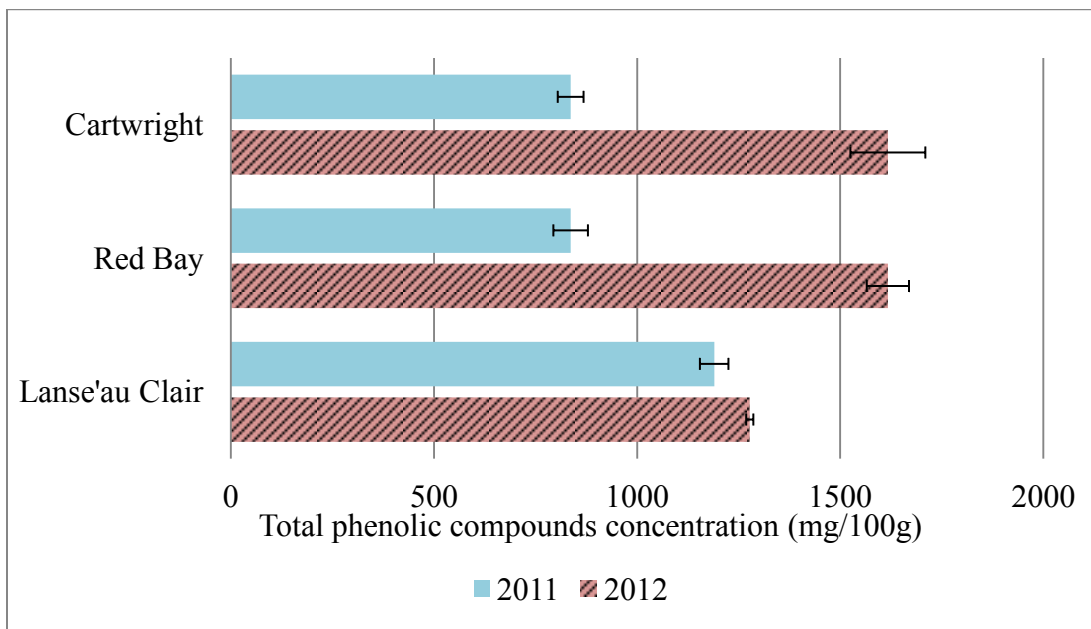
The fruit of both partridgeberry and bakeapple contained the highest ( $P < 0.05$ ) amount of phenolic compounds at the Red Bay location (Figures 5.13 and 5.14). Approximately 4,000 mg/100g fruit was determined at this location compared with 3000 mg/100g in fruit collected at Cartwright and Lanse'au Clair.

Although in partridgeberry the total phenolics content was affected by both the location and the growing season, these were only single effects and no interaction was observed (Table 5.5, Figure 5.13). Fruits harvested in 2012 contained significantly ( $P < 0.05$ ) more total phenolics compared to berries from 2011 (Table 5.5, Figure 5.13).

With regards to bakeapple, the interaction between the growing season and the location was significant (Table 5.6; Figure 5.14). In 2011, berries from Cartwright and Red Bay contained significantly less total phenolics than berries from Lanse'au Clair. In 2012, on the contrary, berries picked at Cartwright and Red Bay contained significantly more total phenolics than fruits collected at Lanse'au Clair, approximately 1500 mg/100g of fruit compared to 1250 mg/100 g in berries from Lanse'au Clair (Figure 5.14). When superimposing the effect of the growing season on the location from where the berries were collected, the amount of total phenolics in bakeapple was affected by both year and the location (Table 5.6 and Figure 5.14). Fruits collected from all three locations contained higher total phenolics in 2012 than in 2011 (Figure 5.14).

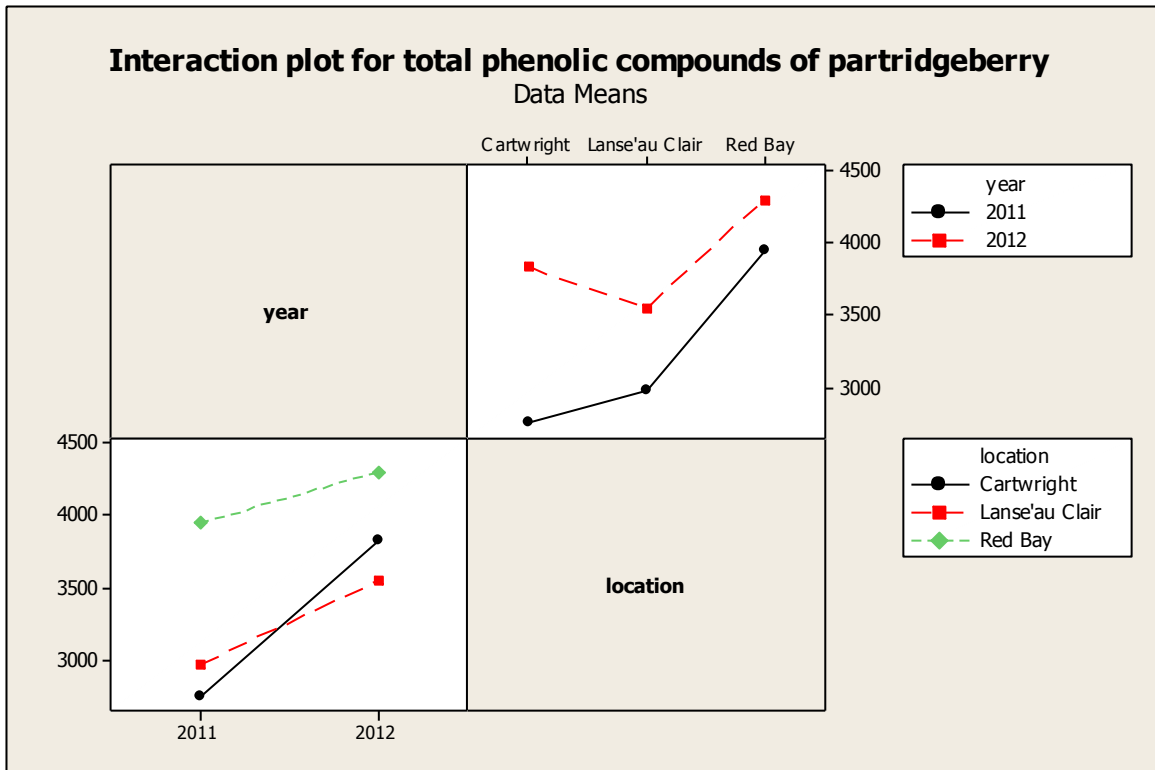


**Figure 5.13 Total phenolic compound content (mg/100g) in partridgeberry juice (Bars represent standard error)**

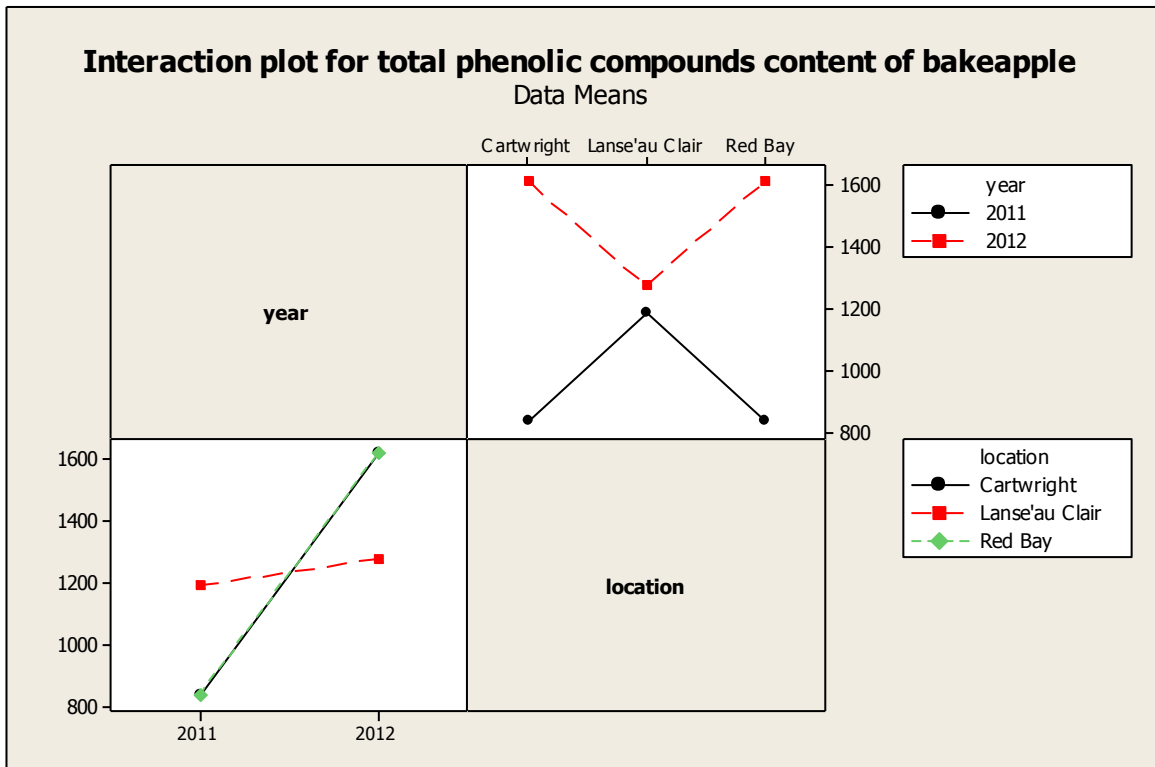


**Figure 5.14 Total phenolic compound content (mg/100g) in bakeapple juice (Bars represent standard error)**





**Figure 5.15** Interaction plot for total phenolic compound content in partridgeberry juice



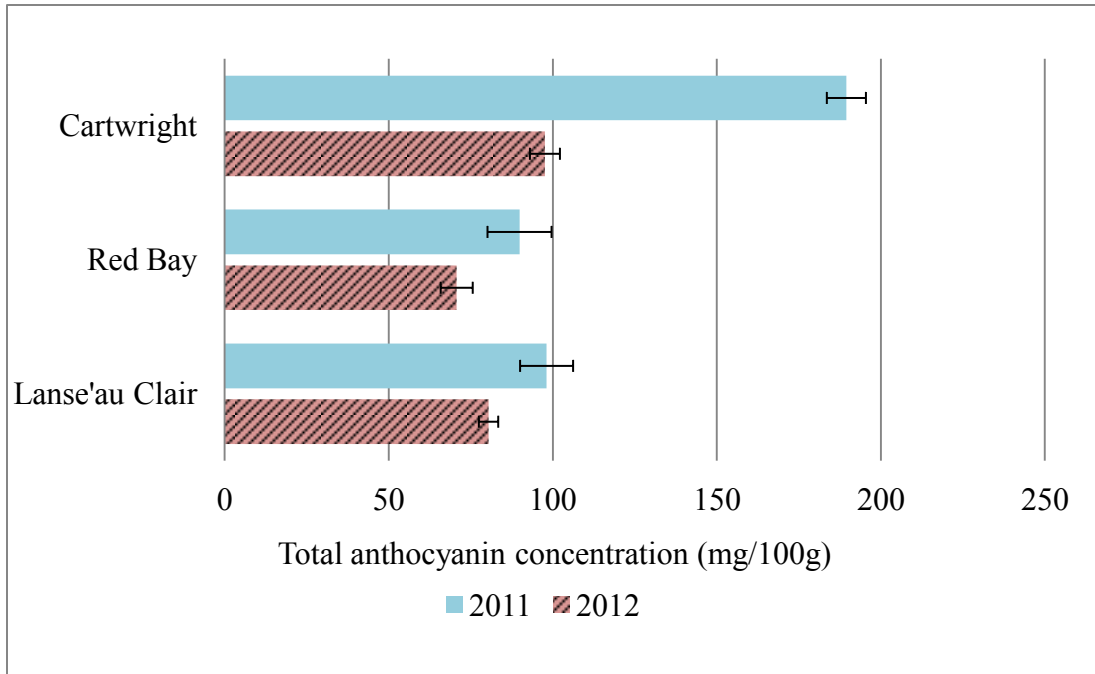
**Figure 5.16** Interaction plot for total phenolic compound content in bakeapple juice

#### **5.6.4 TOTAL ANTHOCYANIN**

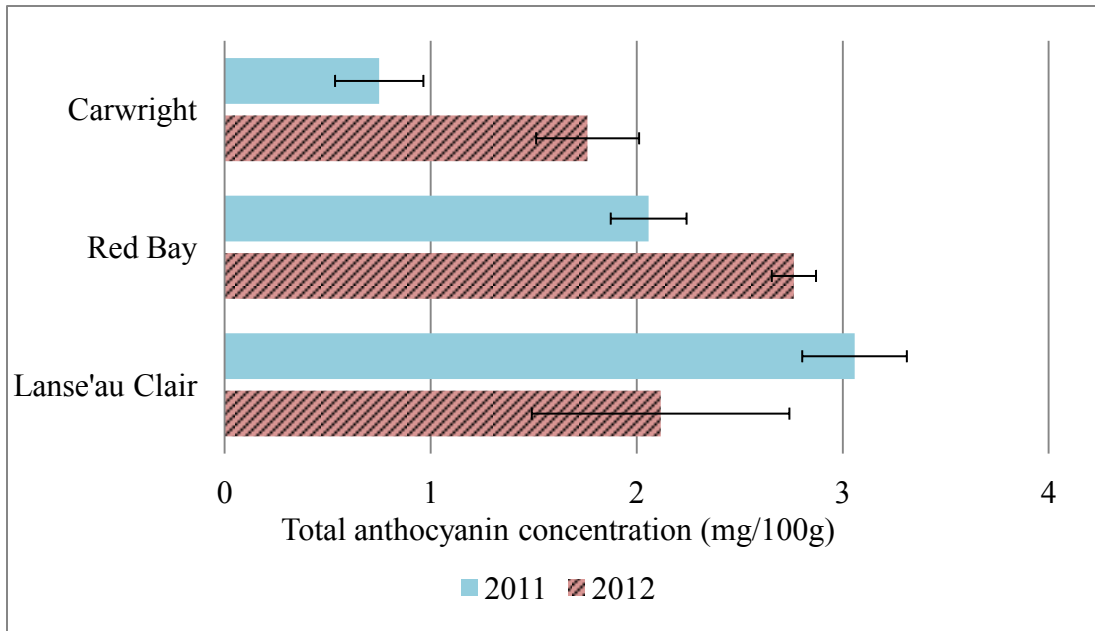
The total anthocyanin concentrations of partridgeberry were significantly ( $P < 0.05$ ) higher in 2011 than 2012 (Table 5.5 and Figure 5.17). The interaction effect of the location and the growing season on total anthocyanin concentrations of partridgeberry was significant ( $P < 0.05$ ) (Table 5.5) so were the single factors. Partridgeberry fruits samples picked in 2011 from Cartwright contained the highest ( $P < 0.05$ ) (~190 mg/100g) amount of total anthocyanins. The remaining of samples contained from 70 mg/100g to 100 mg/100g. The lowest (~80 mg/100g) anthocyanin concentrations were observed in partridgeberries picked from Red Bay during 2011 and 2012 (Figure 5.17). The interaction plot for anthocyanin of partridgeberry (Figure 5.19) indicates the effect of location followed roughly the same pattern for growing season. From 2011 to 2012, berries from Lanse'au Clair and Red Bay only show slight decrease, while fruits picked at Cartwright had significantly ( $P < 0.05$ ) lower content.

In bakeapple, the highest (~2.7 mg/100g) total anthocyanin concentrations was observed in fruits picked at Lanse'au Clair as compared to Red Bay (~2.5 mg/100g) and Cartwright (~1.2 mg/100g) (Figure 5.18).

The interaction effect on total anthocyanin concentrations of bakeapple between the location and the growing season was significant ( $P < 0.05$ ) (Table 5.6). Overall, the 2011 growing season was better for the anthocyanin content in fruit picked from Lanse'au Clair location, whereas 2012 was better for the fruit from other two locations (Figure 5.18).



**Figure 5.17 Total anthocyanins content (mg/100g) in partridgeberry (Bars represent standard error)**



**Figure 5.18 Total anthocyanins content (mg/100g) in bakeapple (Bars represent standard error)**

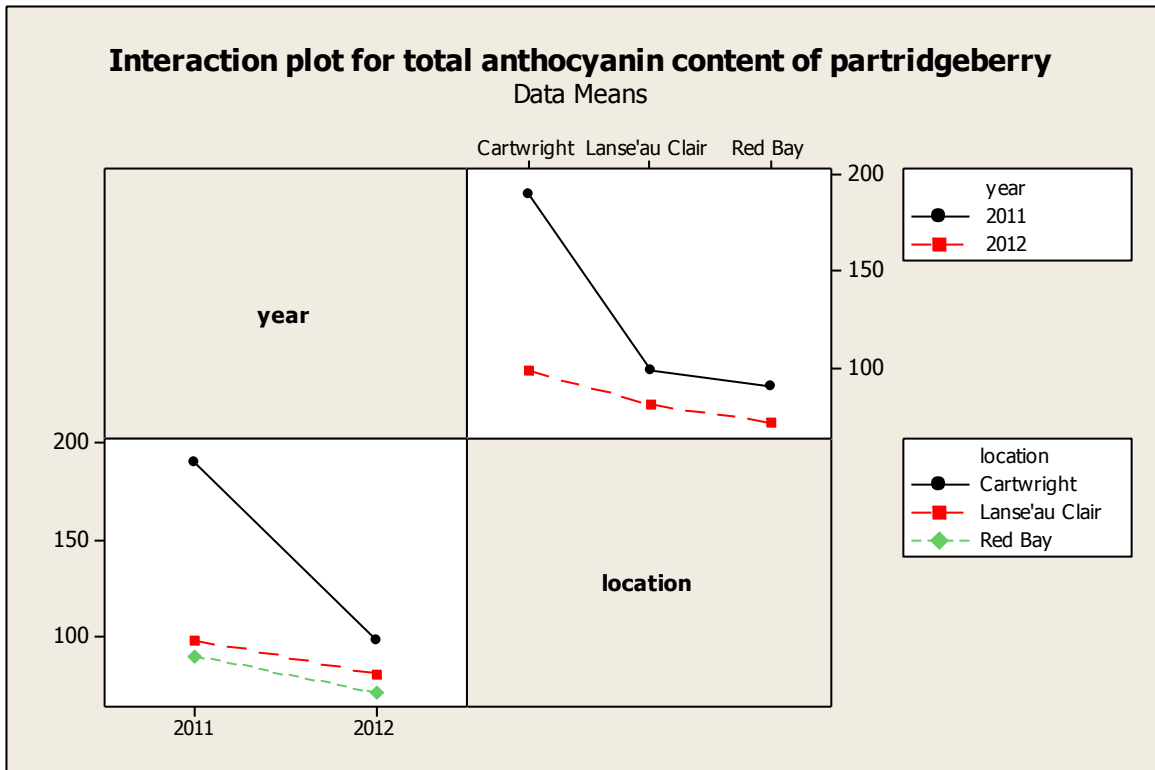


Figure 5.19 Interaction plot for total anthocyanin content in partridgeberry

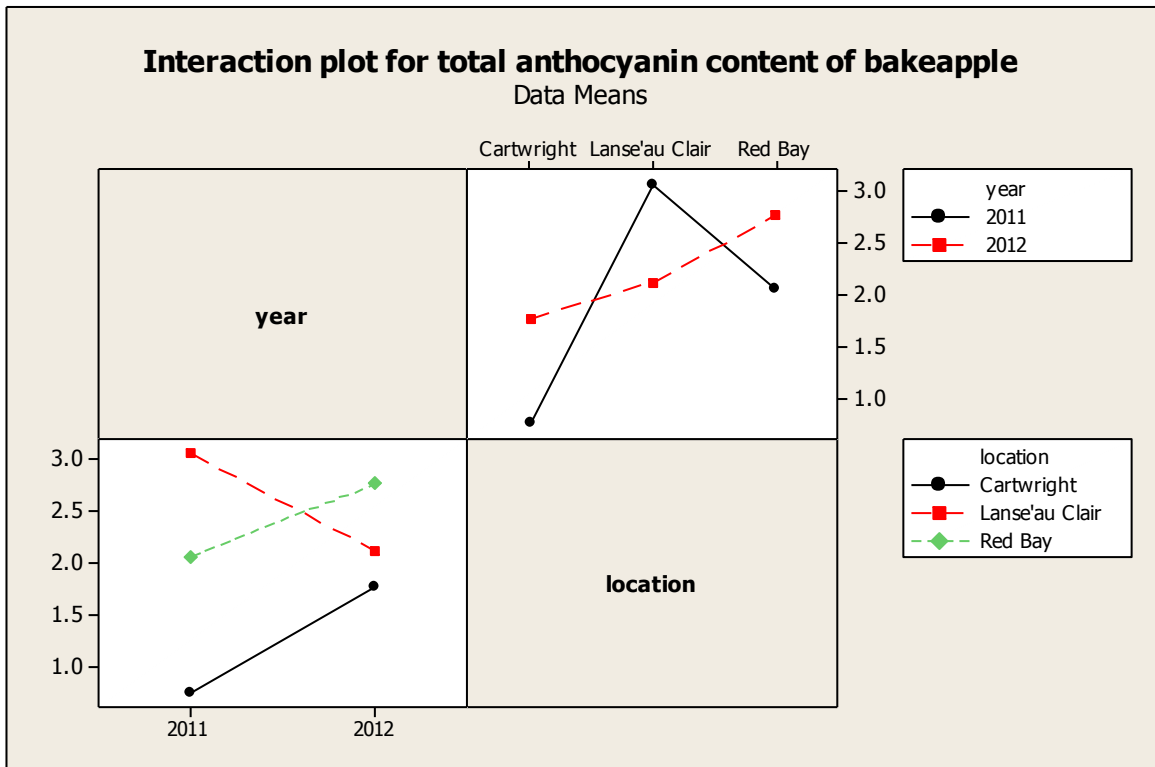
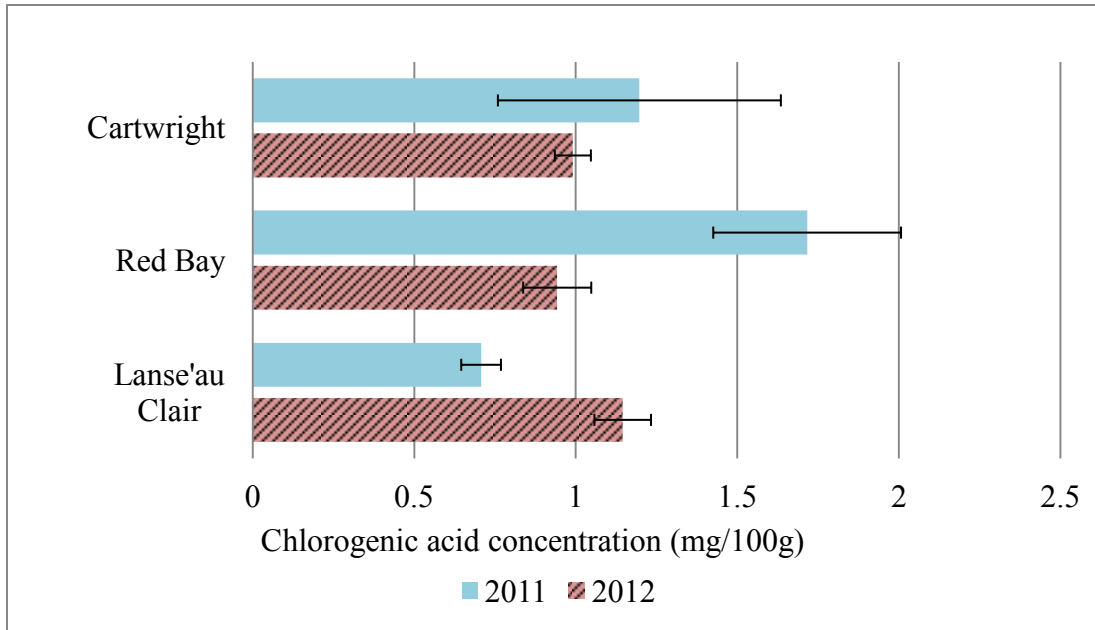


Figure 5.20 Interaction plot for total anthocyanin content in bakeapple

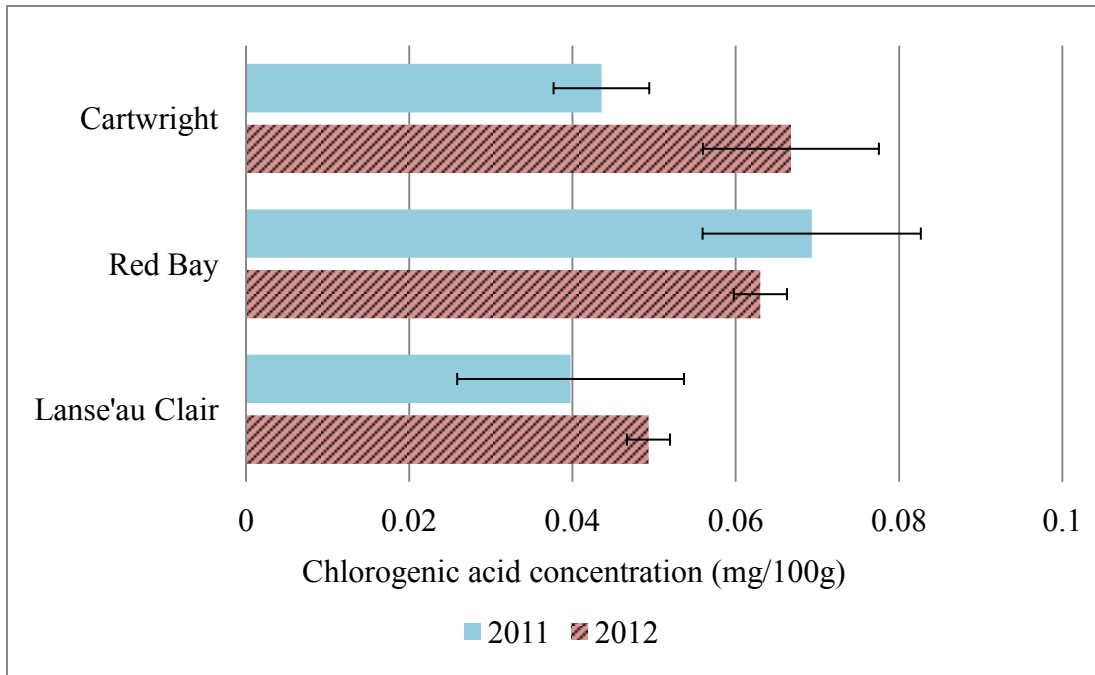
### **5.6.5 CHLOROGENIC ACID**

There was a weak interaction ( $P < 0.058$ ) effect of the location and the growing season that affected the amounts of chlorogenic acid in partridgeberries (Table 5.5; Figure 5.23). The growth season and the location effects were not significant. The highest amount of chlorogenic acid was observed in fruits of partridgeberry (~1.25 mg/100g) and bakeapple (~0.06 mg/100g) collected from Red Bay as compared to berries from Lanse'au Clair and Cartwright (Figure 5.21 and 5.22). In case of partridgeberry, average chlorogenic acid level in fruits from Lanse'au Clair and Cartwright was approximately 1 mg/100g (Figure 5.22).

In bakeapple, chlorogenic acid level in fruits from Cartwright (~0.05 mg/100g) was slightly higher than Lanse'au Clair (~0.04 mg/100g) (Figure 5.22). Fruit from Red Bay contained the highest amount of chlorogenic acid, although the differences were not significant (Figure 5.22).



**Figure 5.21 Chlorogenic acid level (mg/100g) in partridgeberry (Bars represent standard error)**



**Figure 5.22 Chlorogenic acid level (mg/100g) in bakeapple (Bars represent standard error)**

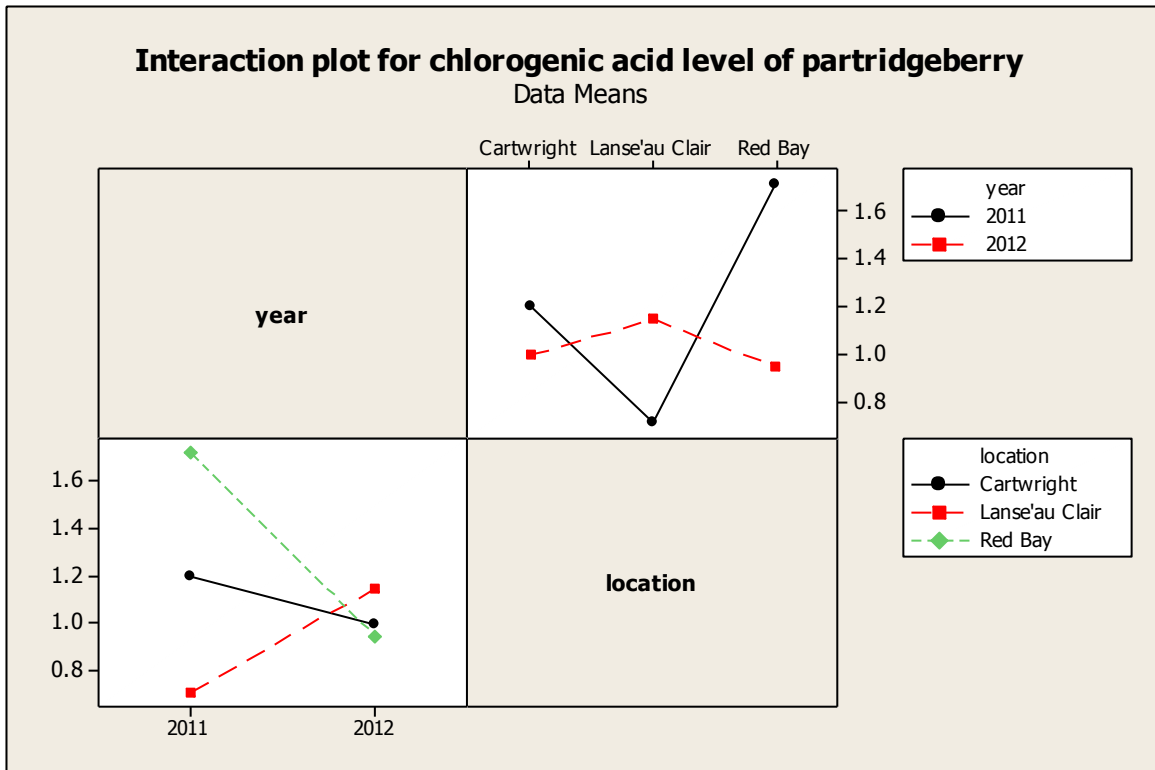


Figure 5.23 Interaction plot for chlorogenic acid level in partridgeberry

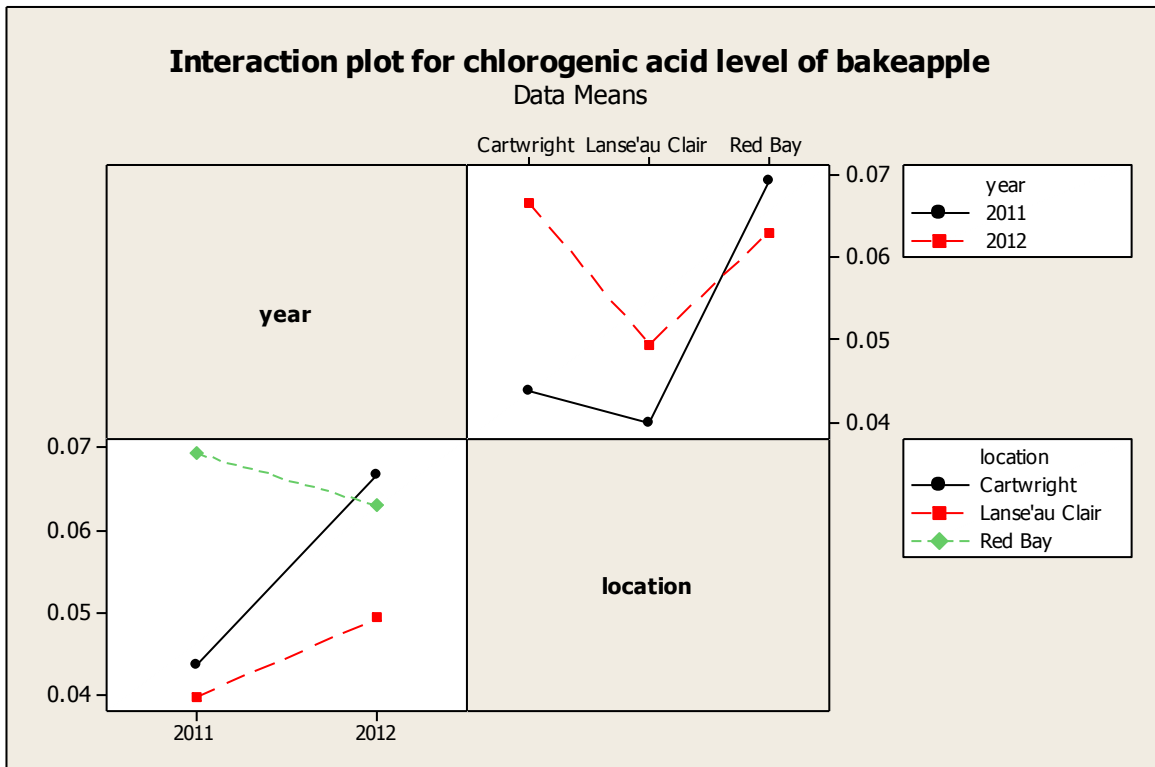


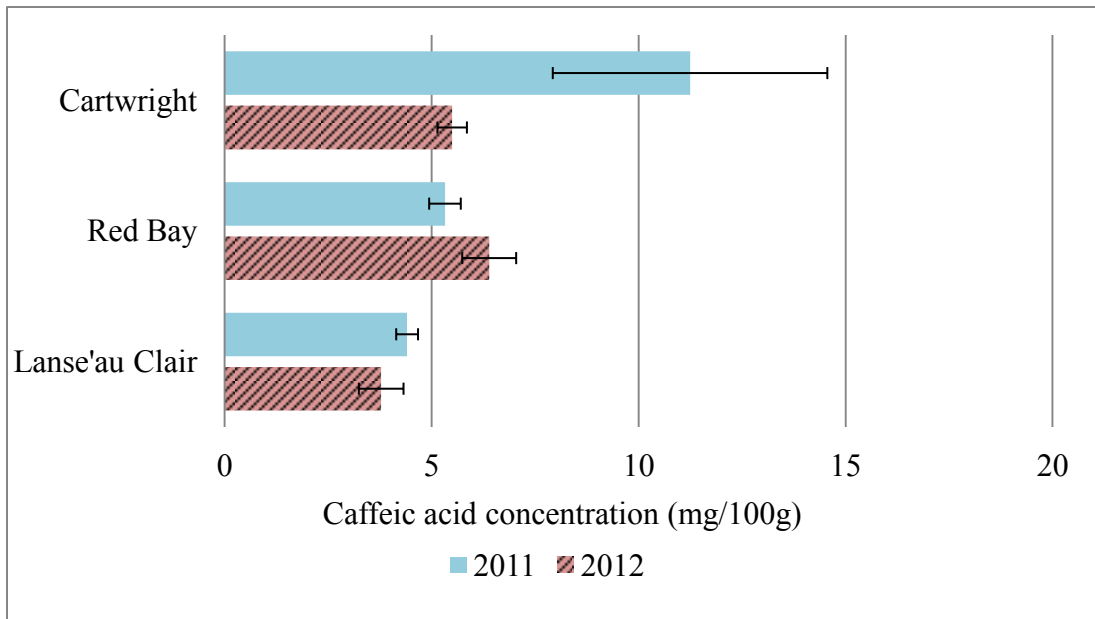
Figure 5.24 Interaction plot for chlorogenic acid level in bakeapple

### **5.6.6 CAFFEIC ACID**

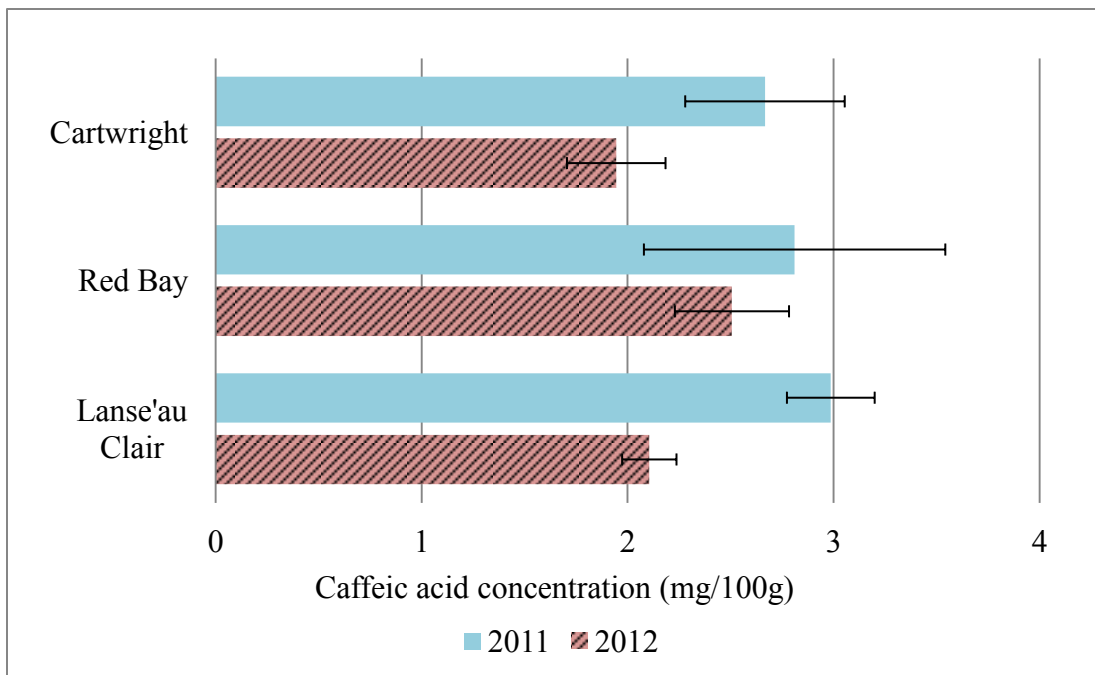
Only the location played a significant role in caffeic acid content of partridgeberries (Table 5.5; Figure 5.25). The highest (11 mg/100g) caffeic acid in partridgeberry was observed in samples picked at Cartwright in 2011 as compared to Lanse'au Clair (~4 mg/100g) and Red Bay (~6 mg/100g) (Figure 5.25).

There were no significant differences observed in caffeic acid content in bakeapple berries collected from all three locations in both seasons (Table 5.6; Figure 5.26). Caffeic acid levels in bakeapple picked at Red Bay and Lanse'au Clair were similar (~2.5 mg/100g) to each other. They were slightly higher than in fruits picked from Cartwright (~2.2 mg/100g) (Figure 5.26).





**Figure 5.25 Caffeic acid level (mg/100g) in partridgeberry (Bars represent standard error)**



**Figure 5.26 Caffeic acid level (mg/100g) in bakeapple (Bars represent standard error)**

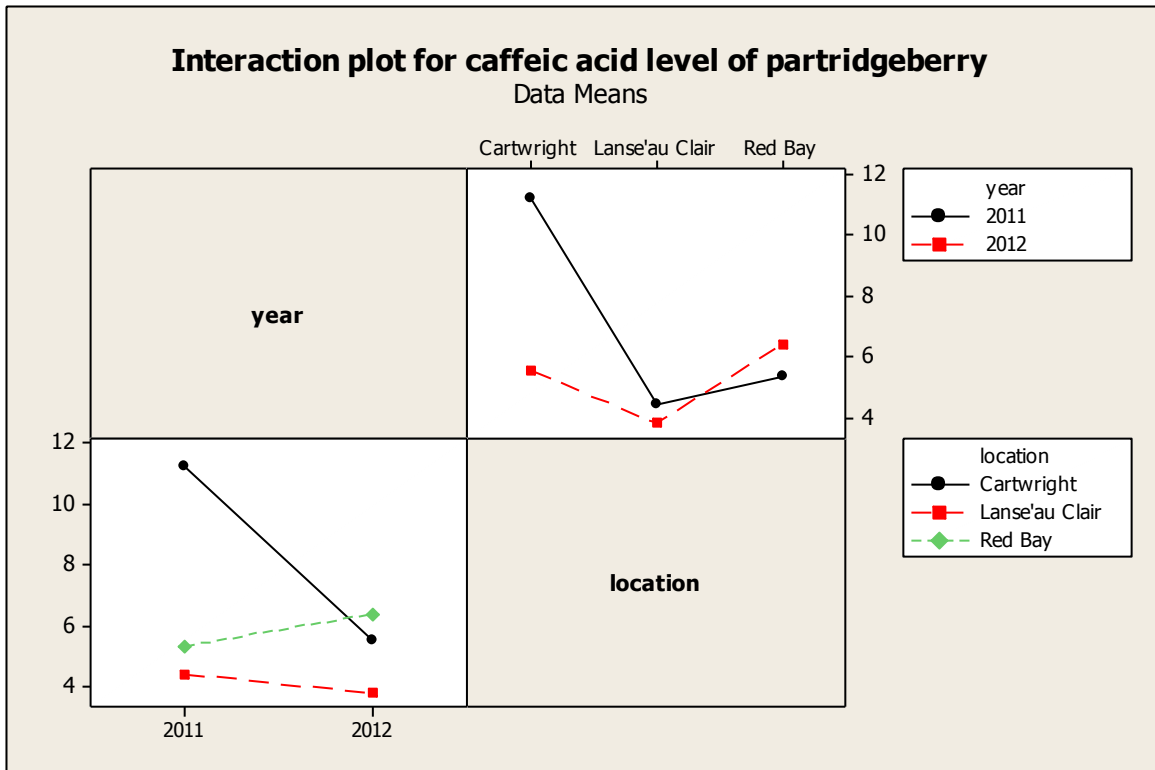


Figure 5.27 Interaction plot for caffeic acid level in partridgeberry

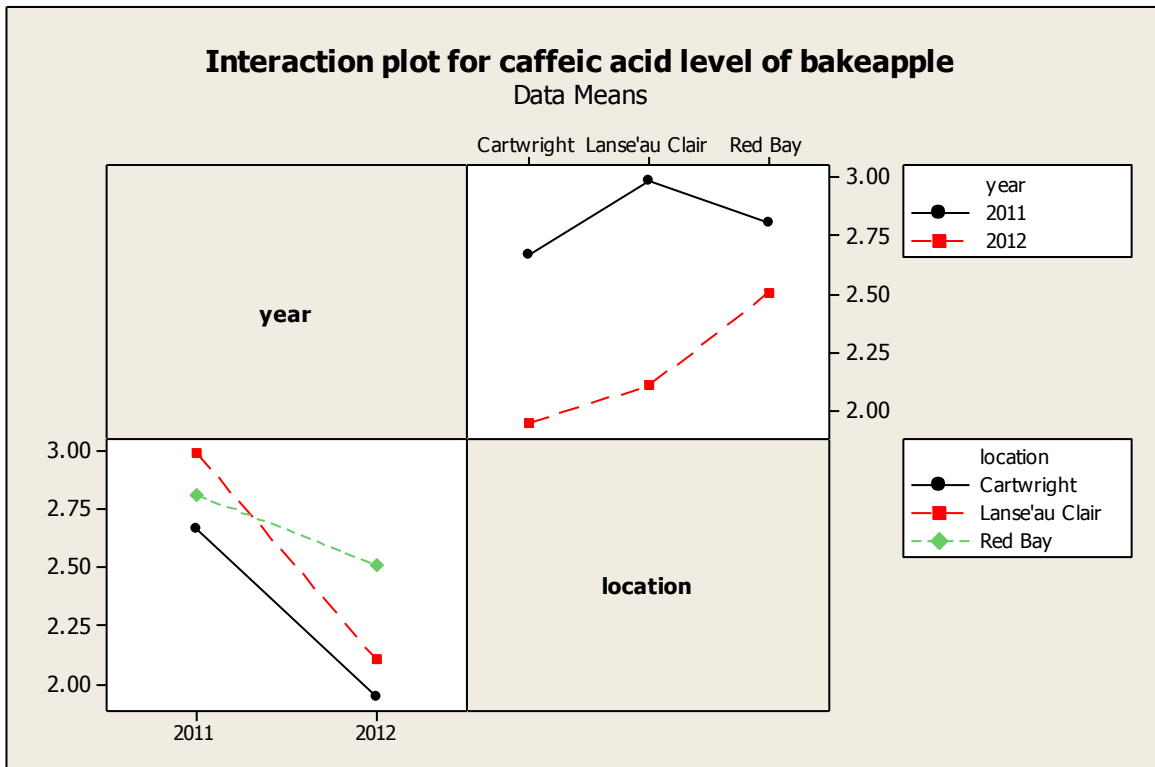
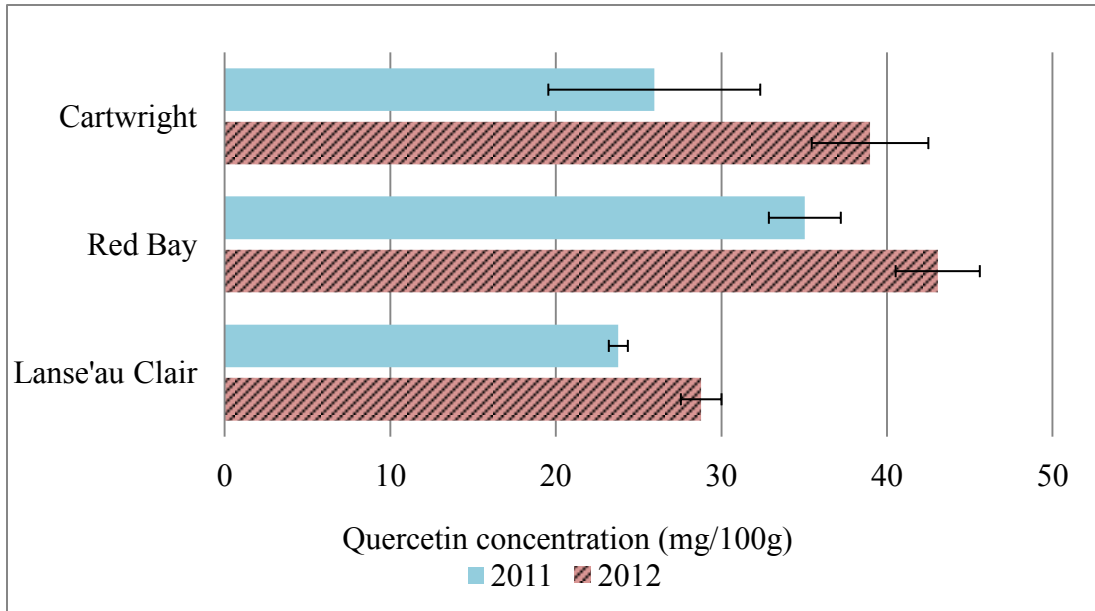


Figure 5.28 Interaction plot for caffeic acid level in bakeapple

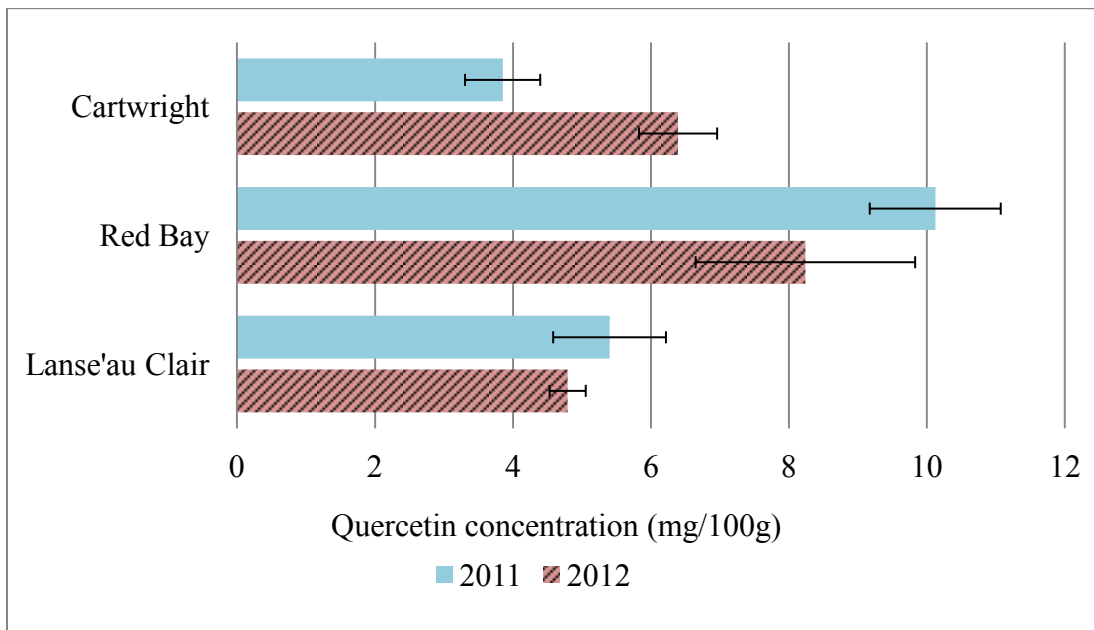
### 5.6.7 QUERCETIN

The location from where the fruit was picked played a significant role in the amount of quercetin 3-O- $\beta$ -D-glucoside content of both partridgeberries and bakeapple (Tables 5.5 and 5.6; Figures 5.29 and 5.30). The fruit of both partridgeberry and bakeapple contained the highest (~40 mg/100g) amount quercetin from Red Bay location compared to partridgeberry fruits collected from Lanse'au Clair (~25 mg/100g) and Cartwright (~30 mg/100g) (Figure 5.31). The range of quercetin concentration in bakeapple was from 4 to 10 mg/100g. Samples from Cartwright and Lanse'au Clair contained lowest amount of quercetin, (average 5 mg/100g) for both years, while the average quercetin of fruits from Red Bay was up to 9 mg/100g (Figure 5.29).

When considering the effect of the growing season on the location of fruit collection, the interaction between growing season and location was not significant while two single factors, growing season and location significantly ( $P < 0.05$ ) affected quercetin level of partridgeberry (Table 5.5). The quercetin levels of partridgeberry picked in 2012 were significantly higher than 2011 (Figure 5.29, Table 5.5). The location of picking bakeapple fruits was the major factor of quercetin level (Table 5.6). Quercetin level of bakeapple picked at Red Bay was the highest (10mg/100g of berries) in both years, followed as Lanse'au Clair then Cartwright (Figure 5.30 and Table 5.6).



**Figure 5.29 Quercetin level (mg/100g) in partridgeberry (Bars represent standard error)**



**Figure 5.30 Quercetin level (mg/100g) in bakeapple (Bars represent standard error)**

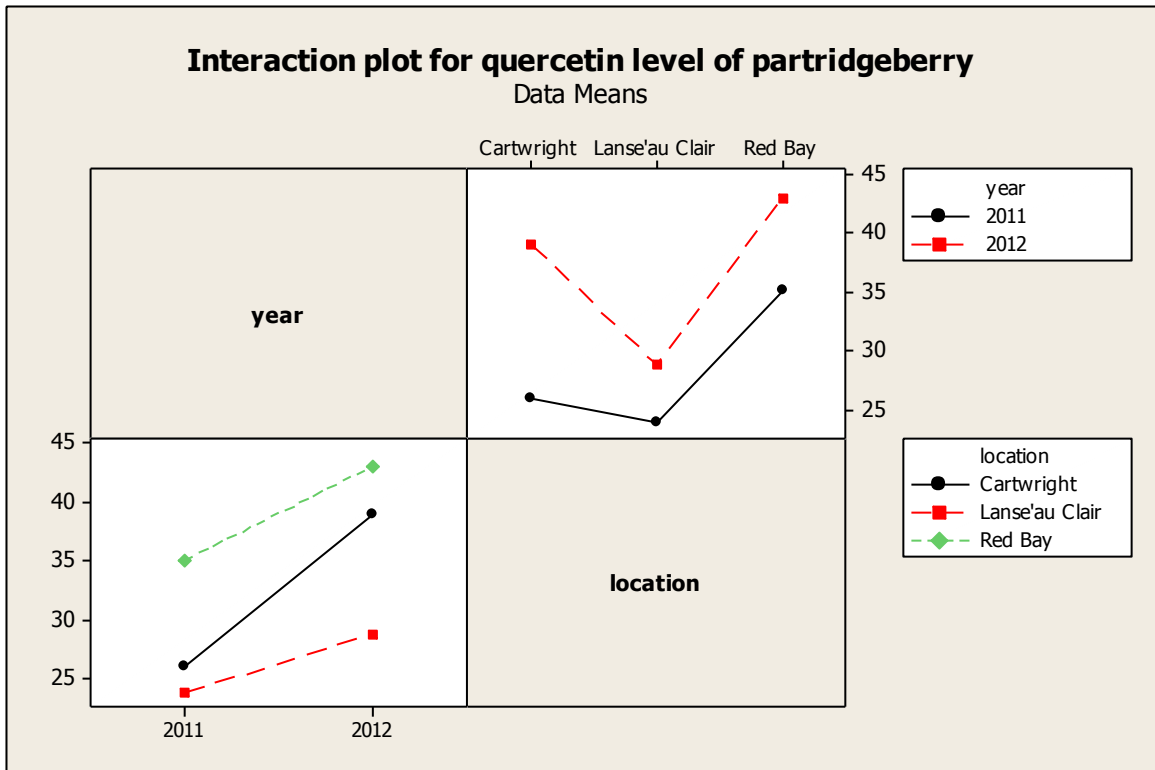


Figure 5.31 Interaction plot for quercetin level in partridgeberry

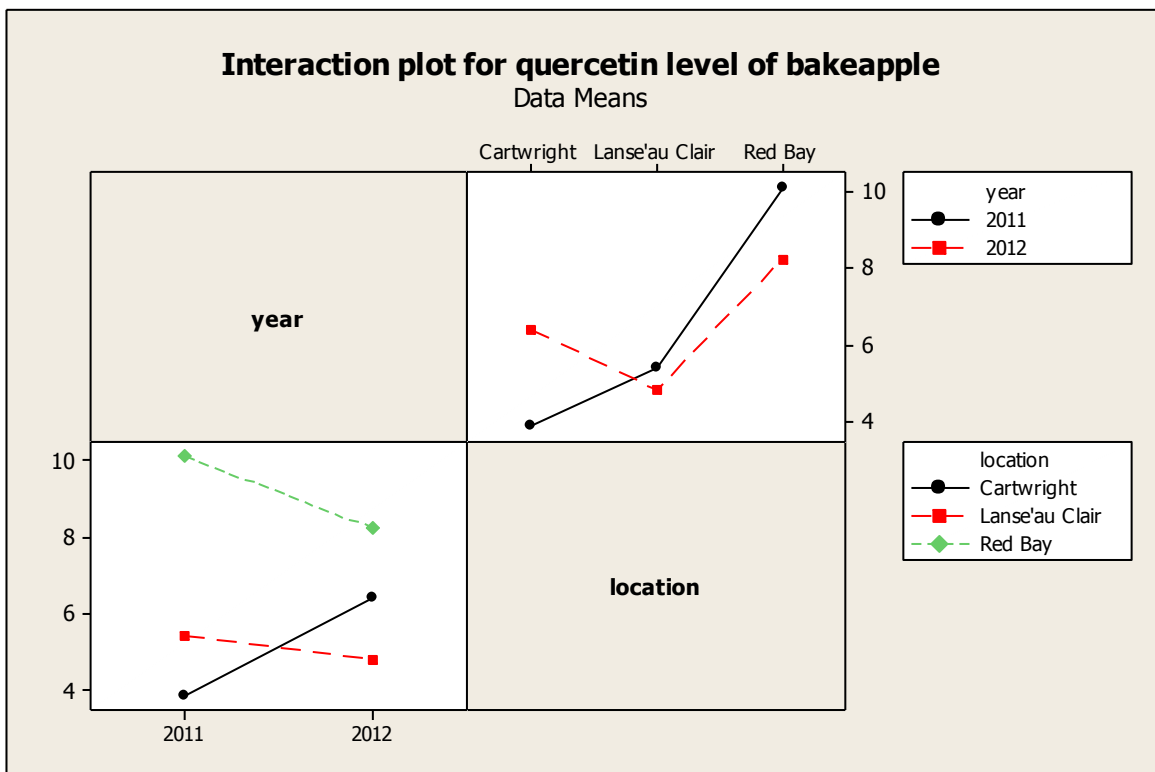


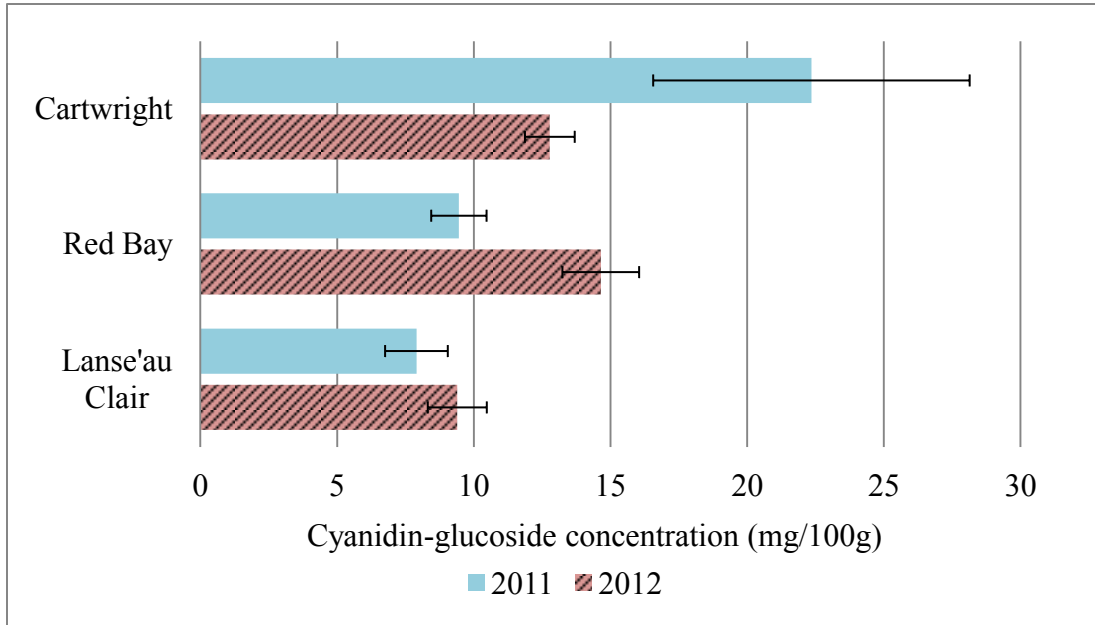
Figure 5.32 Interaction plot for quercetin level in bakeapple

### **5.6.8 CYANIDIN-GLUCOSIDE**

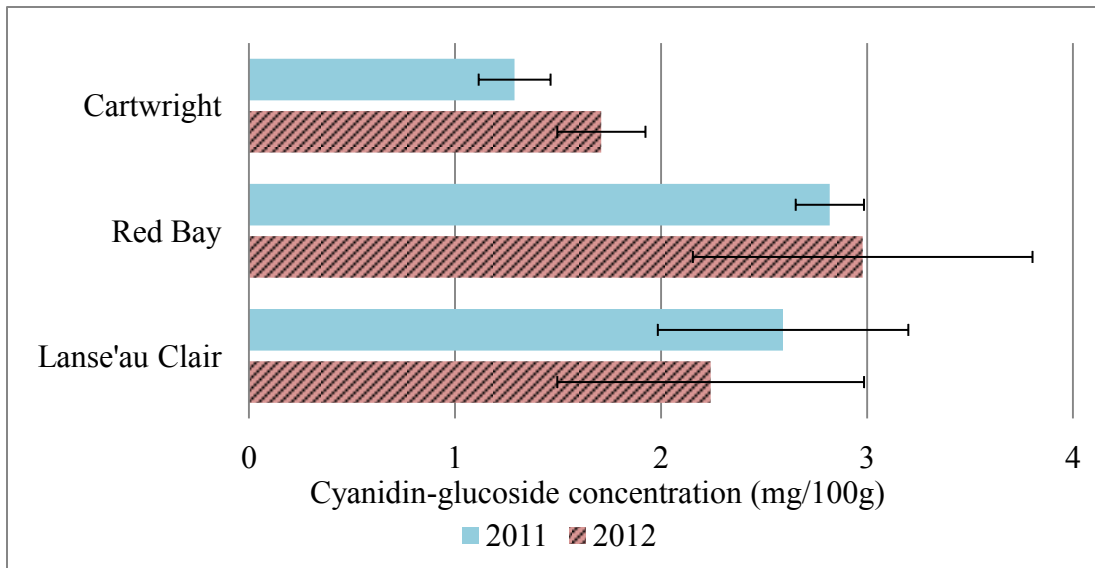
The location and the growing season were together significant factors affecting cyanidin-glucoside content in partridgeberry (Table 5.5; Figure 5.33). Partridgeberry fruits samples picked in 2011 from Cartwright contained the highest (26 mg/100g) amount of cyanidin-glucoside amounts. The cyanidin-glucoside levels in samples from other locations were from 9 mg/100g to 15 mg/100g. The lowest cyanidin-glucoside levels were observed in berries picked at Lanse'au Clair (9 mg/100g) in both years (Figure 5.33).

With regards to bakeapple, the highest (~3.0 mg/100g) cyanidin-glucoside levels of samples were fruits picked at Red Bay as compared to Lanse'au Clair (~2.2 mg/100g) and Cartwright (~1.5 mg/100g) (Figure 5.34). The interaction between location and the season was not significant (Table 5.6).

When superimposing the effect of the growing season on the location from where the fruits were collected, the interaction between location and growing season was a major factor ( $P < 0.05$ ) affecting cyanidin-glucoside level in partridgeberry (Table 5.5).



**Figure 5.33 Cyanidin-glucoside level (mg/100g) in partridgeberry (Bars represent standard error)**



**Figure 5.34 Cyanidin-glucoside level (mg/100g) in partridgeberry (Bars represent standard error)**

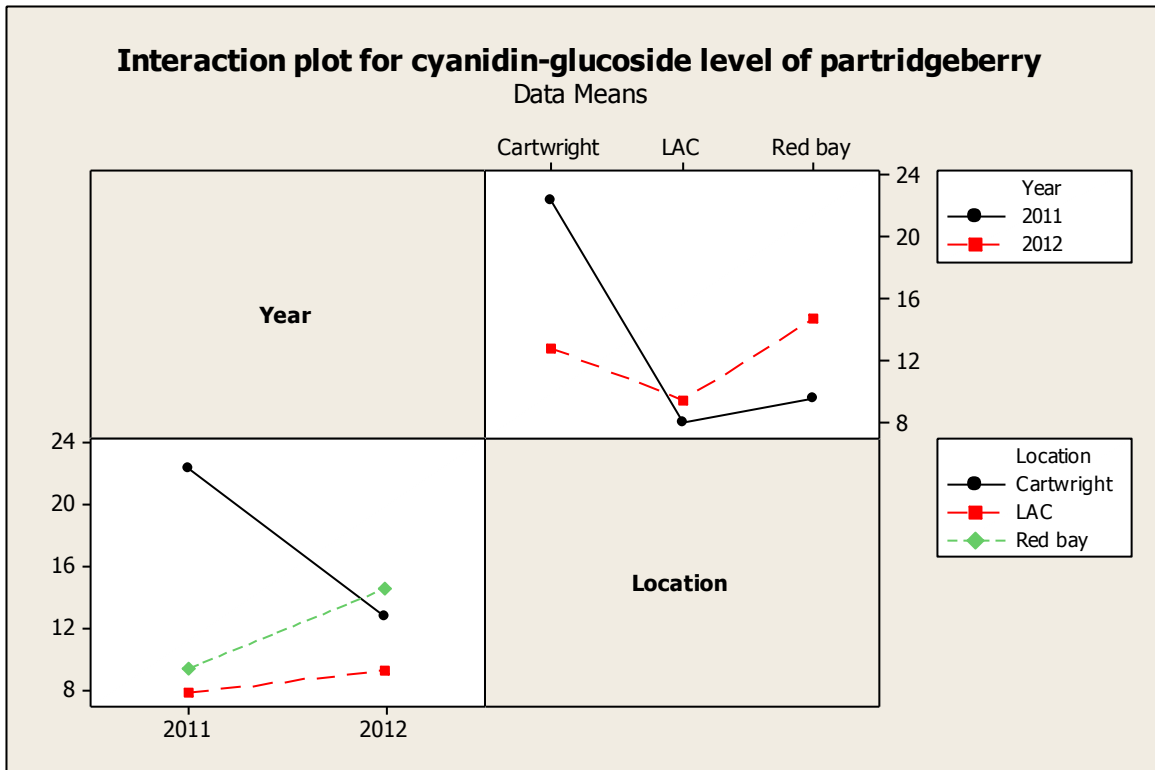


Figure 5.35 Interaction plot for cyanidin-glucoside level of partridgeberry

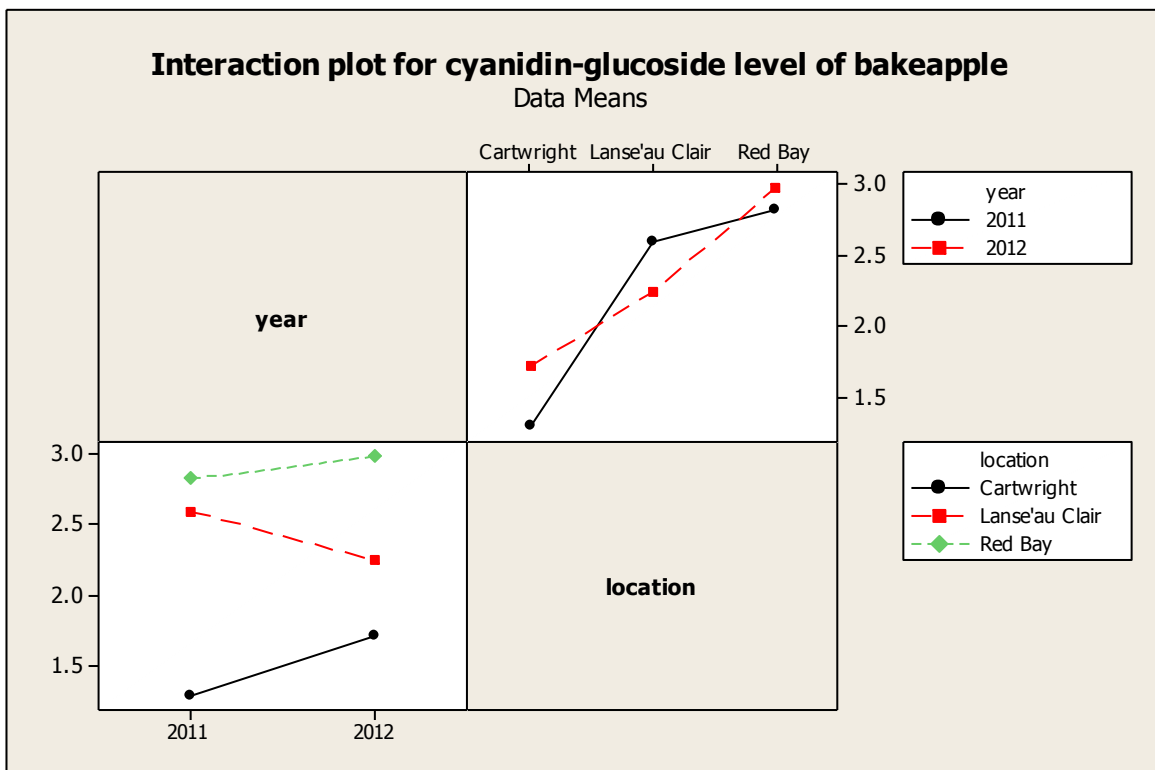


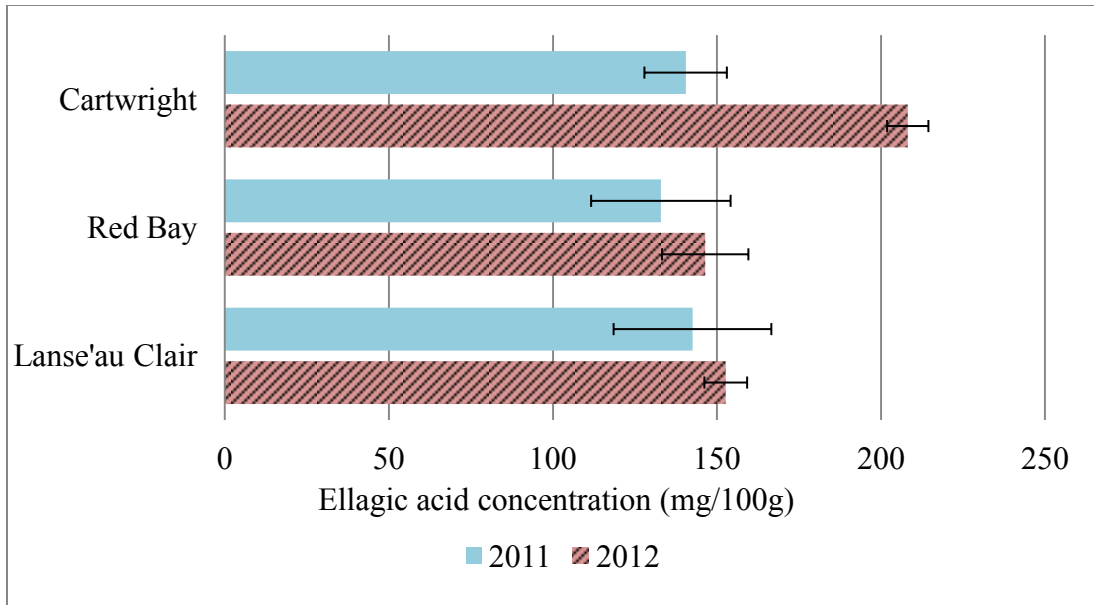
Figure 5.36 Interaction plot for cyanidin-glucoside level in bakeapple



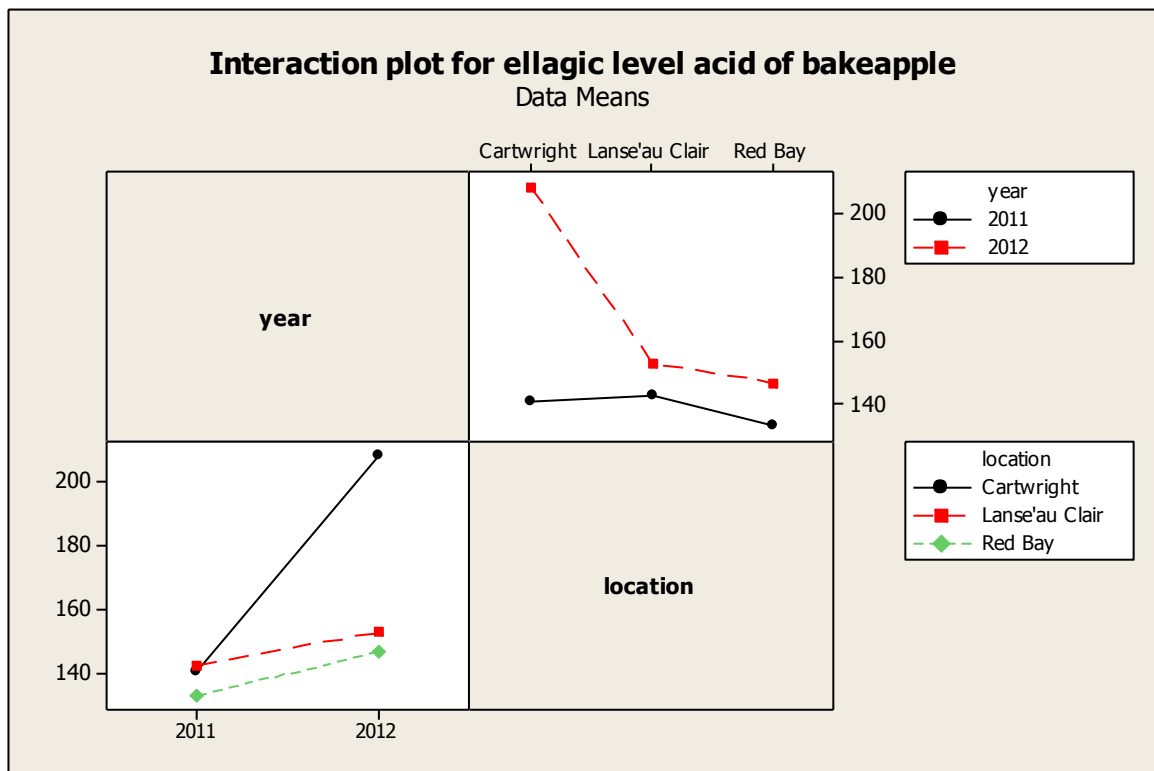
### **5.6.9 ELLAGIC ACID (BAKEAPPLE ONLY)**

Only a single effect of the growing season was significant for the levels of ellagic acid in bakeapple fruit collected from all three locations (Table 5.6; Figure 5.37). The highest concentration of ellagic acid in bakeapple was observed in fruits picked at Cartwright (~160 mg/100g) as compared to samples from other two locations (~150 mg/100g) (Figure 5.37).

When considering the effect of the growing season on the location from where the fruits were picked, the interaction between the growing season and location was not significant. Definitely the growing season was the major factor significantly affecting ellagic acid level in bakeapple fruits, especially when fruit was coming from Cartwright location (Table 5.6).



**Figure 5.37 Ellagic acid level (mg/100g) in partridgeberry (Bars represent standard error)**



**Figure 5.38 Interaction plot for ellagic acid level in bakeapple**

**Table 5.5 P-value for years, locations and interaction effects on nutritional compounds of partridgeberry**

Source of variation	P-values							
	Soluble solids content	Titratable acidity	Total phenols	Total anthocyanin	Chlorogenic acid	Caffeic acid	Quercetin 3-o- $\beta$ -D-glucoside	Cyanidin - 3-o- $\beta$ -glucoside
Years	0.542	0.730	<u>0.001</u>	<u>0.000</u>	0.344	0.152	<u>0.008</u>	0.656
Locations	<u>0.000</u>	0.254	<u>0.001</u>	<u>0.000</u>	0.236	<u>0.032</u>	<u>0.008</u>	<u>0.015</u>
Y*L	<u>0.000</u>	<u>0.003</u>	0.169	<u>0.000</u>	<u>0.058</u>	0.080	0.497	<u>0.036</u>

**Table 5.6 P-value for years, locations and interaction effects on nutritional compounds of bakeapple**

Source of variation	P-values								
	Soluble solids content	Titratable acidity	Total phenols	Total anthocyanin	Chlorogenic acid	Caffeic acid	Quercetin 3-o- $\beta$ -D-glucoside	Cyanidin- 3-o- $\beta$ -glucoside	Ellagic acid
Years	<u>0.000</u>	<u>0.000</u>	<u>0.000</u>	0.347	0.277	0.065	0.981	0.864	<u>0.033</u>
Locations	0.184	<u>0.056</u>	0.989	<u>0.002</u>	0.115	0.653	<u>0.001</u>	0.061	0.105
Years*Locations	0.843	0.426	<u>0.000</u>	<u>0.021</u>	0.332	0.745	0.074	0.767	0.157

## **CHAPTER 6 DISCUSSION**

The hypothesis that the yield and concentrations of selected nutritional compounds in fruits of both partridgeberry and bakeapple differ between the locations from where the fruits are collected (especially with regards to latitude) is supported by the data presented in Chapter 5 (Results). Our data are in agreement with a number of reports describing composition of these two berries (Faragher 1983; Poudel et al. 2009; Steyn et al. 2009; Akerstrom et al. 2010; Martinussen et al. 2010; Zheng et al. 2012). The Apidae family was the major pollinator group for partridgeberry and Syrphidae family for bakeapple (Table 5.2). When bakeapple flowers were in bloom (approximately day 160 Julian date), there were approximately ten to twenty pollinators in each pan, and the population slightly increased when partridgeberry flowers came to bloom (approximately day 200 Julian date).

There is a number of reports on partridgeberry/lingonberry and bakeapple from northern Europe including Finland and Norway (Hakkinen and Torronen 2000; Viljakainen et al. 2002; Greene 2009; Martinussen et al. 2010; Naess and Chagnon 2011; Lashmanova et al. 2012), and on partridgeberry and/or bakeapple in Canada, mostly focusing on pollinators, phenology, and botany of the plants (Bellemare et al. 2009; Brown and McNeil 2009). Research data on nutritional characteristics of these two species in northern Canada are scarcely available, even in Newfoundland and Labrador where both partridgeberry and bakeapple are of the major interest. Moreover, most of the studies and reports were compiled based on monthly observations because of the remote locations of the sites where these species grow (White 2011). The study presents data

collected on weekly basis in three southern Labrador locations, over a period of two growing seasons, 2011 and 2012, between June and October.

There were two major factors affecting the concentration of sugars, phenolic compounds and anthocyanins in the berries: location where the berries were grown and the growing season associated with weather conditions. Compare to the data of sugars, phenolic compounds and titratable acidity from other studies (Table 6.1), our results were much higher. The major reason for that is the fruit juice (approximately 60% of fruit weight) was used for analysed of these three compounds. Plant factors may also affect the nutrition levels of fruits, for example, the number of fruits on one plant can affect the nutrition contents in the fruits (Karak et al. 2012). Research indicated that increasing fruit yield of eggplants would reduce sugar and protein content in fruits, but increase the total phenolic compounds (Karak et al. 2012). However, the fruit yields effect on the level of compounds was not considered in this project. The influence of the soil type in southern Labrador on berry composition was not studied due to the open field setup. The research plots were located at different latitudes, with different temperatures, humidity, day length and wind exposure. In general, higher latitudess had lower temperatures and longer days during summer. Surprisingly, in this study, the most northern location Cartwright (latitude 53.72°, altitude 10 m) was the warmest during both years. Red Bay is about 2°C lower (latitude 51.71°, altitude 2 m) than Cartwright and Lanse'au Clair (latitude 51.45°, altitude 10 m) is about 2.3°C lower. The latitude had a significant effect on most of the berries' nutritional compounds, such as soluble solids, total phenolics, total anthocyanins, and individual compounds, including chlorogenic acid, caffeic acid, quercetin, cyanidin and ellagic acid (in bakeapple only).

**Table 6.1 Compounds concentrations from other studies**

Composition	Bakeapple	Partridgeberry	Reference
Soluble sugar (g L <sup>-1</sup> ) *	52.21	85.85	Viljakainen, et al. 2002
TA (Citric acid) (%) **	1.16 - 1.23	2.32 - 2.77	White 2011
Total phenolics (mg/100g) *	500 - 1000	700	Northern Periphery Programme 2005
Total anthocyanin (mg/100g) *	1.0	77.5	Koponen et al. 2007
Chlorogenic acid (mg/100g) **	0.11	0.16 - 0.18	White 2011
Caffeic acid (mg/100g) *	1.8	4.5	Mattila et al.2006
Quercetin (mg/100g) *	0.6	7.4 -14.6	Hakkinen et al. 1999
Cyanidin glucoside (mg/100g) *	1.0	76.9	Koponen et al. 2007
Ellagic acid (mg/100g) *	55.9 - 60.6	ND	Hakkinen et al. 2000

\* Fruits from Finland \*\* Fruits from Labrador

## 6.1 PLANT OBSERVATIONS

Observations of plant growth were taken weekly during the entire growing season. Weekly photos, presented in Appendices B and C), capture growth and developmental stages of both partridgeberry and bakeapple.

Growth patterns in both species were similar over the approximately 90 (often less) days of the growth cycle, including production of fruits. Partridgeberry set buds beginning of June, Plants were in full bloom mid-July, fruits were formed by the end of July and were ready for harvest by September. Similar to bakeapple, the yield of partridgeberry was difficult to determine due to the natural/wild stand conditions with diverse species and density of their population. The plant density in the wild field could vary from field to field, so did the yields from different locations. Average yield of

partridgeberry on commercial farms in North America varies from 50 to 340 kg ha<sup>-1</sup>, depends on cultivar (Penhallegon 2009). In this study, the average yield of wild partridgeberry was from 190 to 310 g m<sup>-2</sup> (1900 to 3100 kg ha<sup>-1</sup>), which was ten times higher than the commercial production in USA. The yield of wild partridgeberry can also be affected by the harvest date and location (Penney et al. 1985). This study was done at Eastern Newfoundland and the total yield of partridgeberry was from 72 to 143 g m<sup>-2</sup>, with 100% of red fruit by late September in the report. Different locations had different sun exposure and precipitation that likely affected harvest date and yield (Penney et al. 1985). In the study reported here, the weather conditions differed between years and locations. Average temperature in 2012 was higher than 2011 by approximately 3°C (Appendix D), and Cartwright was the warmest location in both years compared to Lanse'au Clair and Red Bay.

Fruit set of partridgeberry in this study and was approximately 73%. Report from Sweden indicated the fruit set 64% for cross-pollination and 58% for open-pollination (Hjalmarsson 1997). Fruit set was affected by temperature with 15 to 20°C being the best for pollination. Temperatures above 25°C lower the pollination of partridgeberry due to higher temperatures reducing pollinators activities (Hjalmarsson 1997). However, it has been proven cross-pollination provided the best fruit set and pollinators were essential for good fruit set in partridgeberry (Hjalmarsson 1997). In the present study, partridgeberry grown in open and coastal areas, open-, cross- and self-pollination were combined together, so the fruit set of partridgeberry was higher (72% - 74%) (Table 5.4) than the study from Sweden (Hjalmarsson 1997).

Bakeapple plant buds are present on underground rhizomes and resume growth and development early June. In a following week, plants sprout on the surface of the bog, and two weeks later leaves develop. Plants are in full bloom at the beginning of July and fruits are ready to harvest by August (Figure 5.3). After harvest, leaves turn brown and dry out within two weeks. Since the plants grow in natural habitat, it is difficult to predict and assess the fruit yield. A number of studies conducted in northern Europe reported varying yields of bakeapple fruit in natural stands. Kortesharju (1984) studied bakeapple yield in northern Finland over 5 years. The results showed a wide range in yield variation, from 2.1 to 10.7 kg ha<sup>-1</sup> flower damage by frosts and fruit damage by *Galerucella* sp. (a leaf beetle) in some years (Kortesharju 1984). In the present study, the average yield varied from 2.4 g m<sup>-2</sup> to 2.8 g m<sup>-2</sup> (24 to 28 kg ha<sup>-1</sup>), which was significantly higher than in the report from Finland (Table 5.3). Although our study was only conducted over two years during no late spring frosts were recorded, and no disease or insects damage occurred. In order to determine the average yield of bakeapple more accurately in southern Labrador, a longer term observations are needed.

## **6.2 COMPETITION OF NATIVE PLANT SPECIES WITH BAKEAPPLE AND PARTRIDGEBERRY IN FIELDS OF LABRADOR**

In our belt transects we found 6 families of native plants, with 9 species in the square meter in total. Even greater plant diversity was observed in the open wild fields. Plants in arctic and sub-arctic regions are always facing a very short growing season, low temperatures and limited availability of nutrients for growth and development (Mulder 1999).



Partridgeberry plants in Labrador are also competing with other native species for light and nutrients. Although partridgeberry is a shrubby plant, most of the time it expands horizontally. This may result in the leaf exposure to low light intensity as it is shaded by higher plants such as birch and Labrador tea. Holloway et al. (1982) demonstrated that partridgeberry plants produce longer stems and more leaves under higher light intensities, over two growing seasons. Although the authors indicated that in the natural stands moisture and nutrient availability may modify stem length and leave number. Contrary to Holloway et al. (1982), Saario and Voipio (1997) demonstrated that the yield of partridgeberry was not significantly affected by different levels of fertilizer in soil.

Bakeapple is the only dioecious member in rose family. A good pollination is absolutely essential for its fruit set. This, however, is often limited due to the early blooming time, when pollinators are scarce. Although we did not find any other plant species blooming at the same time as bakeapple in our belt transect, a study by Brown and McNeil (2009) indicate that the flowering time of *Cassandra calyculata* L., *Kalmia* spp., and *Andromeda glaucophylla* overlapped with bakeapple, competing for pollinators at northern Quebec which borders Labrador. Poor pollination results in low number of drupelets per fruit. Besides problems with adequate pollination, bakeapple is also coping with limited nutrient resources due to other plant species growing in bogs. Jean and Lapointe (2001) have shown that bakeapple plants require large amounts of carbon during ramet growth, flowering, fruit initiation and development. In addition, the carbohydrate storage capacity of bakeapple rhizomes is low (23% of its dry biomass) and requires more carbon resources from soil (Jean and Lapointe 2001).

### **6.3 POLLINATORS**

In this study, pollinators were monitored for 30 minutes at all three locations once a week during the growing season. Most of the collected insect specimens belonged to the order Diptera (Table 5.2). Potentially, these could be the best choice insects for pollination of bakeapple in southern Labrador. Pelletier et al. (2001) indicated that insects played an important role in bakeapple pollination even if the plants bloom for only short period of time (approximately 10 days). On the other hand, a study by Brown and McNeil (2009) demonstrated that the muscid and syrphid flies were the most important pollinators for bakeapple as their activities and flying time overlapped with its flowering time. Muscid flies belong to Muscidae family and are commonly known as house flies or stable flies. Syrphid flies belong to family Syrphidae and are often called hover or flower flies due to their hovering or nectaring at flowers what has been observed at all three locations in southern Labrador. In our study, the apid and halictid bees were rare during bakeapple bloom, but they were abundant during the flowering of partridgeberry, similarly to the study by Brown and McNeil (2009). Similar to bakeapple, insects are essential for pollination of partridgeberry (Penhallegon 2006). Bumble bees were the major pollinators for partridgeberry from the observations recorded in this study (Figure 5.3).

Pollinators activity have been strongly related to temperature. A warmer climate in spring increases general insect activity (Pelletier et al. 2001). From the weather data presenteded in this thesis, the average temperature in 2012 was higher than 2011, so were the fruit yields in both species. This can be explained by higher temperatures positively affecting pollinators' activity which in turn affected the fruit set. Honeybees were not

found in Labrador due to the cold climate, although it has been demonstrated that honeybees could contribute to the pollination of bakeapple (Naess and Chagnon 2011). The anticipated climate change in the northern parts of the country will likely bring higher temperature to Labrador and create conducive conditions for commercial pollinators, such as honeybees and bumble bees.

#### **6.4 SOLUBLE SOLIDS CONTENT**

Glucose and fructose are the major sugars in both partridgeberry and bakeapple (Viljakainen et al. 2002). Concentrations of sugars in both fruits were affected by the growing season and the latitude in this study (Table 5.5 and 5.6). The soluble solids content in partridgeberries is significantly different between locations and the interaction of year and location was significant ( $p < 0.05$ ). In bakeapple, however, the location did not play a significant role, only the season ( $p < 0.05$ ). According to Poudel et al. (2009), the higher temperature decreases sugars accumulation rate. In a study by Zheng, et al. (2012) demonstrated that total sugar contents in sea buckthorn berries grown at higher latitude were lower than those grown at lower latitude.

In present study, the total sugar content of partridgeberry was significantly higher at Cartwright location (higher latitude) while bakeapple was not. This can be explained by the different fruiting dates and number of days to reach fruit maturity. Bakeapple fruits matured between 200 to 230 Julian days, which was the warmest period of the growing season in Labrador. Partridgeberries produced fruit from 215 to 270 Julian dates, which lasted until the end of the growing season. In the middle of summer, Cartwright was the warmest location in both years, but also the coldest by the end of summer. Moreover, the average temperature in 2012 was significantly higher than 2011. The low

temperature in Cartwright at the harvest date can be used to explain why the total sugar contents in partridgeberry were higher than at other locations. However, lower temperatures in 2011 also resulted in higher total sugar content in bakeapple than 2012. In this study, temperature may be the major factor affecting total sugar content in both berries (Appendix D).

## **6.5 TITRATABLE ACIDITY**

Titratable acidity for partridgeberry in our study was from 30 to 38 g L<sup>-1</sup> and bakeapple between 11 and 16 g L<sup>-1</sup> (Figure 5.10 and 5.11). Organic acids in fruits can be affected by different latitude and temperature (Zheng, et al. 2011). A study on wild Chinese sea buckthorn recorded a negative correlation between latitude and total acid concentration. Fruits picked in southern China contained more total acid than fruits picked in northern China (Zheng, et al. 2011). Other research on the relationship between metabolic efficiency and radiation indicated that shading can increase organic acids content in grapes (Iannini et al. 1989).

Our results have shown that interaction between the location and the growing season significantly affected titratable acidity of partridgeberry (Fig 5.9). The titratable acidity of bakeapple was strongly affected by the growing season but weakly by the location (Fig 5.10). The explanation for the results may be organic acid concentrations in partridgeberry and bakeapple were only slightly influenced by temperature. The major difference between the three locations during the growing season was temperature. On the other hand, organic acid in these two fruits may be affected by other environmental condition such as precipitation.

## 6.6 TOTAL PHENOLIC COMPOUNDS

For partridgeberry, the concentration of total phenolic compounds significantly differed from year to year and between locations (Table 5.5). Berries from Cartwright (the highest latitude) had the lowest concentration of total phenolic compounds in both years. Berries from Lanse'au Clair and Red Bay had higher total phenolics than from Cartwright, particularly the Red Bay berries contained the highest amounts of phenolic compounds (Fig 5.13). A study done on blackcurrants also indicate that the value of total phenolics in berries decreased with the increasing latitude (Zheng et al. 2012). Similarly, the higher temperature stimulates accumulation of phenolics (Zheng et al. 2012). Our results confirms this finding as the berries from Cartwright were low in total phenolic compounds. Temperatures in Red Bay were lower than Lanse'au Clair in both years and the latitude of Red Bay is slightly higher than Lanse'au Clair. Total phenolic compounds were still the highest in berries collected in Red Bay compared to other two locations. The field in Red Bay is very close to the ocean, while the field in Lanse'au Clair is about 2 kilometers away from the shore.

In bakeapple, the total phenolics were significantly different between the growing seasons ( $p < 0.05$ ), so was the interaction year\*location ( $p < 0.05$ ). This could be linked to the higher temperatures in 2012 compared to 2011. Although it has been reported that the influence of temperature on phenolics concentration in bakeapple can be determined by the plant genotype (Martinussen et al. 2010). For example, in cultivar 'Nyby', the highest phenolic concentration in berries was observed at 15 °C while in cv 'Fjellgull' the same was observed at 9 °C. On the contrary, in our study, the higher temperature resulted in higher concentration of total phenolic compounds in berries. The latitude did not play a

significant role. The major differences between high and low latitude areas were attributed to day length and solar radiation. The similar concentration of phenolics may be explained by the short days. The flowers only lasted less than 5 days and it only took about 2 weeks to bear the fruits.

In plants, phenylalanine is a precursor of phenolics. Phenylalanine is one product from the shikimate pathway. In the pathway, phenylalanine is synthesised from sugars, which are the products photosynthesis (Macheix et al. 1990). Temperature can strongly affect photosynthesis and thus the synthesis of phenolics (Macheix et al. 1990). For example, grapes synthesize most phenolics under 15°C (Macheix et al. 1990). Study indicated the optimum temperature for bakeapple was 9 to 15°C (Marks and Taylor 1978) and for partridgeberry was 10 to 18°C (Karlsson 1985). In our study, the highest temperature was 24°C among the three locations. The average temperature was less than 20°C at most of the time during growing season. However, the average temperature was slightly higher than optimum temperature for bakeapple, but most of the time was in the optimum range for partridgeberry.

The concentration of caffeic acid was approx 7.0 mg/100g in partridgeberry and 2.5 mg/100g bakeapple and this of chlorogenic acid was around 1.0 mg/100g in partridgeberry and 0.05 mg/100g in bakeapple. The results have shown that the location was significantly affecting concentration of both acids in partridgeberry. Chlorogenic acid concentrations were higher in berries from Red Bay than other two locations, while berries from Cartwright contained highest caffeic acid concentrations. Both caffeic acid and chlorogenic acid belong to hydroxycinnamic acid group. However, research of Zheng et al. (2012) have shown the hydroxycinnamic acid increasing with the increasing latitude.

Caffeic acid concentrations in partridgeberry have shown a similar pattern in our study. The reason for this may be the interaction of the location and climatic conditions.

## **6.7 ANTHOCYANINS**

Varying latitude may result in varying anthocyanin concentration in fruits due to the differences in day length, temperature and solar radiation. Numerous studies have focused on temperature and/or latitude effects on fruits' anthocyanin concentration in northern berries (Akerstrom et al. 2010; Borochoy-Neori et al. 2011). Martinussen et al. (2010) indicated the total anthocyanins in bakeapple were negatively related to high temperature, similarly to the studies in apple and pear peel (Faragher 1983; Steyn et al. 2009; Martinussen et al. 2010). On the other hand, reports on anthocyanin content in pomegranate have shown decreasing anthocyanin content with rising temperature (Borochoy-Neori et al. 2011). *Vaccinium myrtillus* contained less total anthocyanins with increasing altitude due to low temperature (Rieger et al. 2008). In addition, increasing temperature during the growing season caused a significant increase of total anthocyanin content in strawberry (Wang and Zeng 2001). Although the total anthocyanin content would change with different temperature, the content of cyanidin remained unaffected (Akerstrom et al. 2010). Changing light (day length) did not have any effect on anthocyanin content in these reports (Akerstrom et al. 2010). For blackcurrant, it was found the positive correlation between total anthocyanins and temperature, but not for cyanidin-glycoside (Zheng et al. 2012). The authors also reported the total anthocyanins were lower in blackcurrant grown in more northern regions of Finland (Zheng et al. 2012). On contrary, Akerstrom et al. (2010) indicated the bilberries grown in higher latitude contained more anthocyanins due to longer day length and solar radiation. They

also reported the anthocyanin content in fruits was strongly affected by interactions between climate conditions and genotype of studied plants (Akerstrom et al. 2010).

In the study presented here, the total anthocyanin concentrations in partridgeberry were significantly different in berries coming from different locations. Also the year and the interaction of location and year were significant. The cyanidin-glucoside concentration in berries was also significantly different between the locations and the interaction between location and growing season was significant as well. Concentration of total anthocyanins of partridgeberry from Cartwright was the highest which is most probably due to the higher latitude. However, the concentrations of cyanidin-glucoside were not significantly different which may suggest lack of the effect of temperature on cyaniding concentration. It has been proven the anthocyanins in partridgeberry include cyanidin-3-galactoside (88.0%), cyanidin-3-arabinoside (10.6%), and cyanidin-3-glucoside (1.4%) and delphinidin-3-glucoside less than 0.1 % (Andersen 1985). The above might explain why the P- values of total anthocyanin concentrations for partridgeberry in this thesis are different from cyanidin-3-glucoside (Table 5.5).

For bakeapple, the total anthocyanin concentrations were significantly different between locations and the interaction of year and location was also significant, while for cyanidin-3-glucoside content only the location played a significant role. The HPLC results confirm that the major anthocyanin in berries in this study was cyanidin-3-glucoside, and the P-value results of total anthocyanin concentration and cyanidin were very close. However, since anthocyanin level did not differ much with changing temperature, the latitude might be the factor here. In our study, plants grown in the lower latitude showed higher concentrations of total anthocyanins in berries.



## **6.8 DISCUSSION SUMMARY**

Considering a very short length of the growing season and fluctuations of daily temperatures in southern Labrador, both investigated species produce satisfactory fruit yield which brings additional income for the local people. Weekly observations of growth of the plants and the pollinators' activities helped to identify main stages in the annual cycle of both species and helped to determine and interpret fruit yields at the locations the berries were harvested.

In Labrador, temperature varied during the growing season at different locations. In this present study, soluble solids content in partridgeberry was mainly affected by interaction between the location and the growing season, but for bakeapple it was only affected by the growing season. Titratable acidity of both fruits was affected by the location and the growing season. Environmental factors such as radiation, temperature and precipitation affect titratable acidity of fruits. Phenolics are one of the major products from the plant secondary metabolite pathways. Anthocyanins, caffeic acid, chlorogenic acid, quercetin and ellagic acid belong to phenolics. Amount of phenolic compounds in fruits will change during their growth and maturation (Macheix et al. 1990). External factor such as light, temperature and radiation will affect phenolic compounds accumulation by regulation of enzyme activity (Macheix et al. 1990).

The results of our study demonstrate that most of nutritional compounds detected in partridgeberry and bakeapple during the course of this study were affected by the growing season, the location and/or the interaction between the two. The above supports our hypothesis, that the nutritional compounds levels of partridgeberry and bakeapple were affected by the latitude and the growing season.

## CHAPTER 7 CONCLUSION

Most locals in southern Labrador do not clearly understand plant growth and nutritional value of partridgeberry and bakeapple, although collecting them from native stands is part of their culture. From my personal experience, they know where and when to harvest these fruits, but they are not familiar with fruit structure, pollination, pollinators, yield and nutritional compounds of the two fruits. This study provides a blueprint for partridgeberry and bakeapple, as a guideline for people to help them understand partridgeberry and bakeapple.

### 1. Pollination and pollinators

Although partridgeberry can be pollinated by wind and some degree of self-pollination exists, cross pollination is the major method for both partridgeberry and bakeapple. During summer plant observation, it was found the major pollinators for bakeapple were Syrphidae family, and both *Bombus* ssp. and Syrphidae family were major pollinators for partridgeberry. There were no other flowers in bloom at the same time with bakeapple, but wild blueberry and Labrador tea flowered at the same time as partridgeberry. It indicated that pollinator competition for bakeapple was low but high for partridgeberry. However, there were many more pollinators during partridgeberry bloom than that of bakeapple.

### 2. Weekly plant observation and yield

The yield of partridgeberry was from 190 to 310 g m<sup>-2</sup> (1900 to 3100 kg ha<sup>-1</sup>), and yield of bakeapple was from 2.4 to 2.8 g m<sup>-2</sup> (24 to 28 kg ha<sup>-1</sup>) in our present study. However, the yield data were not highly accurate for the whole areas of southern Labrador due to the environmental conditions. Weekly observation results also provide

the harvest date for both fruits. In this study, the harvest date was strongly affected by temperature, with a higher temperature resulting in plants blooming and ripping earlier. In addition, Cartwright was the most northern field in this study, but the harvest dates were the earliest for both bakeapple and partridgeberry. At all three location, wild blueberry and Labrador tea were considered as pollinator competitors for partridgeberry due to the blooming at the same time. There was no pollinator competitor for bakeapple since the plants were flowering earlier.

### 3. Nutritional levels of partridgeberry and bakeapple

Growing season significantly affected nutritional compounds in both fruits. Growing season was a complex factor, including temperature, precipitation, humidity and other such as wind. Latitude also significantly affected nutritional compounds in both fruits. Different locations also had different temperature, precipitation, humidity and other environmental conditions. Fruits in northern location usually were considered more nutritious due to more radiation and longer day time, but it was not strongly proved in the present study. Nutritional level in fruits was also affected by growing conditions and genotype. Growing conditions such as temperature can strongly affect enzymes activities during plant growth.

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## **APPENDIX A**

### **Heavy metals**

It has been documented that heavy metals can accumulate in *Vaccinium* and *Rubus* species (Kuklova et al. 2010). Although the fruits described in this study have been harvested in the presumably pristine environments of the Canadian Far North, the locals are concerned that some may contain traces of heavy metals. Lead, copper, cadmium and arsenic are of particular concern for the fruit samples coming from sites close to refuse dumps (Jane White personal communication). Although heavy metal analyses in the collected fruit have not been included in this thesis, the issue is of importance for the future research.

Heavy metals pollution is of the major concern to human health. Heavy metal ions frequently are present in water, vegetables, fruits and other foods. Heavy metals are always found in the areas surrounding metal processing factories, dumps and smelters. Although, tolerance to heavy metals was found in the most plant species, especially among the vascular plants (Salemaa, et al. 1999) once the amount of heavy metals exceeds the limit threshold it can cause catastrophic damages to human health and physiological processes in the biological systems (Demirbas 2008).

Cadmium exists in natural environment like earth's crust and water. In Canada, most of cadmium emission sources are from mine drainage, smelters and heavy industries (Baker and Matheson 1980). Cadmium usually exists as divalent cation combined with other ions such as cyanide, ammonia, chloride, carbonate, hydroxide, and many organic radicals (Santillan-Medrano 1976).

Copper toxicity to humans was first reported about 200 years ago (Owen 1981). Copper is an essential micronutrient both for animals and plants, but it is toxic to cells when the concentration is higher than trace amount (75-100 mg). Copper usually exists as free ions in fruits. If bound to proteins it damages the macromolecule by oxidation (Ratkevicius et al. 2003).

Arsenic is another heavy element that widely exists in a complex form in the earth's crust. Arsenic complex can be separated into organic and inorganic forms. Most of inorganic forms are highly toxic but only a little or no toxicity was found in organic forms (Chappell 1994).

Inductively Coupled Plasma (ICP) has been used for determination of metals in solutions for over 40 years (Bradford and Cook 1997). Many techniques with ICP were developed, but inductively coupled plasma - optical emission spectroscopy (ICP-OES) and inductively coupled plasma - mass spectrometry (ICP-MS) are most widely used. ICP can determinate all heavy metal elements except argon (Bradford and Cook 1997).

The heavy metal pollution of the berries might be an issue in areas where berries are collected near dump sites in Southern Labrador (Jane White, personal communication). So far, no such issues were detected. However, a number of dump sites in Southern Labrador are accepting old car batteries and other decommissioned equipment and a possibility of heavy metal pollution in the Canadian North became a reality (Krista Head, personal communication).

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## APPENDIX B



Stages of Bakeapple plant growth and pollinators activity records during two consecutive growing seasons 2011 and 2012 in southern Labrador; observations and photos taken on weekly basis



Date	Growing Season (year)		Photos**	
	2011	2012		
Julian date: 157-160	Plant growth	Some leaves come out, most of plants stay underground, produce shoots underground	No records*	 
	Stem length	~1 cm	No records	
	# of flower /m <sup>2</sup>	None	No records	
	Pollinators	Limited number of pollinators, few Syrphidae, one <i>Bombus</i> spp. found	No records	
	# of leaves / plant	1~2	No records	
Julian date: 164-170	Plant growth	Rhizomes emerging; first leaves above-ground, most plants stay underground	No records	 
	Stem length	~2 cm	No records	
	# of flower /m <sup>2</sup>	None	No records	
	Pollinators	Limited number of pollinators, few Syrphidae, none <i>Bombus</i> spp. found	No records	
	# of leaves / plant	1~2	No records	

Date		2011	2012	Photos
Julian date: 170-176	Plant growth	Leaves come out from underground and grew up to about 2 cm tall, limited leaves with flowers in bloom or open, or flowers open without leaves, with 4-5 petals	Most flowers in bloom, few flowers were post bloom, flowers have 4-5 petals, about 2-3 cm diameter	
	Stem length	~ 4-5 cm	7~10 cm	
	# of flowers/m <sup>2</sup>	2~5	15~25	
	Pollinators	Limited number of pollinators, few Syrphidae, one <i>Bombus</i> spp. found	Pollinators were present in field, most from Syrphidae family	
	# of leaves / plant	1~2	1~2	
Julian date: 177-182	Plant growth	More flowers coming out from underground, 50% in bloom, 50% open, one or two already dried out	50% plants started form fruits, the rest were in bloom or post bloom, male flowers almost dry out	
	Stem length	5~7 cm	10~15 cm	
	# of flower /m <sup>2</sup>	3~7	2~5	
	Pollinators	Limited number of pollinators, a few Syrphidae family	Limited number of pollinators, most from Syrphidae family, more in Cartwright	
	# of leaves / plant	1~2	1~4	







Date		2011	2012	Photos
Julian date: 183-190	Plant growth	Most flowers were open, 25% were in bloom	Fruits were covered by sepals, and grew inside	
	Stem length	10~15 cm	10~15 cm	
	# of flower /m <sup>2</sup>	5~10	~1	
	Pollinators	Limited number of pollinators, few from Syrphidae family, couples of <i>Bombus</i> spp. found	Large number of pollinators, include family Syrphidae and <i>Bombus</i> spp.	
	# of leaves / plant	1~3	1~4	
Julian date: 191-197	Plant growth	Limited number of fruits unripe and covered by sepals, flowers in bloom	Fruits can be seen; fruits were hard and enlarged under sepals cover	
	Stem length	10~15 cm	10~15 cm	
	# of flower /m <sup>2</sup>	2~5	Less than 1	
	Pollinators	Few of pollinators found in Lanse'au Clair and Red Bay, large numbers of <i>Bombus</i> spp. at Cartwright	Number of insects from Syrphidae family, few <i>Bombus</i> spp.	
	# of leaves / plant	1~3	1~4	

Date		2011	2012	Photos
Julian date: 198-204	Plant growth	Most flowers are post bloom, unripe fruit covered with sepals, fruits were green	Fruits were red and grew, about 1-1.5 cm in diameter, few fruits still covered	
	Stem length	10~15 cm	10~15 cm	
	# of flower /m <sup>2</sup>	~1	None	
	Pollinators	Large numbers of <i>Bombus</i> ssp. and Syrphidae family found in all three locations	Many pollinators were seen, most were from Syrphidae family, few <i>Bombus</i> spp.	
	# of leaves / plant	1~3	1~4	
Julian date: 205-212	Plant growth	All flowers are post bloom, fruit unripe. 1/3 fruits in red	Most fruits red, fruit size about 1.5 cm (diameter), fruits were still hard and not ready for harvest	
	Stem length	10~15 cm	10~15 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Numbers of pollinators in Lanse'au Clair and Red Bay, few in Cartwright	Pollinators were present in all three locations	
	# of leaves / plant	1~3	1~4	

Date	2011	2012	Photos	
Julian date: 213-217	Plant growth	50% fruits red, remaining were covered, sepals start to separate from fruits	One or two fruits could be picked, most of fruits were turning from red to orange, and soften	
	Stem length	10~15 cm	10~15 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Limited number of pollinators, few from Syrphidae family, <i>Bombus</i> spp. found	Few pollinators found in fields	
	# of leaves / plant	1~4	1~4	
Julian date: 220-226	Plant growth	Fruits were almost ripen, but not soft enough for harvest	Fruits ready to harvest; orange in color and soft	
	Stem length	10~15 cm	10~15 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Limited number of pollinators, few Syrphidae, none <i>Bombus</i> spp. found	Limited number of pollinators, few from Syrphidae family, none <i>Bombus</i> spp. found	
	# of leaves / plant	1~4	1~4	

Date	2011	2012	Photos
Julian date: 227-232	Plant growth	Numbers of fruits were soft and ready for harvest	Fruits orange in color and soft, most of them already harvested, fruits remained on plants started to dry out
	Stem length	10~15 cm	10~15 cm
	# of flower /m <sup>2</sup>	None	None
	Pollinators	Limited number of pollinators, few from Syrphidae family and <i>Bombus</i> spp.	Limited number of pollinators, few from Syrphidae family, none <i>Bombus</i> spp. found
	# of leaves / plant	1-4	1~4
Julian date: 233-240	Plant growth	No records*	Few fruits left, started to dry out
	Stem length	No records	10~15 cm
	# of flower /m <sup>2</sup>	No records	None
	Pollinators	No records	Limited number of pollinators, few from Syrphidae family and <i>Bombus</i> ssp.
	# of leaves / plant	No records	1~4 or dead




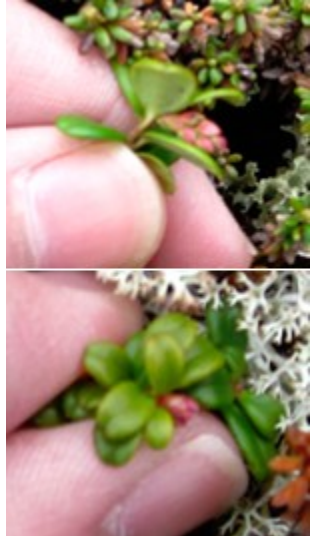
Date		2011	2012	Photos
Julian date: 247-251	Plant growth	No records	Most of leaves dead, brown in color and fall	
	Stem length	No records	10~15 cm or dead	
	# of flower /m <sup>2</sup>	No records	None	
	Pollinators	No records	No pollinators were seen	
	# of leaves / plant	No records	1~4 or dead	
Julian date: 254-256	Plant growth	No records	Most of leaves were dead, brown in color and fall	
	Stem length	No records	10~15 cm or dead	
	# of flower /m <sup>2</sup>	No records	None	
	Pollinators	No records	No pollinators were seen during observations	
	# of leaves / plant	No records	1~4 or dead	



\* No records due to delayed start of data collection (late arrival to Labrador) or early departure from the site (travel arrangements)



\*\* Two pictures are provided for each week, 2011 (top) and 2012 (bottom)

## APPENDIX C

Stages of Partridgeberry plant growth and pollinators activity records during two consecutive growing seasons 2011 and 2012 in southern Labrador; observations and photos taken on weekly basis

Date		Growing Season (year)		Photos**
		2011	2012	
Julian date: 157-160	Plant growth	Both flower and leaves buds were tight,	No records*	
	Stem length	~ 7 cm	No records	
	# of flower /m <sup>2</sup>	None	No records	
	Pollinators	Limited number of pollinators, few Syrphidae, one <i>Bombus</i> spp. was found	No records	
	# of leaves / plant	7-15	No records	
Julian date: 164-170	Plant growth	Buds swell and stems grew	No records	
	Stem length	~ 7 cm	No records	
	# of flower /m <sup>2</sup>	None	No records	
	Pollinators	Limited number of pollinators, few from Syrphidae family, none <i>Bombus</i> spp. was found	No records	
	# of leaves / plant	7-15	No records	



Date		2011	2012	Photos
Julian date: 170-176	Plant growth	Buds enlarged, stems elongated, leaves buds started open	Flower buds were red in color, leaves buds were open	
	Stem length	~ 7.5 cm	~ 7.5 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Limited number of pollinators, few from Syrphidae family, one <i>Bombus</i> spp. found	Limited number of pollinators, few Syrphidae family seen	
	# of leaves / plant	7-15	Up to 20	
Julian date: 177-182	Plant growth	New leaves came out, flower buds grew and turn green	Red petals can be seen, some flowers in bloom in Cartwright	
	Stem length	~ 7.5 cm	~7.5 cm	
	# of flower /m <sup>2</sup>	None	10~20	
	Pollinators	Limited number of pollinators, few from Syrphidae family	Limited number of pollinators, major were from Syrphidae family, more in Cartwright	
	# of leaves / plant	Up to 20	Up to 20	



Date		2011	2012	Photos
Julian date: 183-190	Plant growth	Flower buds enlarge, in some can see the petals. Leaves buds open	Most of flowers in bloom	
	Stem length	~ 7.5 cm	~ 7.5 cm	
	# of flower /m <sup>2</sup>	Less than 5	~50	
	Pollinators	Limited number of pollinators, few from Syrphidae family, two of <i>Bombus</i> spp. found	Large number of pollinators, includes Syrphidae family and <i>Bombus</i> spp.	
	# of leaves / plant	Up to 20	Up to 20	
Julian date: 191-197	Plant growth	20% flowers in bloom, the rest stay closed with petals in red color	Flowers in bloom, 25% post bloom, few plants started to form fruits	
	Stem length	~ 7.5 cm	~ 7.5 cm	
	# of flower /m <sup>2</sup>	0~15	~60	
	Pollinators	Few pollinators were found in Lanse'au Clair and Red Bay, large numbers of <i>Bombus</i> spp. at Cartwright	Numbers of Syrphidae family, few <i>Bombus</i> spp.	
	# of leaves / plant	Up to 20	Up to 20	





Date	2011	2012	Photos
Julian date: 198-204	Plant growth	90% flowers in bloom	Most flowers were post bloom and berries started to enlarge, about 5 mm diameter
	Stem length	~ 7.5 cm	~ 7.5 cm
	# of flower /m <sup>2</sup>	30~50	~10
	Pollinators	Large numbers of <i>Bombus</i> ssp. and from Syrphidae family were found in all three locations	Many pollinators were seen, most were from Syrphidae family, a few <i>Bombus</i> spp.
	# of leaves / plant	Up to 20	Up to 20
Julian date: 205-212	Plant growth	50% flowers were in bloom, the rest were post bloom	Berries started to turn red, about 5~7mm diameter
	Stem length	~ 7.5 cm	~ 7.5 cm
	# of flower /m <sup>2</sup>	10~15	None
	Pollinators	Many pollinators in Lanse'au Clair and Red Bay, few in Cartwright	Few pollinators found in fields
	# of leaves / plant	Up to 20	Up to 20



Date		2011	2012	Photos
Julian date: 213-217	Plant growth	Flowers done, unripe green fruits, 2~5 mm in diameter	A half of berry surface was in red, size kept growing, about 7 mm in diameter	
	Stem length	~7.5 cm	~7.5 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Limited number of pollinators, few from Syrphidae family, <i>Bombus</i> ssp. found	Few pollinators found in fields	
	# of leaves / plant	Up to 20	Up to 20	
Julian date: 220-226	Plant growth	Berries grew, wide range of color green to red	Fruits almost reached the maximum size, 75% skin in light red color	
	Stem length	~ 7.5 cm	~ 7.5 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Limited number of pollinators, few from Syrphidae family, none <i>Bombus</i> ssp. found	Limited number of pollinators, few from Syrphidae family, none <i>Bombus</i> ssp. found	
	# of leaves / plant	Up to 20	Up to 20	

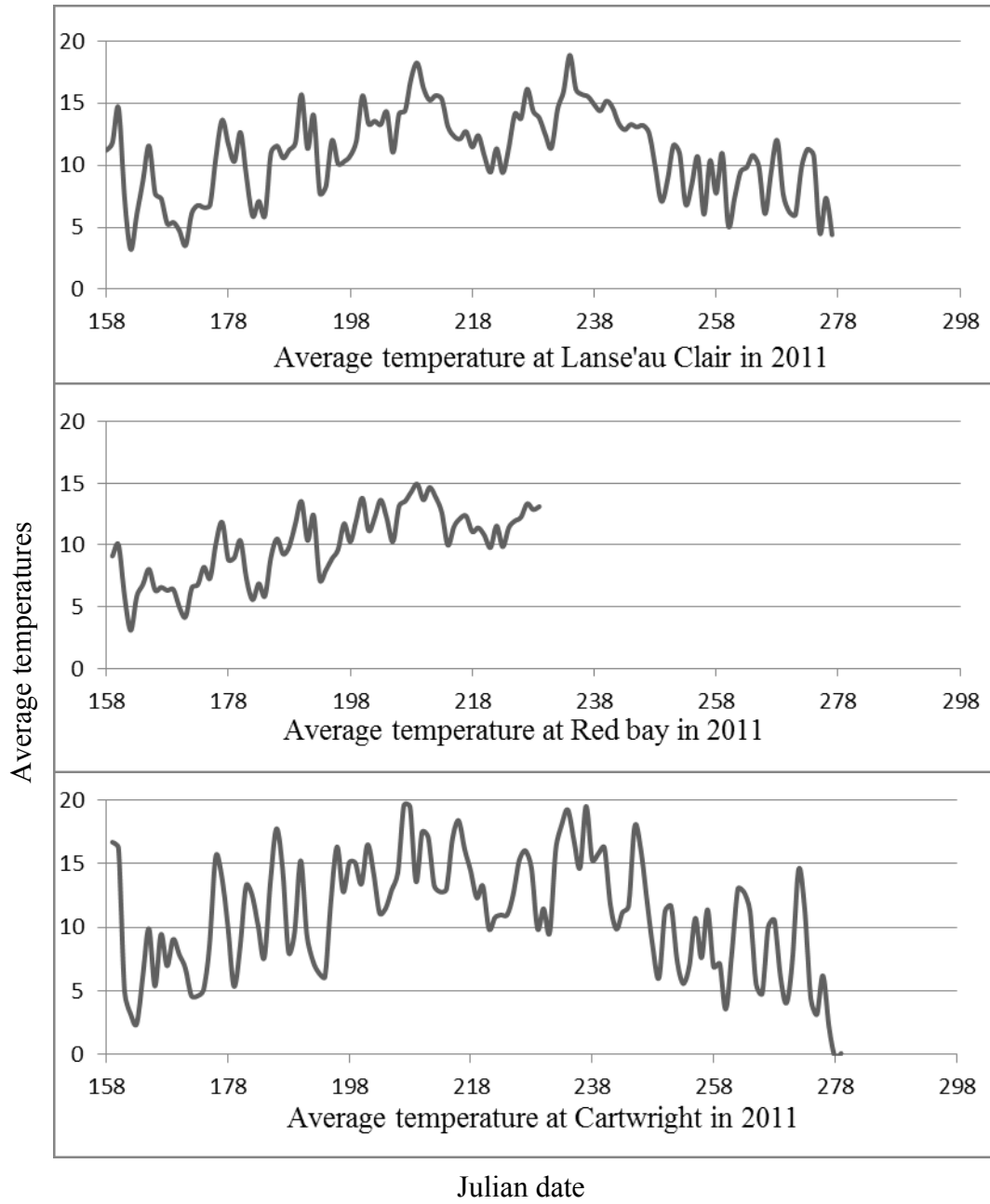
Date		2011	2012	Photos
Julian date: 227-232	Plant growth	Berries grew, wide range of colors, green to red. Wide range of sizes, 2~7mm in diameter	Berries were almost in red, less than 25% of berry surface was in green	
	Stem length	~ 7.5 cm	~ 7.5 cm	
	# of flower /m <sup>2</sup>	None	None	
	Pollinators	Limited number of pollinators, few from Syrphidae family and <i>Bombus</i> ssp.	Limited number of pollinators, few from Syrphidae family, none <i>Bombus</i> ssp. found	
	# of leaves / plant	Up to 20	Up to 20	
Julian date: 233-240	Plant growth	No records*	Berries in light red color, few berries could be picked	
	Stem length	No records	~ 7.5 cm	
	# of flower /m <sup>2</sup>	No records	None	
	Pollinators	No records	Limited number of pollinators, few from Syrphidae family and <i>Bombus</i> ssp.	
	# of leaves / plant	No records	Up to 20	

		2011	2012	Photos
Julian date: 247-251	Plant growth	No records	Fruits in red color, some were ready for picked	
	Stem length	No records	~ 7.5 cm	
	# of flower /m <sup>2</sup>	No records	None	
	Pollinators	No records	No pollinators were seen	
	# of leaves / plant	No records	9-14	
Julian date: 254-256	Plant growth	No records	Berries in dark red color, most of ready for harvest	
	Stem length	No records	~ 7.5 cm	
	# of flower /m <sup>2</sup>	No records	None	
	Pollinators	No records	No pollinators were seen during observation	
	# of leaves / plant	No records	9-14	

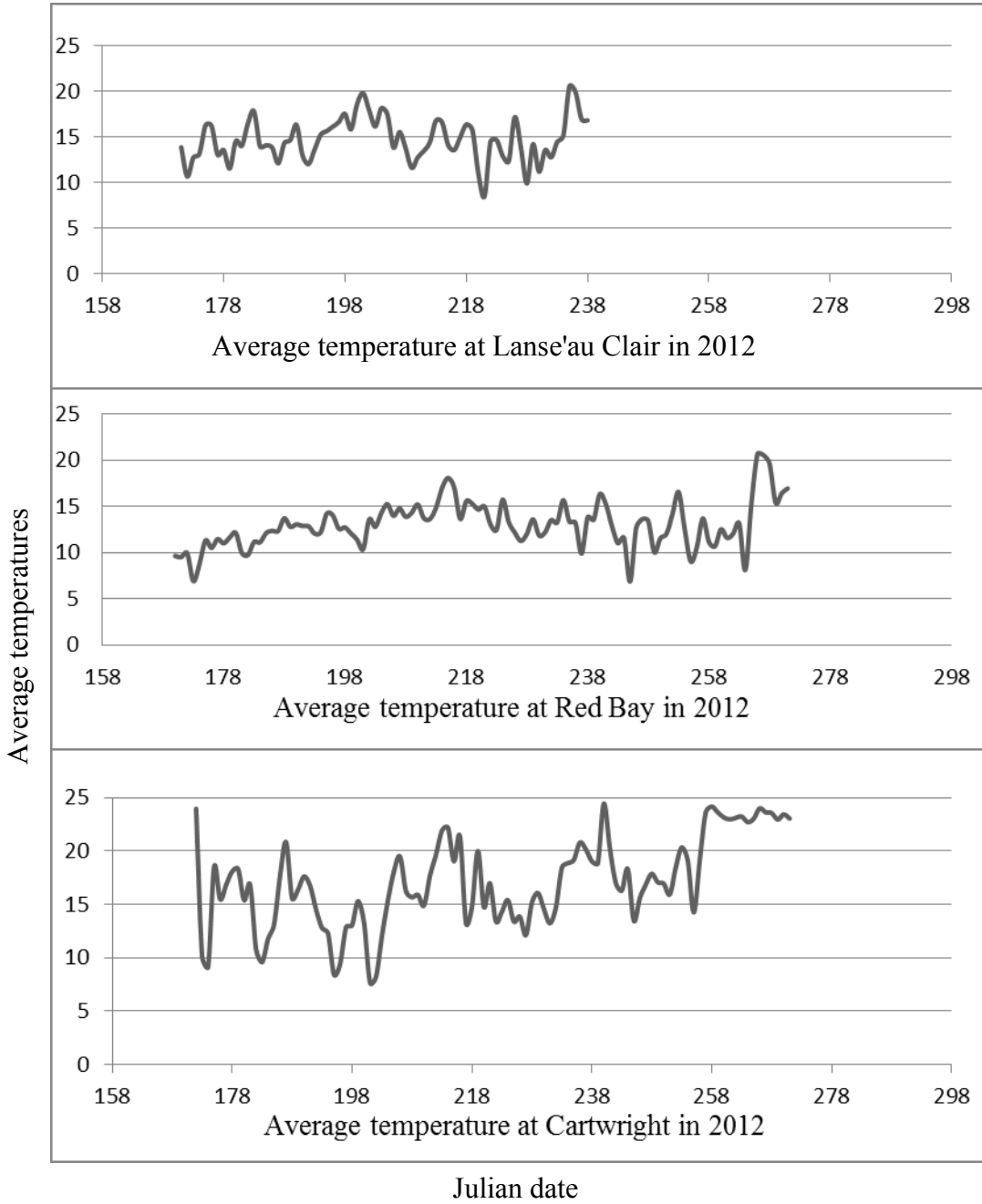
\* No records due to delayed start of data collection (late arrival to Labrador) or early departure from the site (travel arrangements)

\*\* Two pictures are provided for each week, 2011 (top) and 2012 (bottom)

## APPENDIX D

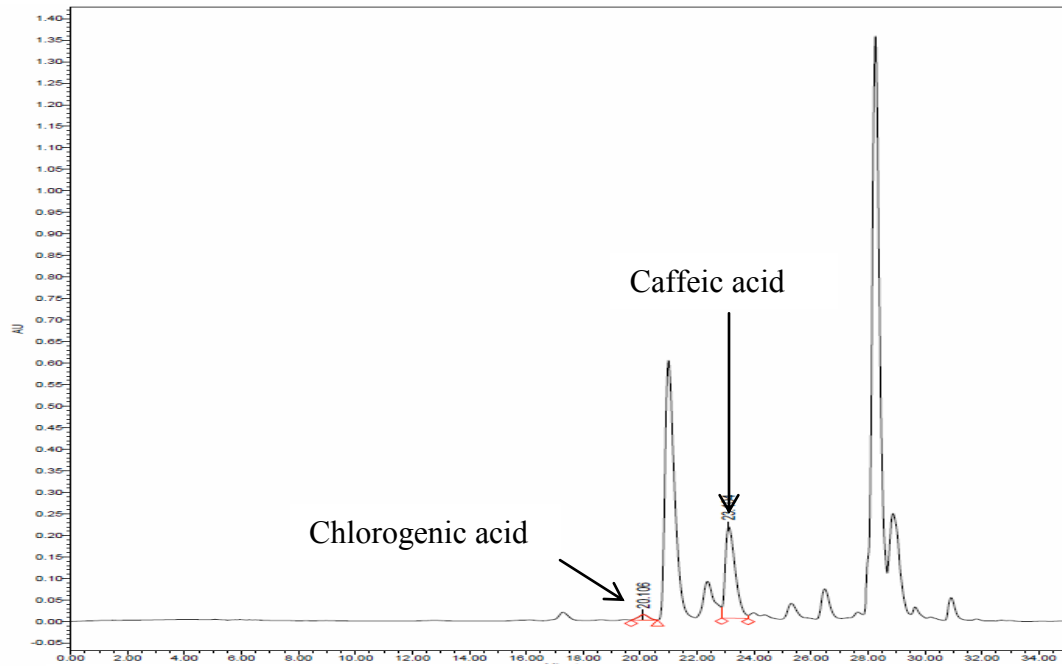


Mean temperatures in 2011 at Lanse'au Clair, Red Bay and Cartwright

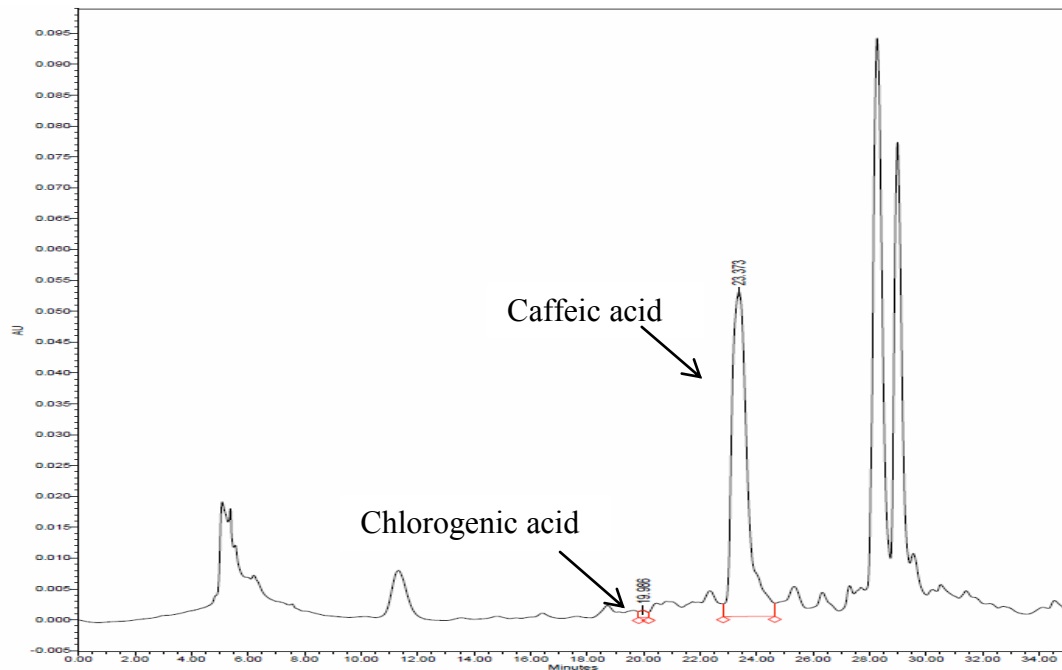


Mean temperatures in 2012 at Lanse'au Clair, Red Bay and Cartwright

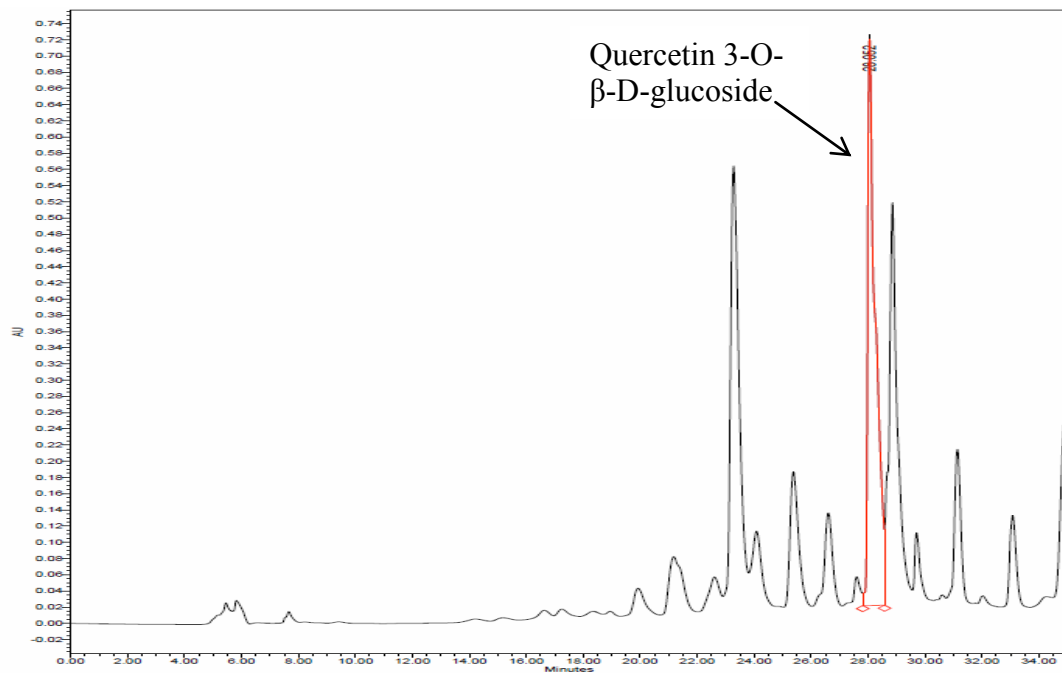
## APPENDIX E



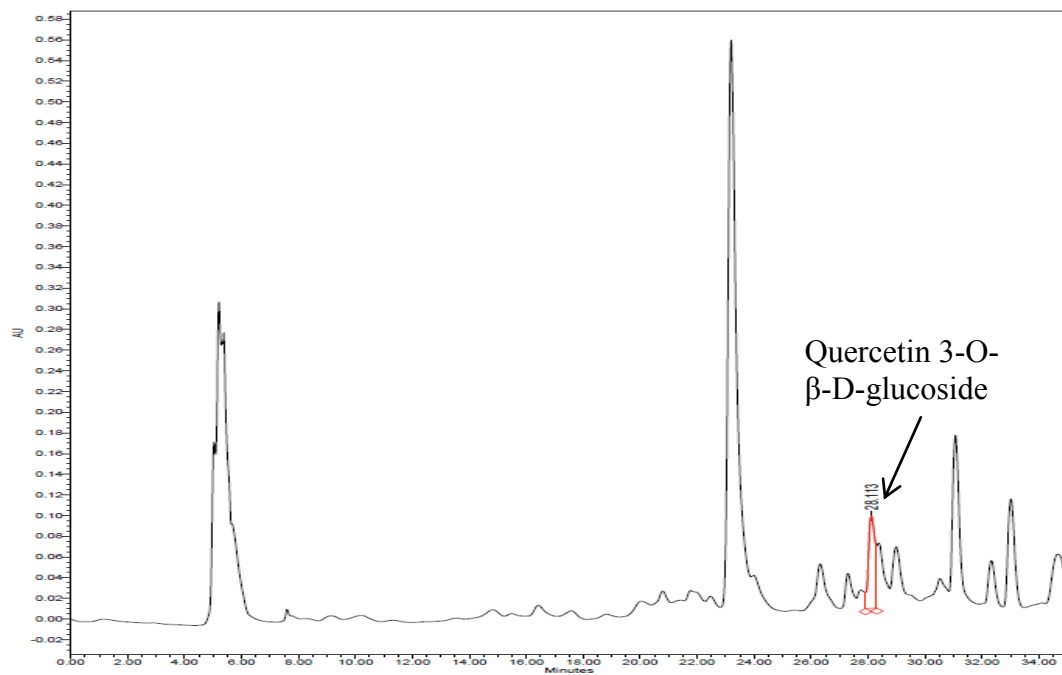
HPLC chromatogram of partridgeberry recorded at 325 nm



HPLC chromatogram of bakeapple recorded at 325 nm

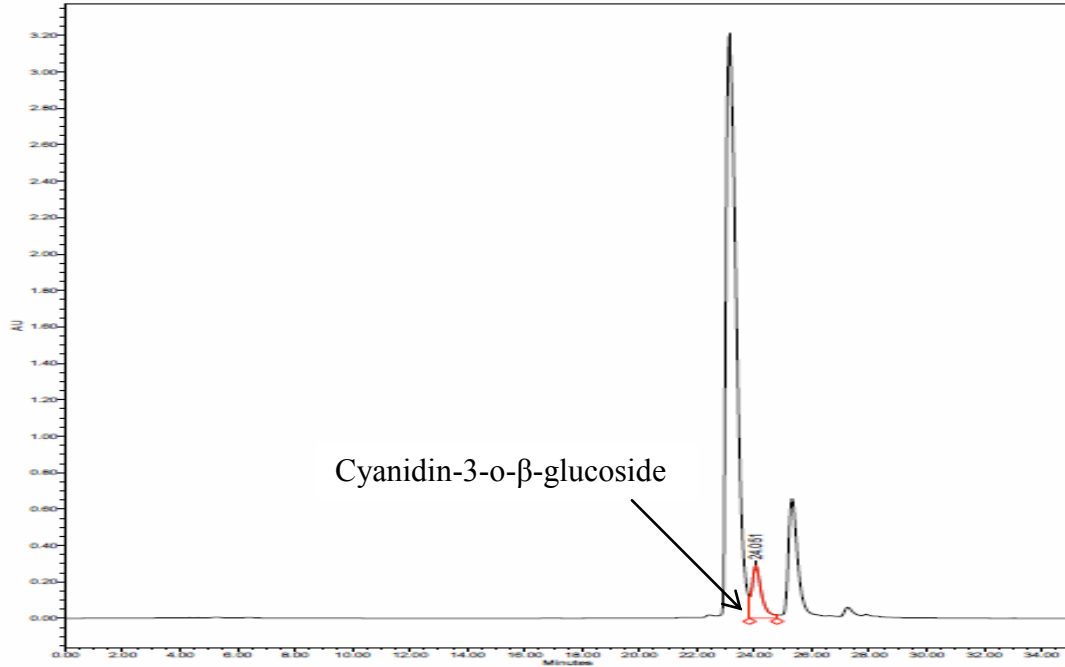


HPLC chromatogram of partridgeberry recorded at 260 nm

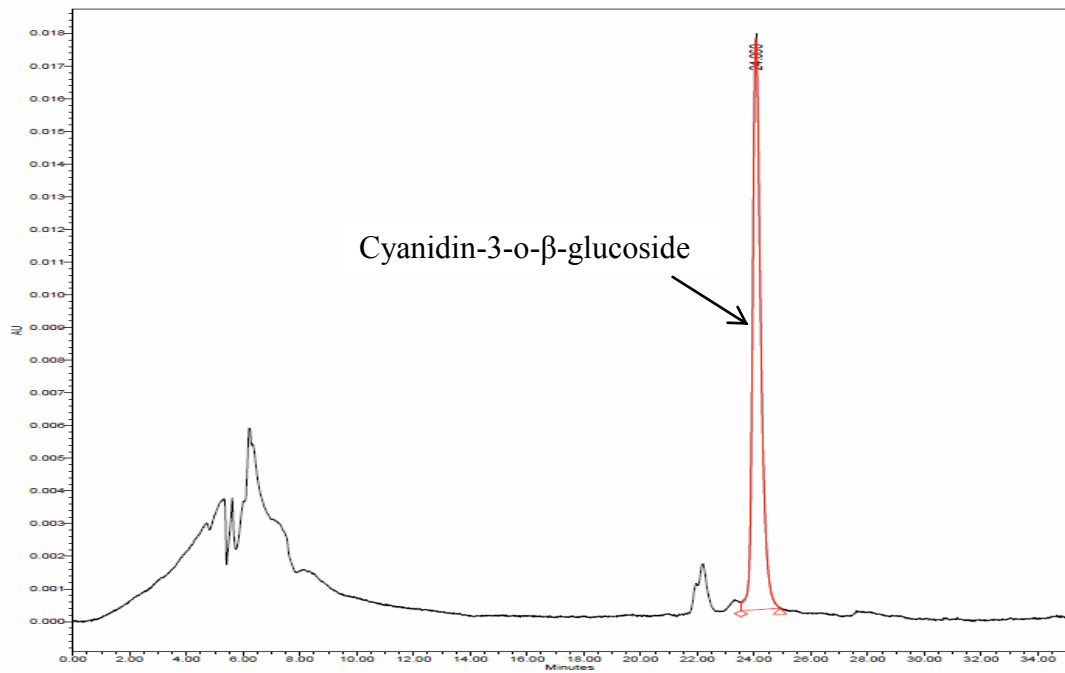


HPLC chromatogram of bakeapple recorded at 260 nm

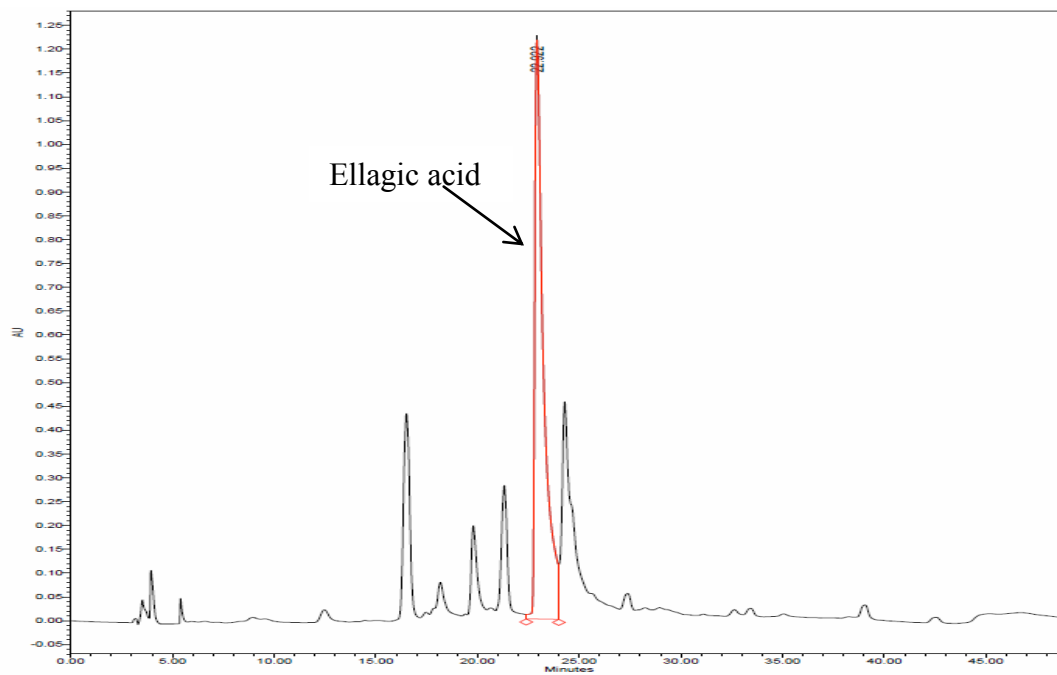




HPLC chromatogram of partridgeberry recorded at 520 nm



HPLC chromatogram of bakeapple recorded at 520 nm



HPLC chromatogram of ellagic acid extracts baked apple recorded at 260 nm