Pre-harvest factors affecting quality and shelf-life in raspberries and blackberries (*Rubus* spp. L.)

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Cover: Picture of the blackberry trial in greenhouse and raspberry trial under high tunnels.

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Abstract

Fruit including berries have been demonstrated to exhibit a broad spectrum of benefits including protection against cardiovascular, neurological, and lung diseases, as well as having antioxidant, antimicrobial, anti-inflammatory, anti-diabetic and anti-aging properties. These protective effects are reported to be due to their high content of bioactive compounds, such as vitamin C, vitamin E, phenolic acids, ellagitannins, flavonoids and carotenoids. This thesis investigated the effect of pre- and postharvest factors on the concentrations of bioactive compounds in raspberries and blackberries. The factors studied included genetic variability, organic and synthetic fertilizers, seasonal variation (harvest-to-harvest and annual variation) in greenhouse, high tunnel and open field production, and post-harvest storage. Concentrations of bioactive compounds (anthocyanins, vitamin C, ellagic acid, carotenoids) in the berries, or in the leaves, were quantitatively analysed by high-performance liquid chromatography, and total phenolics were analysed by a spectrophotometric method. Generative parameters (yield, fruit size) investigated for the cultivar difference and organic nitrogen, responded differently to the nitrogen level within cultivars and varied significantly in primocane raspberries. Taste compounds, such as total acidity and different sugars, varied during the season and with nutrient regimes. Time of harvest also affected the ellagic acid content, which was high in early season, and the vitamin C content, which was high in late season in primocane raspberries. Significant changes were also found between different years. Vitamin C decreased significantly with high synthetic N and with low K application, but the application of high N with high K showed positive influence in the level of nutrients and bioactive compounds in blackberries except for ellagic acid. Increased level of organic fertilization (12-17 g N/plant) in primocane raspberries caused only minor changes in the analysed compounds. The post-harvest performance of raspberries regarding the levels of bioactive compounds was less dynamic than in blackberries. In addition, raspberries harvested in late season showed comparatively less changes in sugar content during storage as compared to early harvest, indicating less enzymatic activity in the late season.

Keywords: anthocyanins, antioxidants, cultural regime, flavonoids, fructose, glucose, post-harvest storage, seasonal influence, sucrose, and vitamin C.

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Dedication

This thesis is dedicated to both my parents: My father, who not only raised and encouraged me, but also challenged himself greatly over the years to provide for my education and scholarly growth; and my mother, who was a source of great love, motivation and strength during moments of despair and discouragement.

Finally,

this thesis is dedicated to all those who believe in the fortune of learning.

Allah is the one who created everything out of nothing by the strength of his word. He brought everything into being, and to him we shall all return. He creates life and causes death (Quran, Sura al-A`raf 7:44).

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Ali, L., Svensson, B., Alsanius, B.W. & Olsson, M.E. (2011). Late season harvest and storage of Rubus berries - major antioxidant and sugar levels. *Scientia Horticulturae* 129(3), 376-381.

II. Ali, L., Alsanius, B., Rosberg, A., Svensson, B., Nielsen, T. & Olsson, M. E. (2012). Effects of nutrition strategy on the levels of nutrients and bioactive compounds in blackberries. *European Food Research and Technology* 234(1), 33-44.

III. Ali, L., Svensson, B., Alsanius, B.W. & Olsson, M.E. Influence of time of harvest, annual variation and storage on quality of raspberries cultivated in open field, high tunnel and greenhouse. *(Manuscript)*

IV. Ali, L., Svensson, B., Alsanius, B.W. & Olsson, M.E. Quality of primocane raspberries - cultivar and organic nitrogen interactions in late summer and autumn production. *(Manuscript)*

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Abbreviations

ANOVA	Analysis of variance
DMC	Dry matter content
DW	Dry matter
EA	Ellagic acid
FW	Fresh weight
GH	Greenhouse
HRV	Harvest
HT	High tunnels
K	Potassium
N	Nitrogen
NADPH	Nicotinamide adenine nucleotide phosphate
OP	Open field
PCA	Principals component analyses
ROS	Reactive oxygen species
SOD	Superoxide dismutase
Spp.	Species
TSS	Total soluble solids
UV	Ultraviolet light

1 Objectives and key questions

The main objective of this thesis was to investigate the effect of environmental factors and cultivation methods on the level and chemical composition of bioactive and antioxidant compounds in Rubus berries (raspberries, *Rubus idaeus* L.; blackberries, *Rubus fruticocus* L.). The bioactive and antioxidant compounds analysed were vitamin C, total phenolics, anthocyanin, ellagic acid and carotenoids (lutein, β-carotene). In addition, compounds contributing to taste such as sugars (fructose, glucose, and sucrose) and acidity were analysed.

The individual research questions addressed in Papers I-IV were:

1) How does the late season with its low light intensity and short day length affect the bioactive and antioxidant compounds in raspberries and blackberries at harvest and during their shelf-life? (Paper I)
2) How does plant fertilisation strategy affect the content of nutrients and bioactive compounds in blackberries at harvest and during storage? (Paper II)
3) How do the level and chemical composition of bioactive compounds vary in floricane raspberries with seasonal changes? (Paper III)
4) How do seasonal variations during berry development affect the phytochemical changes during the shelf-life of floricane raspberries? (Paper III)
5) How does organic fertilizer level affect the cultivation potential for yield and bioactive compound content in primocane raspberries? (Paper IV)

2 Introduction

2.1 Plant material

Raspberries and blackberries are classified botanically as follows:

Kingdom – Plantae, plants
Subkingdom – Tracheobiota, vascular plants
Superdivision – Spermatophyta, seed plants
Division – Magnoliophyta, dicotyledons
Class – Magnoliophyta
Subclass – Rosidae
Order – Rosales
Family– Rosaceae, Rose family
Genus – *Rubus*
Species – *Rubus idaeus* L. (raspberries) and *Rubus fructicocus* L. (blackberries)

2.2 Biology, origin and distribution

Rubus berry species are closely related to roses, one of the most diverse genera in the plant kingdom. Different species in the Rubus genus are native to six continents and have been found from the tops of mountains to coastal locations at sea level (Daubeny, 1996; Thompson, 1995). They grow especially well as cool climate plants, but will also produce worthwhile crops in the subtropics. Rubus berries are not true berries but aggregate fruits and have a number of culinary uses in the modern era, *e.g.* as a fresh fruit, processed into jams, as a yogurt flavouring, pie filling *etc*. (Rao & Snyder, 2010; Daubeny, 1996).
The Rubus plant forms range from completely self-fruitful to completely self-unfruitful and are often referred to as brambles. Bramble fruit are generally

separated into two groups, raspberries (*Rubus idaeus* L.) and cultivated blackberries (*Rubus fructicocus* L.) (Industries, 2002). Raspberry and blackberry are closely related to strawberry in the subfamily *Rosoideae*. The plant is an erect, semi-erect or trailing, generally thorny, shrub, producing renewal shoots from the ground called 'canes'.

There are more than 200 raspberry species and most of these have red berries (European), although some have black berries (American). The fruit is a collection of 'drupelets', each containing a seed. The red raspberries are thought to originate from Asia Minor and there are Roman records of its use (Troy and the foothills of Mount Ida, Turkey) dating back to the 4th century AD. Hence the Romans probably spread raspberry cultivation throughout Europe. The species was named after Mount Ida by Carl Linnaeus. References to raspberries as food and widespread cultivation in the European countries was reported by the 16th century, although it was the English who cultivated, hybridised and improved the species throughout the Middle Ages (Hummer, 2010; Trager, 1995; Jennings 1988; McGregor, 1976).

The formal name of the ancient blackberry was *Rubus eubatus* and it was always considered wild, so in the early days it was not cultivated. Development of the modern blackberry is relatively recent and was mainly done in America. There are now more than 40 blackberry species. The European blackberry (*Rubus fructicocus* L.) is centred in the Caucasus region and has been introduced into Asia, Europe, North and South America. The use of blackberry as a food source and medicinal plant is well documented from prehistory (Early Stone Age, 40,000 BCE) to the modern era (21st century) (Shiow & Lin, 2000; Connolly, 1999; Tutin *et al.*, 1980).

2.3 Bioactive compounds

More recently, several field of research including epidemiology, human medicine and nutrition have shown that high intake of fruit and vegetables is associated with good health and prevention of diseases (Seeram, 2008; Maynard *et al.*, 2003; Joshipura *et al.*, 2001). This protection has been attributed to the level of nutrients in fruit and vegetables, as well as non-nutrient molecules called 'bioactive compounds'. These have antioxidant or other biochemical effects against pathological conditions such as several forms of cancer and cardiovascular disease, as well as inhibiting DNA synthesis, inflammation and microbial activities (Paredes-Lopez *et al.*, 2010; Kraft *et al.*, 2008; Shukitt-Hale *et al.*, 2008; Cho *et al.*, 2004; Joshipura *et al.*, 2001). Rubus berries, including raspberries and blackberries, are among the soft fruits considered a healthy and nutritious part of the human diet. Rubus berries contain a number of nutrient and non-nutrient molecules such as vitamin C (Ali *et al.*, 2011; Borges *et al.*, 2010), phenolics (Nigel *et al.*, 2000), ellagic acid (de Ancos *et al.*, 2000), ellagitannins (Clifford & Scalbert, 2000; de Ancos *et al.*, 2000), flavonoids (Kalt *et al.*, 1999), dietary fibre (Acosta-Montoya *et al.*, 2010; Schmeda-Hirschmann *et al.*, 2005), carotenoids (Mertz *et al.*, 2009; Parry *et al.*, 2005), vitamin E (Van Hoed *et al.*, 2009; Xu *et al.*, 2006; Parry *et al.*, 2005; Bushman *et al.*, 2004), calcium (Lefevre *et al.*, 2011; Ganhao *et al.*, 2010), magnesium (Plessi *et al.*, 2007) and linoleic acid (Kim *et al.*, 2011; Bakowska-Barczak *et al.*, 2007; Parry *et al.*, 2005; Bushman *et al.*, 2004).

Box 1. Definitions of the words used in the thesis

Bioactive compounds — "Bioactive compounds are essential and non-essential compounds (e.g., vitamins or polyphenols) that occur in nature, are part of the food chain, and can be shown to have an effect on human health" (Biesalski *et al.*, 2009).

Phytochemicals — "Phytochemicals are non-nutritive constituents produced by secondary metabolism in plants. They are known to defend plants against predators, microbial infections and ultraviolet light, to regulate metabolic pathways and provide color and flavor to the plant" (DeBoer, 2005).

Antioxidants —"Any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delay or prevents oxidation of that substrate" (Halliwell & Gutteridge, 1985).

Free radicals —"A free radical is any species capable of independent existence that contains one or more unpaired electrons. (An unpaired electron is one that occupies an atomic or molecular orbit)" (Halliwell & Gutteridge, 1985).

2.4 Antioxidants and reactive oxygen species (ROS)

Reactive oxygen species (ROS), including free radicals and other reactive oxygen molecules, are formed in normal metabolism, and are today considered to have important roles in cell signaling and homeostasis (Hancock et al., 2001; Apel & Hirt, 2004). ROS are species of oxygen, which are in more reactive state than molecular oxygen. ROS are formed in metabolism, e.g. in mitochondria (Murphy, 2009), and the formation of superoxide ($O_2^{\cdot -}$) through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a known example of ROS generation during normal respiration. NADPH oxidase enzymes catalyze this process with electron supplied by the NADPH and leading further reduction to hydrogen peroxide (H_2O_2) (Anna *et al.*, 2007; Balaban *et al.*, 2005; Geiszt *et al.*, 2001)

$O_2 + e^{\cdot -} \rightarrow O_2^{\cdot -}$ (superoxide)
$\downarrow$
$2O_2 + NADPH \rightarrow 2O_2^{\cdot -} + NADP + H^+$ (electron supplied by NADPH)
$\downarrow$
$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2$ (hydrogen peroxide)

Further reactions may lead to hydroxyl radicals ($OH^\bullet$), especially in the presence of metal ions. There is a family of enzymes involved for these reactions such as superoxide dismutase (SOD) and myeloperoxidase. The superoxide may also react with nitric oxide (NO•) and can generate another reactive molecule, peroxynitrite.

$NO^\cdot + O_2^{\cdot -} \rightarrow OONO^-$

There are also some non-mitochondrial sources, which produce ROS in various cell types. There are several multi-protein based enzymes reported that generate ROS in response to various stimuli (Hideki *et al.*, 2005; Miklós & Thomas, 2004). However, they can also be formed as physiological metabolites as a result of respiration burst in the phagocytic cells in human body (Lluis *et al.*, 2005). The formation of ROS is seen at high rates in many diseases, including cardiovascular disease, cancer, diabetes, and other neurodegenerative disorders, and have been suggested to be a part of the progression, and sometimes the initiation of the disease (Uttara et al., 2009; Chrissobolis et al., 2011; Babizhayev et al., 2011), though recently ROS has been suggested also to have a protective role in autoimmune disease and act as regulators of autoimmune inflammation (Hultqvist et al., 2009). Antioxidants are compounds that posses the ability to delay or prevent oxidation of these species (Halliwell & Gutteridge, 1985).

2.4.1 Oxidative stress

The balance between different forms of ROS, *i.e.* free radicals or other reactive forms of oxygen, and elimination of these by the antioxidative defence mechanism are very important in maintaining normal metabolism. Oxidative stress occurs when ROS concentrations increase and cannot be eliminated by the antioxidative defence system (Figure 1). These high ROS levels cause damage to cell constituents such as lipids, proteins and nucleic acid, and eventually result in cell death. Examples of oxygen-derived free radicals include superoxide, hydroxyl hydroperoxyl, peroxyl and alkoxyl radicals. Other common reactive species produced in the body include nitric oxide and the peroxynitrite anion (Prior & Cao, 2000). The level of stress induced is determined by the rate at which oxidative damage is provoked and the rate at which it is efficiently repaired or removed by compounds such as antioxidants. Most ROS come from endogenous sources such as by-products of normal food metabolism and the body's immune system.

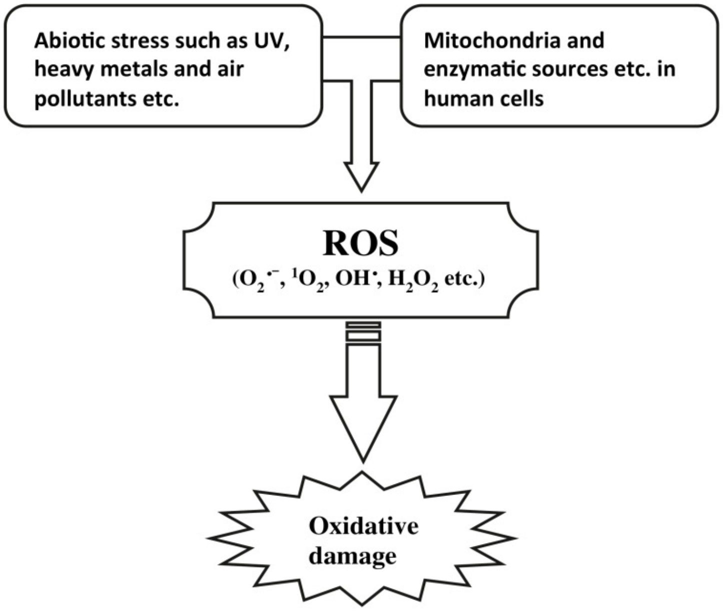

Figure 1. Stages in oxidative stress. Diagram adapted and reprinted with the kind permission of the authors (Scalbert *et al.*, 2002).

External sources such as environmental pollution, radiation, cigarette smoke and herbicides can also generate ROS (Orient *et al.*, 2007; Lluis *et al.*, 2005; Lievre *et al.*, 2000).

2.5 Anti-oxidative defense

To deal with these ROS, the body is equipped with anti-oxidative defence mechanisms that include various kinds of enzymes (e.g. superoxide dismutase, glutathione peroxidase, catalase) and high and low molecular weight antioxidants. These anti-oxidative defence compounds neutralise free radicals by donating one of their own electrons before the free radicals get a chance to create disorder in the body (Schuelke *et al.*, 2012; Lievre *et al.*, 2000).

A number of plant compounds have been recognised as having positive effects against the free radical compounds in biological systems. These compounds are reported to stop cancer at its inception by preventing the various carcinogens that initiate cancer, or by blocking enzymes that potentiate cancer. Certain phytochemicals such as anthocyanins, carotenoids and ellagitannins stop carcinogens from damaging cells, tissues and organelles by helping the body to produce enzymes that destroy carcinogens. One class in particular, the plant phenolics, can prevent DNA adduction, presumably by presenting alternative targets for attack by carcinogens (Teel & Castonguay, 1992; Newmark *et al.*, 1984). A number of reducing agents such as vitamin C, sulphites, glucose oxidase, erythorbic acid and ascorbyl palmitate are reported to be oxygen scavengers by transferring hydrogen atoms. Plant compounds such as carotenoids, some vitamins, phenols, flavonoids and glutathione have also been reported to be oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibiters and synergists (Wang & Hongjun, 2000).

2.6 Physiological effects of bioactive compounds

The array of bioactive compounds found in berry fruits recently represents a broad spectrum of biological and medicinal properties. These properties are not necessarily dependent on the antioxidant values, but in fact are produced by currently unknown molecular mechanisms. Beyond their antioxidant functions, these compounds have long been known to play a role as natural remedies for the respiratory, digestive, circulatory, and urinary system (Szajdek & Borowska, 2008). They have been e.g. found to seal capillary vessels, improving their elasticity and the peripheral circulation of the blood and boosting the body's resistance to infections. Owing to those properties, they are also applied in the production of ophthalmic preparations to improve microcirculation of the blood in the capillary vessels of the eyeball, thus improving vision at dusk and at night (Kramer, 2004; Martin-Aragon *et al.*, 1998).

Berry phytochemicals may act to change the genomic expression of cells regarding their ability to modulate initiation, promotion and progression of cancer. The possible anticarcinogenic mechanisms include antiproliferation, induction of apoptosis, antiangiogenic and detoxification activity, in addition to antioxidant activity (Kresty *et al.*, 2006; Prior *et al.*, 2005). Anthocyanins have been reported to induce enzyme activation to inhibit possible DNA damage by the carcinogens. Ellagic acid from raspberries is also reported to exert biological effects as an antiproliferative agent through induction of apoptosis (Ross *et al.*, 2007). In *in vitro* studies, the phenolic compounds found in raspberries have been shown to inhibit tumour metastasis by reducing expression of vascular endothelial growth factor (VEGF), a promoter of angiogenesis (Huang *et al.*, 2006; Liu *et al.*, 2005).

A growing body of research shows that berry fruits have positive effects on age-related neurodegenerative diseases such as Alzheimer's disease (Miller & Shukitt-Hale, 2012; Joseph *et al.*, 2009; Shukitt-Hale *et al.*, 2009). In animal models, the capability of berries for enhancing both cognition and motor control has been demonstrated. In addition, dietary supplementation with berry fruits has been shown to reduce serum oxidative and inflammatory markers in humans and, in some cases, improve cognition among older adults.

The role of phytochemicals in diabetes and obesity has also been widely researched. Polyphenolic fractions from raspberry extract, *e.g.* anthocyanins, have been shown to have the potential to interact with the enzyme R-amylase, and ellagitannins can interact with R-glucosidase, thus improving the therapeutic potential effect on post-meal blood glucose levels (McDougall *et al.*, 2005b; McDougall & Stewart, 2005). Anthocyanins have also been demonstrated to play a role in improvement of adipocyte function (a factor in obesity), and to induce insulin secretion (Tsuda, 2008; Jayaprakasam *et al.*, 2005).

Berry phenolics have been widely studied as antimicrobials for their possible role in several infections, *e.g.* in urinary system, causing diarrhoea, inflammation, colonisation of the tooth surface by oral streptococci and periodontitis (Westhoff *et al.*, 2012; Yamanaka *et al.*, 2004; Howell, 2002; Mills & Bone, 2000). The complex phenolic polymeric structures of ellagitannins are reported to be effective in inhibiting growth of the pathogenic bacteria *Salmonella, Clostridium, Staphylococcus* and *E. coli* (Westhoff *et al.*, 2012; Labrecque *et al.*, 2006; Ryan *et al.*, 2001).

The polyphenols from *Rubus* berries are also reported to be chelating agents for metal ions such as iron, aluminium and copper. At neutral pH, tannin compounds, such as proanthocyanidins or the galloyl ester of glucose, form complexes with metal ions and these complexes precipitate easily at neutral pH through the gut barrier (Santos-Buelga & Scalbert, 2000; Kennedy & Powell, 1985).

2.7 Metabolism and bioavailability

The polyphenols are widely distributed in coloured fruits, including Rubus berries, and possess antioxidant activities. They have also been suggested to scavenge reactive species. The bioavailability of these compounds is the concentration that reaches the circulatory system and is available to the target site, and is distributed to organs and tissues (Paredes-Lopez *et al.*, 2010). Bioavailability studies of polyphenols in humans are scarce, but more studies have been performed *in vitro* or in animals.

The polyphenols are metabolised through a common pathway (Scalbert & Williamson, 2000) (Figure 2).

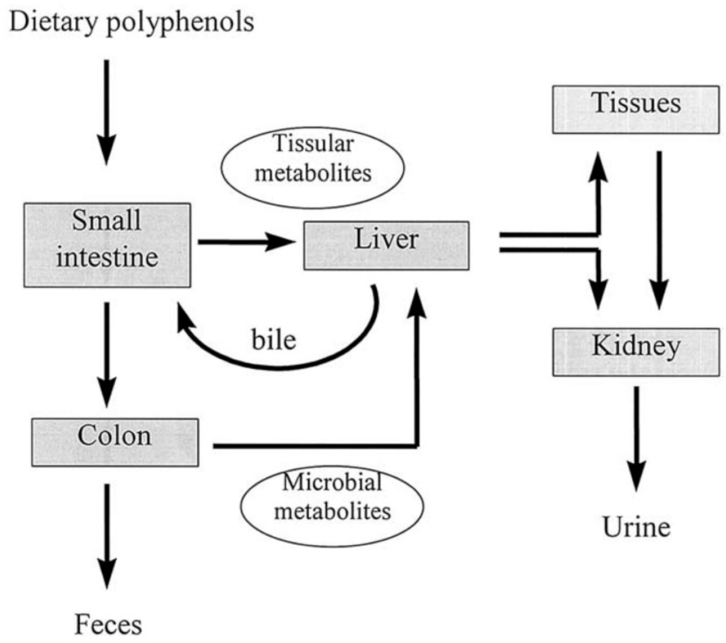

Figure 2. Routes for dietary polyphenols and their metabolites in humans. Reprinted with the kind permission of the authors (Scalbert *et al.*, 2002).

The majority of the polyphenols in food are in the form of esters, glycosides or polymers and need hydrolysis by the intestinal enzymes or intense metabolism by the colon microflora before they are available to the body. However, the aglycones (the non-sugar component of a glucoside molecule) are primarily hydrophobic compounds and are easily absorbed through biological membranes (Colin, 2006; Claudine *et al.*, 2004). The absorption and metabolism of different polyphenols is minimal on entering the body, but is mainly supported by the liver through cytochrome P450 enzymes (Konishi *et al.*, 2004; Lei *et al.*, 2003). The chemical structure of polyphenols determines their rate and extent of intestinal absorption and the nature of the metabolites in the plasma. During the progression of absorption, the polyphenols are conjugated in the small intestine and later in the liver. In previous studies, they have been reported to be mainly found in methylated, sulphated, glucuronidated and glycated form (Rao & Snyder, 2010; Lhoste *et al.*, 2003; Donovan *et al.*, 2001). This conjugation is a metabolic detoxification process for xenobiotic impurities, restricting their toxic effects and facilitating their elimination through biliary and urinary excretion. The polyphenols are secreted via biliary routes into the colon (large intestine), where they are intensively metabolised by the microflora and the resulting aglycones are reabsorbed by the system or removed through faeces (Scalbert *et al.*, 2002).

The flavonoids present in Rubus berries are mainly glycosides, which cannot passively diffuse through the membranes. Glycosidated flavonoids such as flavones, flavonoles, isoflavones and anthocyanins are attached to sugars and need deglycosylation before they can be absorbed (Colin, 2006; Gee *et al.*, 2000). Bioabsorption and metabolism of anthocyanins are very rapid , and the concentration in plasma peaks within 15 to 60 minutes. Excretion is typically complete within 6 to 8 hours (Holst & Williamson, 2008). The acylated flavonoids, such as epicatechin, are attached with the galloyl substitutions (attachment of gallic acid) and appear to be easily absorbed in aglycone form by the tissues. They are not very common in *Rubu*s berries (Walgren *et al.*, 2000). The bioavailability of anthocyanins in general is reported to be very low, between 0.01 and 1.8 % of the ingested amount (McGhie & Walton, 2007; Donovan *et al.*, 2001). In human studies where volunteers were fed 960 µM anthocyanins (200 g fresh blackberries) (cyanidin-3-glucoside), only 0.16% of the original anthocyanin ingested and >60% as metabolites were found in the urine. In animal studies, only 0.088% the ingested dose of cyanidin-3-glucoside fed as Marion berry was recovered, conjugated as glucuronide and methylate (Rao & Snyder, 2010; McGhie & Walton, 2007; Wu *et al.*, 2004).

Ellagitannins are reported to be partially hydrolysed in the gut to release ellagic acid (Scalbert & Williamson, 2000). Animal studies have shown that the metabolism of ellagitannins from raspberries is not catalysed by enzymes in the gut but occurs optimally at pH 8, or after 1 hour of exposure to the microflora in the caecum (Daniel *et al.*, 1991). A study in humans investigating the pharmacokinetic properties of ellagitannins found that after consumption of strawberries, raspberries, walnuts and oak, neither ellagitannins nor ellagic acid were found in the urine, although these were metabolised to urolithin B conjugated with glucuronic acid. In that study the level and time for maximum excretion varied between different fruits, but in general occurred after 16 hours of consumption and lasted until 40 hours. The same trend has been found in studies analysing excretion from clonal metabolites. For raspberries, it ranges from 0.2 to 7.6% (Cerda *et al.*, 2005). In another study, treatment with ellagic acid, obtained after hydrolysis, showed that plasma levels were highest 1 hour after eating, and recovery in urine was maximum between 0 to 4 hours after eating (Paredes-Lopez *et al.*, 2010; Stoner *et al.*, 2005). The bioavailability of the ellagitannins varies depending on the type of derivatives and microbiota in the human body(Stoner *et al.*, 2005.

The phenolic acids, *e.g*. benzoic acid and cinnamic acid, are present in both free and esterified forms. Berries often contain high levels of benzoic acid, often as hydroxybenzoic acid, which is not common in other fruit and vegetables. Hydroxybenzoic acids are components of complex structures such as hydrolysable tannins (gallotannins and ellagitannins) (Mullen *et al.*, 2002a). The hydroxycinnamic acids are more common in fruit such as blueberries, kiwis, plums, cherries and apples rather than in Rubus berries (Claudine *et al.*, 2004). In the human body, complex molecules such as flavonoids and tannins undergo hydrolysis or are digested by microflora and rapidly converted into their corresponding phenolic acid or aldehydes. The bioavailability and antioxidant activity depend on the number and position of hydroxyl group bound to the aromatic ring. Hydroxycinnamic acid has been found to be more bioavailable than hydroxybenzoic acid (Sroka & Cisowski, 2003).

2.8 Bioactive components in berries

The bioactive compounds include:

2.8.1 Vitamin C

Ascorbic acid and dehydroascorbic acid are collectively named vitamin C. The name 'ascorbic acid' is derived from *a*-, meaning 'no' and '*scorbutus*' meaning 'scurvy', the disease caused by a deficiency of vitamin C. Dehydroascorbic acid is the oxidised form of ascorbic acid. Dehydroascorbic acid is most often only a minor part of the vitamin C content, and normally it comprises only 5-10% of total vitamin C in fruit crops, although exposure to air, the presence of metal ions or light treatment can increase the dehydro- form by oxidation or activation of ascorbate oxidase enzyme. Dehydroascorbic acid can be reduced back to ascorbic acid by dehydroascorbate reductase, or irreversibly oxidised to ketoglonic acid. Due to this reduction ability of ascorbic acid, it is considered a scavenger of reactive oxygen species. However, humans cannot synthesise ascorbic acid due to lack of the enzyme L-gluconolactone oxidase (Davey *et al.*, 2000). In plants ascorbic acid is synthesised from D-glucose or D-galactose (Naidu, 2003).

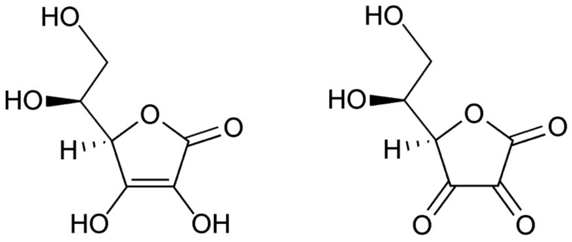

Figure 3. Ascorbic acid (left) and dehydroascorbic acid (right). Diagram taken from the free internet encyclopaedia (Wikipedia)

2.8.2 Phenolic compounds

The berry fruits are characterised by high contents of the phenolics, such as phenolic acids, flavonoids, tannins and stilbenes (Paredes-Lopez *et al.*, 2010) (Figure 4).

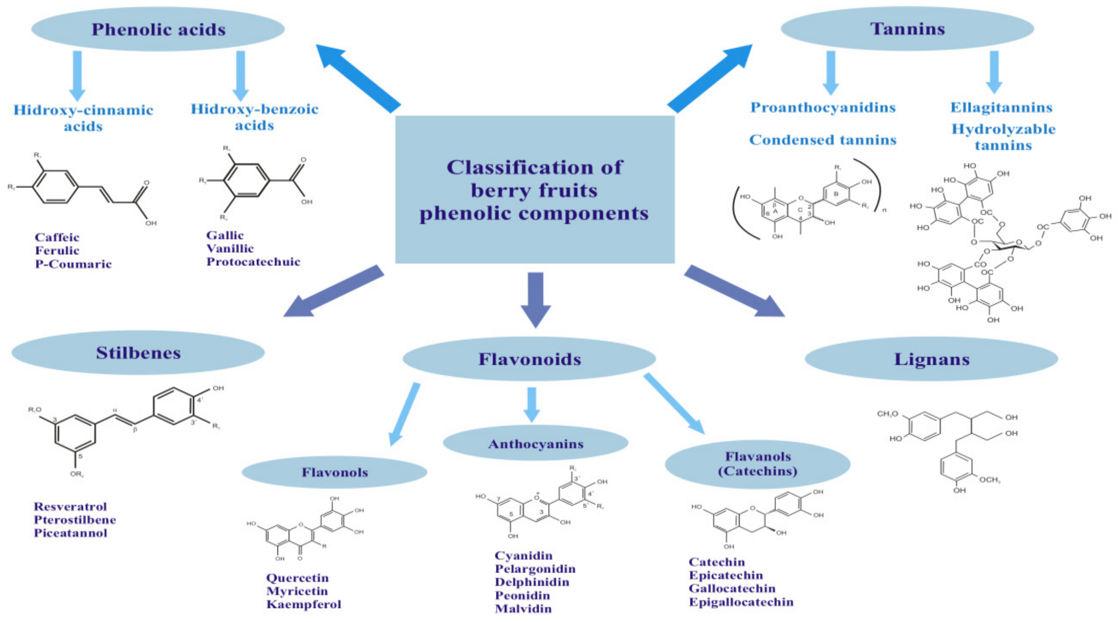

Figure 4. Major phenolic compounds in berry fruit. Figure reprinted with the kind permission of the authors (Paredes-Lopez *et al.*, 2010).

Phenolic acids

Phenolic acids in berry fruits are represented by e.g. cinnamic acid and benzoic acid derivatives (Clifford, 2000b) (Figure 5).

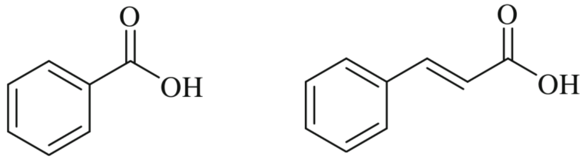

Figure 5. Basic structure of phenolic acid. Diagram reprinted with the kind permission of the author (İlhami, 2012).

The hydroxybenzoic acids are found in both free and esterified form, and consist of p-hydroxybenzoic acid, salicylic acid, gallic acid and ellagic acid, the last only found in a few red fruits such as strawberries, raspberries and blackberries. Furthermore, hydroxybenzoic acids are components of complex structures such as hydrolysable ellagitannins (Scalbert & Clifford, 2000; Tomas-Barberan & Clifford, 2000). Ellagic acid is also the predominant phenolic acid in raspberries, blackberries and strawberries, where it is present in free form or esterified to glucose (Häkkinen *et al.*, 1999).

The hydroxycinnamic acids are more common than the hydroxybenzoic acids, and are mostly found as *p*-coumaric, caffeic, ferulic and sinapic acids. They are glycosylated as derivatives or esters of quinic acid, shikimic acid and tartaric acid. The highest content is found in fruits such as blueberries, cherries, apples and plums. Hydroxycinnamic acids are most commonly found as derivatives of caffeic acid (both in free and esterified form), representing up to 75% of the total amount, and are also the most abundant phenolic acid in fruit (Singh & Ramassamy, 2008). These are found in all parts, are responsible for the tart taste of fruit and in the presence of polyphenol oxidase they are easily oxidised and transformed into brown-coloured compounds. The highest concentrations are seen in the outer parts of ripe fruit (Manach *et al.*, 2004; Clifford, 2000c).

Flavonoids

Flavonoids are low molecular weight polyphenolic substances (C6–C3–C6) and are mostly derived from benzo-γ-pyrone. They are classified according to the substitution group such as hydroxyl, methoxy and glycosides. The major flavonoid classes include flavonols, flavanols and anthocyanins (Heim *et al.*, 2002). These compounds are present in glycosylated form. The associated sugar moiety is very often glucose or rhamnose, but other sugars may also be involved (*e.g*. galactose, arabinose, xylose). In plants they are normally found in the outer and aerial tissues (skin and leaves) because their biosynthesis is stimulated by light (Huda-Faujan *et al.*, 2009; Heim *et al.*, 2002). During plant growth they act as a defence system against stressors such as ultraviolet radiation, pathogens and herbivore attack. As a constituent in human food they help to prevent some degenerative diseases (Harborne & Christine, 2000; Bravo, 1998).

Flavonols are the most ubiquitous flavonoids in foods, and the main representatives are quercetin and kaempferol. The richest sources are vegetables such as onions, curly kale, leeks and broccoli, fruits such as blueberries and red wine (Claudine *et al.*, 2004). Flavones, another class of flavonoids, are much less common than flavonols in fruit and vegetables. Flavones consist chiefly of glycosides of luteolin and apigenin (Manach *et al.*, 2004; Harborne & Williams, 2000).

Flavanols exist in both the monomer form such as catechins, and in the polymer form such as proanthocyanidins. Catechins are found in many types of fruit, *e.g*. apricots and grapes used for producing red wine, but green tea and chocolate are by far the richest sources (Lakenbrink *et al.*, 2000). Catechin and epicatechin are the main flavanols in fruit, although gallocatechin, epigallocatechin and epigallocatechin gallate are found in certain seeds of grapes, tea and leguminous plants (Arts *et al.*, 2000). Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers and polymers of catechins that are bound together by C-C and occasionally by C-O-C links (C4 and C8 or C6) (Khanbabaee & van Ree, 2001; Guyot *et al.*, 1998). Proanthocyanidins are common in fruits, such as grapes, peaches, kaki fruit, apples, pears, and also different berry fruit. These are responsible for the astringent character of fruit and may also be beneficial for human health (Santos-Buelga & Scalbert, 2000).

The anthocyanins are pigment compounds dissolved in the vascular sap of the epidermal tissues of the flower and fruit. They exist in different chemical forms, both coloured and uncoloured, according to pH. The anthocyanins differ with respect to the hydroxyl group, the type and number of sugars attached, and the nature and number of aliphatic or aromatic acids attached to sugars (Jin-Ming *et al.*, 2003; Heim *et al.*, 2002). Cyanidin is the most common anthocyanidin in plant food, including raspberries and blackberries. The anthocyanins are known as the anthocyanidins (aglycones) when in glycoside form (bound to a sugar molecule). The most common sugar substitutes on the anthocyanidins (aglycone) are glucose, fructose, galactose, rhamnose, xylose and arabinose (Teresa & Ballesta, 2008; Clifford, 2000a). The dominant anthocyanins in *Rubus* berries (raspberries and blackberries) are cyanidin-3-sophoroside and cyanidin-3-glucoside (González *et al.*, 2003; Mullen *et al.*, 2002a) (Figure 6).

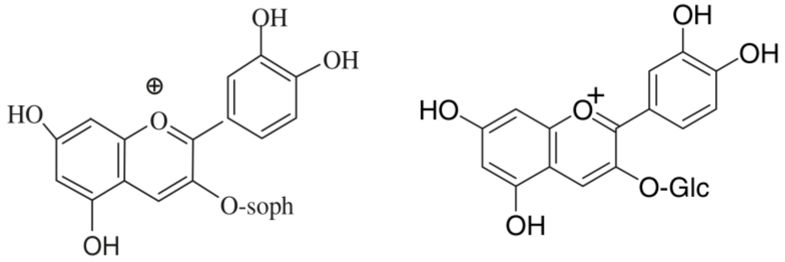

Figure 6. Cyanidin-3-sophoroside (left) and cynidin-3-glucoside (right).

It is the free hydroxy groups on the anthocyanin ring structure that give the molecules their strong scavenging antioxidant properties (Castañeda-Ovando *et al.*, 2009; Kong *et al.*, 2003; Rice-Evans & Miller, 1996).

Tannins

Tannins are ubiquitous in plants, particularly in red fruits. Some of the major contributions of tannins in food in European countries are via soft fruit such as blackberries, raspberries and strawberries, although wine can also be important. During plant growth they protect plant organs from several stress factors and in the human diet they are responsible for the tart taste, possibly due to interaction with proteins in the mucous membranes of the mouth and gustatory receptors (Hager *et al.*, 2008). Tannins are mainly stored in the vacuoles or in the surface wax of plants. In these storage sites they can be maintained in active form, ready to work against undesired stress to the plant. The tannins are found in both hydrolysable form (gallotannins and elligitannins) , *e.g.* esters of gallic acid and ellagic acid, complex tannins and condensed tannins (Khanbabaee & van Ree, 2001). Hydrolysable plant tannins usually attract more attention due to their high antioxidant properties. They are defined as hexahydroxydiphenoyl (HHDP) esters of carbohydrates and cyclitols, the oxidised form of galloyl groups. The most common oligomers in red raspberries and blackberries include lambertianin A and sanguiin H-6 along with casuarictin and potentillin. When ellagitannins are hydrolysed with water, acids or bases, they yield ellagic acid (Landete, 2011), which constitutes 77-88% of the total phenolics in Rubus berries (Häkkinen *et al.*, 1999). They can be present in glycosylated or acylated form, or in ellagitannin derivative form, usually esterified with glucose (Maatta-Riihinen *et al.*, 2004). Ellagic acid is a dimer and is reported to have very high antioxidant properties (Quideau, 2009; Clifford & Scalbert, 2000) (Figure 7).

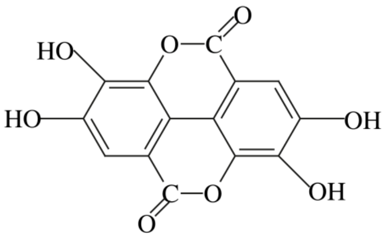

Figure 7. Ellagic acid. Diagram reprinted with the kind permission of the author (Landete, 2011).

Stilbenes

This group of compounds includes resveratrol, which is only present in low quantities in the human diet, although with high wine consumption it can be higher. It occurs as free resveratrol and as piceid, *i.e.* 3-β-mono-D-glucoside (Wang *et al.*, 2002). Small quantities of trans-resveratrol have been found in bilberry, cowberry, red currant, cranberry, strawberry and blackberry (Cvejic *et al.*, 2005; Ehala *et al.*, 2005; Rimando *et al.*, 2004; Wang *et al.*, 2002) (Figure 8).

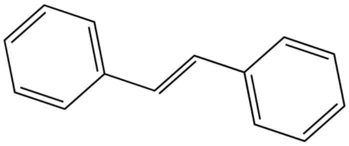

Figure 8. .General form of the trans-stilbenes.

2.9 Fruit quality

Rubus berries, such as raspberries and blackberries, are among the coloured fruits that are a very popular and important part of the food consumed in Northern Europe. These fruit have long been collected and consumed simply because of their taste aspect, but today the increased awareness of health issues has intensified consumer demand. In general, the quality assessment is based on visual aspects of the fruit, texture, flavour and health compounds in the fruit (Pelayo *et al.*, 2003).

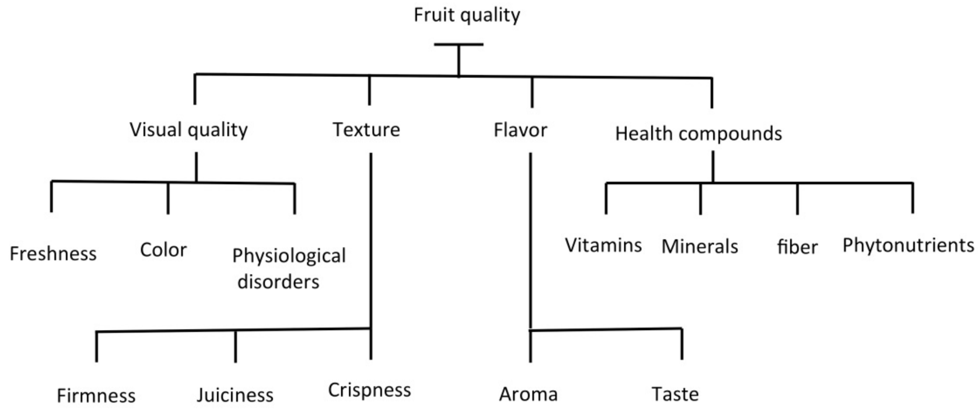

Figure 9. Quality assessment of fruit. Flow chart according to features described features in the literature.

The visual aspect of quality includes freshness, colour and absence of decay or physiological disorders, which play a key role in primary selection by the consumer (Figure 9). The flavour is due to aroma compounds such as ethyl acetate, linalool, 3-methylbutanal and hexanal, as well as taste compounds such as different sugars and acidity. The balance of the two latter attributes determines fruit acceptability for the market (Vazquez-Araujo *et al.*, 2010). Fruit 'texture' is an organoleptic quality appreciated by the consumer that basically includes fruit firmness, juiciness and crispness. The firmness of fruit, commonly defined as the mechanical response essential to the fruit structure, is influenced by the stage of physiological development, degree of ripeness, damage and turgidity, and is understood as an attribute that ought to be maintained during post-harvest handling, transport, storage and processing (Sousa *et al.*, 2007). Recently, the awareness of fruit health compounds has directed consumer preferences more to the nutritional, physiological and antioxidant qualities of fruit, based on the level and composition of various bioactive compounds such as vitamins, minerals, fibre and especially phenolic compounds (Silva *et al.*, 2007).

2.10 Quality variation in Rubus berries

Raspberries and blackberries can be commercially grown in a greenhouse or the open field, often covered with plastic tunnels. Fresh berries are highly perishable and their quality and shelf-life can be greatly affected by different pre-harvest and post-harvest factors.

2.11 Pre-harvest factors

Many aspects such as genetics, environmental factors and cultural practices affect the post-harvest quality of Rubus berries.

2.11.1 Genetic factors

Quality factors are reported to be more or less genetically controlled, as the level and chemical composition of the bioactive compounds vary according to cultivar (Scalzo & Mezzetti, 2010). Increased fruit firmness, resistance to rain damage and resistance to viruses and grey mould (*Botrytis cinera*) have been achieved by Rubus berry breeding and genetic programmes. In recent work, gene expression that encodes for the enzyme responsible for anthocyanin biosynthesis was reported for berry plants (Clark *et al.*, 2007). Anthocyanins are responsible for the red, purple, blue and yellow colour of berries. Colour variation during storage occurs with different raspberry and blackberry cultivars (Dossett *et al.*, 2010; Lo Piero *et al.*, 2005; Haffner *et al.*, 2002). A number of plant characteristics have been improved in recent years, including enhanced yield potential, improved disease resistance, seedlessness, heat tolerance and winter hardiness. More recently, primocane production has been introduced for Rubus berries to extend the harvest season (Clark, 2005). Fruit size has been increased from very small to large in released cultivars (Finn *et al.*, 1998).

2.11.2 Cultural regime

Soil type, compost, mulching and fertilisation influence the water and nutrient supply to the plant and can affect nutritional composition and antioxidant activity of the harvested fruit (Kader, 2002). A deficiency of water can influence the yield and post-harvest quality of berry fruits (Prange & DeEll 1997). High rainfall during the fruit growing season is reported to influence the composition of harvested fruit and its susceptibility to mechanical damage during shipment and storage (Kader, 2002).

Plant nutrition has a major influence on fruit quality. Nitrogen, potassium, phosphorus and calcium in particular have been reported to have pronounced effects on the level of compounds and shelf-life after harvest in berry fruits (Anttonen & Karjalainen, 2009; Goldman *et al.*, 1999; Robert K. & Jennifer R., 1997). Nitrogen is one of the limiting factors for primary production of berry crops, as it is the basic unit for protein synthesis. Nitrogen is the plant nutrient used in the largest quantities and it can regulate cane size and number of Rubus berries. An adequate soil nitrogen supply allows optimal development of fruit colour, flavour, texture and nutritional quality, whereas over-application has been shown to have negative effects on the storage quality of the berry fruit. High nitrogen doses are also reported to decrease some of the antioxidant content, probably due to rapid plant growth and development, and thus preferential allocation of resources are directed to growth processes rather than secondary metabolism (Ali *et al.*, 2012; Anttonen & Karjalainen, 2009; Buskiene & Uselis, 2008; Mitchell *et al.*, 2007; Winter & Davis, 2006; Jeppsson, 2000). The negative effects of high nitrogen on blackberry cultivars are not well documented, apart from a reported decreased level of vitamin C (Ali *et al.*, 2012; Alleyne & Clark, 1997).

Although potassium is not directly involved in the synthesis of bioactive compounds or plant structure in berries, it is involved in numerous physiological and biochemical processes vital for plant growth, yield, quality and stress response. Potassium is the most mobile cation in the berry plant and is involved in photosynthesis and stomata regulation during transpiration. Potassium is also involved in pH regulation of the plant, maintenance of turgour, stress tolerance and enzyme activation (Kowalenko, 1994; Lang, 1983; Morris *et al.*, 1983).

Calcium plays an important role in plant cell elongation and division. It also plays an important role for berry fruit quality, as it is reported to maintain cell wall integration, bind together neighbouring cell walls and control the semi-permeable properties of membranes. Calcium deficiency can manifest itself as early as fruit set and can continue to exist if untreated, resulting in poor fruit quality, especially during the shelf-life after harvest (Ferguson *et al.*, 1999). Excess amounts of calcium are reported to be non-toxic and serve as a detoxifying agent by binding toxic elements and maintaining the cation-anion balance in cells.

2.11.3 Climatic factors

Environmental factors such as light, CO_2, relative humidity, temperature and water availability are major direct or indirect constraints for plant photosynthesis. Environmental factors affect the content of bioactive compounds indirectly by giving the prerequisites for photosynthesis, and thereby providing energy or precursors of the synthesis of the bioactive compounds. Further, the synthesis of these compounds are also affected directly by various environmental factors (Hewett, 2006)

The texture of berry fruits is affected by traits such as number of cellular organelles and biochemical constituents such as water content, cell wall composition and turgour pressure (Sams, 1999). Thus, the external growing environment could lead to changes in the final quality of the berry fruit and its shelf-life after harvest. Temperature and light intensity are reported to have a major influence on the nutritional quality of berry fruits. The location and season of fruit growth, as well as monthly and yearly variations in weather conditions, define the flavour of berries and their content of vitamin C, carotenoids and especially phenolic compounds. Fruit colour is also reported to be affected by the mean temperature in the growing season (Wang & Zheng, 2001; Parr & Bolwell, 2000). Abiotic conditions, *i.e.* soil fertility and water availability, vary from year to year and site to site, and can affect the level and quality of fruit after harvest (Anttonen & Karjalainen, 2009; Wang, 2006; Spiers & Braswell, 2002).

Fruit production in different cultivation systems such as high tunnels or greenhouses can affect the bioactive compounds present due to the various aboveground and belowground factors described above. Different cultural practices such as pruning and thinning define the crop load and fruit size and this may influence the nutritional composition of the fruit (Kader, 2002). Greenhouse cultivation is reported to reduce light availability compared with production in the open field (Cockshull, 1992). The polyethylene cover in the tunnels increases the average temperature from early spring to late autumn, thus extending the season from 6 to 8 weeks, but the light intensity may be reduced by 10 to 21% (PAR) depending on the plastic material (Rohloff *et al.*, 2004).

2.11.4 Physiological factors

Fruit size in general is negatively correlated with firmness and amount of berry phenolics. Smaller fruits are firmer as they have the same number of cells as larger fruit, giving a greater density to the plant tissue. Fruit bearing order may also have a significant effect on the bioactive compounds in berry crops (Figure 10). The phenolics content is reported to be increased by 10-25% from primary to tertiary fruits (Anttonen & Karjalainen, 2009).

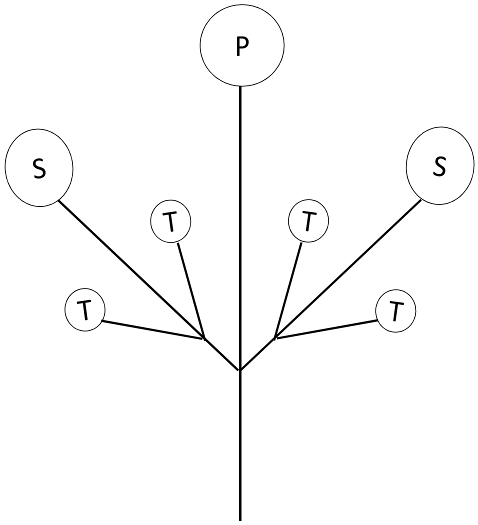

Figure 10. Fruit bearing order in the Rubus berry inflorescence. P= primary fruit, S=secondary fruit and T=tertiary fruit.

2.12 Post-harvest storage

Rubus berries (raspberries and blackberries) are very perishable and have a very short life after harvest (2 to 5 days) due to their natural soft texture and sensitivity to mould and other pathogens. Post-harvest handling and storage conditions such as packaging, relative humidity, temperature, light and storage period may affect the content of the bioactive compounds in Rubus berries (Nunes *et al.*, 2009; Mullen *et al.*, 2002b). Flavour characteristics such as total acidity and total soluble solids as well as pigment compounds such as anthocyanins play an important role for the marketability of Rubus fruit after storage (Krueger *et al.*, 2011; Kafkas *et al.*, 2006). Different sugars, acids and pigment compounds are reported to be affected by photosynthesis and pH level (Pascual-Teresa & Sanchez-Ballesta, 2008; Steyn *et al.*, 2002), and the presence of light and temperature may affect their stability during storage (Wang *et al.*, 2009; McDougall *et al.*, 2005a). Vitamin C is water-soluble and is reported to be very sensitive to temperature during storage (Kalt *et al.*, 1999). The photosynthetic compounds are primary metabolites, but any change during storage can affect other secondary metabolites such as anthocyanins, ellagic acid and total phenolics. However, ellagic acid is quite stable during storage (Zafrilla *et al.*, 2001; Rice-Evans & Miller, 1996).

3 Materials and methods

This part of the thesis gives an overview of the plant material and methodological aspects used for the chemical analyses of bioactive compounds (Papers I-IV).

3.1 Plant material

Raspberries (*Rubus idaeus* L., ‘Polka’, ‘Glen Ample’, ‘Autumn Bliss’ and ‘Autumn Treasure’) and blackberries (*Rubus fruticosus* L., cultivar Loch Ness) were obtained from the university research plots, cultivar trial stations and commercial sites (Table 1).

Table 1. *Location of growing sites in Sweden (incl. geographical co-ordinates) used for production of the raspberries and blackberries analysed in Papers I-IV*

Sample collection	Production system	Data analysed	Latitude	Longitude
Sjöbo, Scania	HT	Paper I and III	55°38'N	13°42’E
Linköping, East Gothia	GH	Paper I	58°19 ′N	15°49′E
SLU, Alnarp, Scania	GH	Paper II	55°39 'N	13°5' E
Ingelstorp, Scania	GH	Paper III	55º25’N	14°1'E
Håslöv, Scania	OF	Paper III	55°20'N	13°2’E
SLU, Rånna West Gothia	HT	Paper IV	58°27 'N	13°49 'E

HT = high tunnels, GH = greenhouse and OF = open field

3.2 Experimental setup

3.2.1 Greenhouse experiment (Paper II)

The experiment described in Paper II was performed in the research greenhouse at SLU Alnarp, Sweden. The blackberries (*Rubus fruticocus* L. 'Loch Ness') were grown in pots with Hasselfors Master coarse sifted sphagnum peat and supplied with N-P-K 14-7-15 + micronutrients. A complete randomised block design, with two blocks and four nutrient treatments, was used in the experiment. The greenhouse was adjusted to temperature 20 ˚C; relative humidity 75% and light intensity 209 (PAR) mol m^{-2} s^{-1}). One-year-old blackberry plants, outdoor vernalised (7 °C for 600 h) and trained to 80 cm height, were transferred to the greenhouse on 5 February 2008. Greenhouse temperature was increased gradually as follows: Day temperature in week 5 = 7 °C, week 8 = 15 °C, week 12 = 20 °C; night temperature in week 5 = 0 °C, week 8 = 5 °C, week 12 = 10 °C. The nutrient regime (T1, T2, T3, T4) comprised combinations of two levels (low, high) of nitrogen (60 and 100 kg ha^{-1}, respectively) and potassium (66.4 and 104 kg ha^{-1}, respectively), along with the micronutrients Ca, P, Mg, S, Mn, Fe, Zn, Cu, B, Mo, Cl, Na, distributed as nutrient solution by drip irrigation.

Table 2. *Nitrogen (low: 60 kg ha^{-1}, high: 100 kg ha^{-1}) and potassium (low: 66.4 kg ha^{-1}, high: 104 kg ha^{-1}) regime used for blackberry plants grown under greenhouse conditions (Paper II). The nutrients were added by drip irrigation. The growing season was grouped into five phases with different nutrient levels to match the requirements and growth of the crop.*

Treatment	N		K	
	Low	High	Low	High
T1	+		+	
T2	+			+
T3		+		+
T4		+	+	

Nutrient levels were tailored to plant growth and development in five distinct phases (phase 1: 5 weeks; phase 2: 6 weeks; phase 3: 5 weeks; phase 4: 6 weeks; phase 5: 4 weeks), where phases 1 and 2 represented vegetative growth and phases 3-5 generative development (Figure 11).

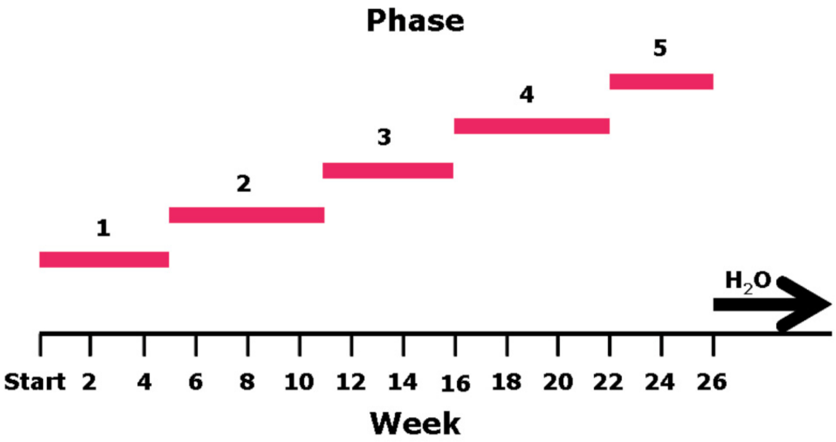

Figure 11. Phases used for nutrient regimes during the plant growing season. The phases were based on plant growth and development stages with respect to nutrient requirements. A high dose of K was applied during phases 2 and 3.

During late season, no additional nutrients were supplied and the plants were irrigated with tap water in the greenhouse (Figure 12).

Figure 12. Blackberry plants grown with different nutrient strategies in the greenhouse (Paper II). Nutrients and water were supplied continuously by drip irrigation (picture reprinted with the kind permission of A.K. Rosberg, 2008).

3.2.2 Commercial greenhouse (Papers I and III)

Blackberries (*Rubus fruticosus* L. ‘Loch Ness’) were collected from an unheated glasshouse near Linkoping (Paper I) and raspberries were collected from Ingelstorp (Paper III) (see Table 1). The experimental setup and cultural regimes were based on common practices for each greenhouse (Table 2). One-year-old potted plants cultivated in substrate were transferred to greenhouse maintaining R×R (row to row) and P×P (plant to plant) distance 2×0.5 m respectively. The nutrients, N, P and K were adjusted to 11.97, 3.03 and 15.07 g/plant respectively, through fertigation (Table 2).

3.2.3 High tunnel (HT) experiment (Paper IV)

The high tunnel experiment described in Paper IV was established at Rånna Experimental Station, Skövde. One-year-old primocane raspberry plants (*Rubus idaeus* L. ‘Autumn Bliss’, ‘Polka’ and ‘Autumn Treasure’), developed from root cuttings in pots, were transferred to the soil with Mypex mulch (0.5 x 2 m) in August 2008. The field was covered with high plastic tunnels from April to October during the study years (Figure 13). The nutrient supply comprised two organic fertilisers; pellets of Biofer (6-3-12) (Gyllebo Gödning, Malmö, Sweden) and the liquid product Bycobact (3.7-0.4-1.7) (Biobact AB, Luleå, Sweden) and these were applied considering the plant requirements and developmental stage. The high and low nitrogen levels tested were 6 g plant^{-1} and 9 g plant^{-1}, respectively, in the first cropping year and 12 g plant^{-1} and 17 g plant^{-1}, respectively, in the second year. The fertilisers were applied on two occasions in 2009 (April and June) and on five occasions in 2010 (April to August). The pellets were spread on the soil surface at each plant and the liquid product was diluted with water and applied manually. The plants were watered manually in connection with fertilisation and otherwise with drip irrigation. The temperature in the plastic tunnels during the growing season was recorded using Tinytag™ data loggers (Gemini Data Loggers, UK).

*Figure 13.*Primocane raspberry plants grown with two levels of organic nitrogen under high tunnels during 2009 and 2010 (picture reprinted with the kind permission of B. Svensson, 2009).

3.2.4 Commercial high tunnel production (Papers I and III)

In Papers I and III the experimental setup was based on commercial production systems in high tunnels at Sjöbo. The experimental setup and cultural regimes were based on common practices of the growers. One-year-old raspberry plants (*Rubus idaeus* L. 'Glen Ample') were transferred to the soil maintaining R×R (row to row) and P×P (plant to plant) distance 2.5×0.5 m respectively. The nutrient supply was made by fertigation with N, P and K 12. 4 and 20 g/plant respectively (Table 3).

Table 3. *Fertiliser application rates in the three different commercial production systems at different sites in Sweden: greenhouse (GH; 55°25'N, 14°1'E), high tunnels (HT; 55°37'N, 13°46'E) and open field (OF; 55°20'N, 13°2'E) in 2008 and 2009*

Farm type	Plants/ha	Method used	Fertilizer		
			N	P	K
			kg/ha (g/plant)	kg/ha (g/plant)	kg/ha (g/plant)
GH	14200	fertigation	170 (11.97)	43 (3.03)	213 (15.00)
HT	5000	fertigation	60 (12.00)	20 (4.00)	100 (20.00)
OF	6600	fertigation	80 (12.12)	0 (0.00)	50 (7.57)

3.2.5 Open field (commercial production system)

For Paper III, raspberry fruit was also collected from a commercial site cultivated open field at Bjärsjö (East Gotland) (Table 1). The available data is presented in Table 2. One-year-old raspberry plants (*Rubus idaeus* L. 'Glen Ample') were transferred to the soil as open field , keeping R×R (row to row) and P×P (plant×plant) distance 4×0.5 m respectively. The nutrients were supplied through fertigation with adjusted amount N and K, 12.12 and 7.57 g/plant respectively.

3.3 Harvest and storage

The raspberries and blackberries were harvested fully ripened, with harvest times ranging from early to late in the season (Papers III and IV). The harvested fruit from each commercial site were placed in a cooler at a temperature around 5 $^{\circ}$C and transported within 24 hours to the post-harvest laboratory at the Department of Horticulture, SLU Alnarp, Sweden (55°39'30"N, 13°5'0"E). There, the samples were stored in plastic containers at 2 °C and 75% relative humidity (Papers I-III). The fruit for Paper IV were not subjected to storage. During shelf-life the contents of different nutritional and bioactive compounds were measured between day 0 and 12 of storage.

3.4 Chemical analyses

The chemical analyses of different compounds were performed on fresh or lyophilised samples depending on the analyte and solvent used for the extraction. The different sugars were measured in fresh samples in Papers I-III and freeze-dried as described in Paper IV. The identification and quantification of different sugars were carried out using liquid chromatography and by comparison against external standards (D-fructose, glucose and sucrose). Titratable acidity and pH were measured according to the standard methods of the Association of Official Analytical Chemists (AOAC). Total soluble solids (TSS) content was measured using a Thermostat Refractometer RFM 80. Vitamin C was measured after a reduction treatment by dithiothreitol (DTT) using a previously described method (Esteve *et al.*, 1997). The identification and quantification were performed using a high-performance liquid chromatography (HPLC) binary gradient method with a UV detector and by comparison with external standard ascorbic acid. Total phenolics were measured by the Folin-C. spectrophotometry method modified by Dewanto *et al.*, 2002. Quantification was made by comparison against external standard gallic acid, 3,4,5-trihydroxybenzoic acid (Sigma-Aldrich, Germany). Ellagic acid and anthocyanins were identified in lyophilised samples by HPLC analysis with a diode array detector. Ellagic acid was quantified by external standard ellagic acid (Sigma-Aldrich, USA) and anthocyanins by comparison with cyanidin-3-glycoside (Polyphenols Laboratories AS, Norway). Modifications of methods were developed by evaluating different columns, binary gradients and speeds of flow. Chlorophylls (chlorophyll a and b), and carotenoids (β-carotene and lutein) were analysed by HPLC using a previously described method (Delgado *et al.*, 2004) with some modifications. Carotenoid content was quantified by external standard all-trans-β-carotene and all-trans-β-lutein, and pre-quantified by a spectrophotometric method (Hartmut & Wellburn, 1983). Chlorophyll a and b were identified and quantified by comparison against external standards chlorophyll a and b (Sigma-Aldrich, Germany).

3.5 Statistics

Statistical analysis of HPLC data was performed by ANOVA using procedure GLM in SAS (SAS Inst. Inc. Cary, NC USA) and Minitab version 15 (Minitab Inc., United States). Differences between means were analysed for each variable, *e.g.* yield and fruit size components (Paper IV) and level of different taste compounds, as well as bioactive compounds (Papers I-IV). Differences were considered statistically significant at $p<0.05$. Pearson correlation coefficients (PROC CORR: SAS Inst. Inc. Cary, NC, USA) were calculated to examine the relationship between phytochemicals (Paper 1). Multivariate statistics followed by PCA (Minitab version 15; Minitab Inc., United States) were performed to test the load of discrimination for different bioactive compounds in relation to nutrient strategies and cultivar differences (Papers II and IV).

4 Results and discussion

4.1 Berry generative characters (fruit yield and weight) (Paper IV)

Generative characters such as fruit weight and crop yield were used in Paper IV to evaluate the optimum level of organic fertiliser for different primocane raspberries. The fruit weight ranged from 3.9 to 6.0 g berry^{-1} and fruit yield from 9.97 to 12.52 tonnes per hectare (corresponding to 1.34 to 1.67 kg plant^{-1}). The results for berry weight are in accordance with previous studies (Milivojevic *et al.*, 2011; Eyduran *et al.*, 2008a). However, the yield in the present study was higher than previously reported for eight primocane cultivars cultivated in open field conditions (Sonsteby *et al.*, 2009) and comparatively less or similar to that of some summer-bearing cultivars (Sonsteby *et al.*, 2009; Rempel *et al.*, 2004). The range of fruit size and yield components presented in this thesis indicate that primocane raspberries have great potential to produce high yield with good quality fruit. While measuring fruit yield, it is important to note differences in key production practices (plant spacing, irrigation, fertilisation), cultivation system (open field, tunnel, greenhouse), and cultivar, as yield is reported to be affected by these pre-harvest factors (Hanson *et al.*, 2011; Thompson *et al.*, 2009).

4.2 Range of compounds analysed (Paper I to IV)

In this thesis, the main sugars found in raspberries and blackberries were fructose, glucose and sucrose. Fructose and glucose were found to be the predominant sugars in all samples analysed, especially blackberries. The ratio of different sugars in raspberries was similar (fructose 35-45%, glucose 30-35% and sucrose 30-35%), but in blackberries the sucrose content was low (10-15%). The fructose content detected in raspberries ranged from 76.19 to 332.03

mg g DW^{-1} (corresponding to 0.68 to 2.78 g (100 g FW)$^{-1}$), the glucose content from 56.52 to 252. mg g DW^{-1} (corresponding to 0.51 to 2.05 g (100 g FW)$^{-1}$)) and the sucrose content from 88.56 to 188.87 mg g DW^{-1} (corresponding to 0.79 to 1.59 g (100 g FW)$^{-1}$). In blackberries the fructose content ranged from 162.22 to 226.06 mg g DW^{-1} (corresponding to 1.61 to 2.50 g (100 g FW)$^{-1}$), the glucose content from 160.07 to 225.93 mg g DW^{-1} (corresponding to 1.59 to 2.491 g (100 g FW)$^{-1}$)) and the sucrose content from 12.23 to 13.52 mg g DW^{-1} (corresponding to 0.12 to 0.13 g/100g FW). The concentrations for different sugars analyzed in present study in raspberries was comparatively lower (study 1) or in the range (study 3 and 4) of previously analyzed raspberry genotypes collected from different regions or cultivated under conventional as well as organic methods (Skupien *et al.*, 2011; Cekic & Ozgen, 2010). The blackberry sugar contents are in accordance with previous studies (Fan-Chiang & Wrolstad, 2010). The TSS content in raspberries ranged from 7.46 to 10.54 % FW and the titratable acidity (TA) from 1.74 to 4.61 % FW. Whereas in blackberries the corresponding ranges were 7.00-8.10 and 1.08-1.39 % FW, respectively. These values are comparable with previous investigations for the post flowering temperature influence and cultivation method effects on fruit quality in raspberries (Remberg *et al.*, 2010; Pantelidisa *et al.*, 2007) and blackberries (Pantelidisa *et al.*, 2007; Josefina *et al.*, 2005). A summary of generative, sensory attributes and level of phytochemicals with respect to genetics, climate and cultural regimes is presented in table 4, 5 and 6. The purpose of this information is to give an overview in the area, relevant to the investigated regimes in this thesis. The values are taken from review studies and research papers. The climate and cultural regimes cited provides limited compositional data based on factorial studies but not a full literature review.

Vitamin C concentration in different cultural and climate regimes in this thesis varied from 1.77 to 3.11 mg g DW^{-1} (corresponding to 19.47 to 34.34 mg/100g FW) in raspberries and 0.83 to 1.96 mg g DW^{-1} (corresponding to 10.49 to 21.67 g (100 g FW)$^{-1}$) in blackberries, which is in agreement with previous results (Skupien *et al.*, 2011; Pantelidisa *et al.*, 2007; Vool *et al.*, 2007).

The qualitative composition of polyphenols showed a wide range between different cultivars, cultural regimes and climatic factors. The ellagic acid content in raspberries and blackberries ranged from 3.89 to 11.6 mg g DW^{-1} (corresponding to 43.13 to 103.91 g (100 g FW)$^{-1}$) and 8.68 to 15.23 mg g DW^{1} (corresponding to 88.102 to 184.76 mg/100g FW), respectively. These contents are similar to those reported in previous studies (Bobinaite *et al.*, 2012; Vrhovsek *et al.*, 2008). Anthocyanins are present in high proportions in red-purple-crimson coloured fruit and vegetables. In this thesis the anthocyanin content ranged from 2.56 to 10.21 mg g DW^{-1} (corresponding to 32.15 to 124.76 g (100 g FW)$^{-1}$) in raspberries (paper I, III and IV) and 16.43 to 20.46 mg g DW^{-1} (corresponding to 199.37 to 225.13 g (100 g FW)$^{-1}$) in blackberries (paper I and III). These results are in agreement with previous studies (Bobinaite *et al.*, 2012; Lugasi *et al.*, 2011; Siriwoharn *et al.*, 2004). The total phenolic content in raspberries and blackberries ranged from 4.45 to 21.52 mg g DW^{-1} (corresponding to 66.83 to 300.20 g (100 g FW)$^{-1}$) and 18.49 to 26.55 mg g DW^{-1} (corresponding to 182.31 to 322.10 g (100 g FW)$^{-1}$) respectively. Again, these are similar to ranges reported in other studies (Skupien *et al.*, 2011; Remberg *et al.*, 2010; Perkins-Veazie & Kalt, 2002).

Table 4. *Summary of generative and sensory attributes of raspberries with respect to genetics and pre-harvest climatic and cultural regime*

Compounds	Effect of genetic variation	Reference	Effect of climate and cultural regime	Reference
Yield (g/plant)	*33.3-2644.0*	*(Milivojevic et al., 2011; Yao & Rosen, 2011; Stephens et al., 2009)*	*1000-2200*	*(Rempel et al., 2004)*
			1487-3853	*(Sonsteby et al., 2009)*
Berry wt. (g)	*0.7-8.9*	*(Milivojevic et al., 2011; Remberg et al., 2010)*	*2.3-8.9*	*(Remberg et al., 2010)*
			1.4-2.9	*(Vool et al., 2007)*
Dry matter (%)	*9.5-15.4*	*(Bobinaite et al., 2012; Remberg et al., 2010; Weber et al., 2008)*	*9.4-12.9*	*(Remberg et al., 2010)*
			12.8-13.7	*(Skupien et al., 2011)*
TSS (%)	*9.26-14.0*	*(Vrhovsek et al., 2008; Weber et al., 2008)*	*9.9-13.3*	*(Malowicki et al., 2008)*
pH	*2.8-3.5*	*(Malowicki et al., 2008; Weber et al., 2008)*	*2.8-3.2*	*(Remberg et al., 2010)*
			2.6-3.1	*(Malowicki et al., 2008)*
Total acidity (%)	*2.8-3.6*	*(Milivojevic et al., 2011; Remberg et al., 2010)*	*1.7-3.7*	*(Remberg et al., 2010)*
			1.5-2.3	*(Vool et al., 2007)*
Total sugars (g/100g FW)	*2.07-7.7*	*(Ali et al., 2011; Cekic & Ozgen, 2010)*	*4.7-7.6*	*(Skupien et al., 2011)*

Table 5. Summary of generative and sensory attributes of blackberries with respect to genetics and pre-harvest climatic and cultural regime

Compounds	*Effect of genetic variation*	*Reference*	*Effect of climate and cultural regime*	*Reference*
Yield (g/plant)	*56.8-8360.0*	*(Wu et al., 2012; Eyduran et al., 2008b)*	*56.4-95.2*	*(Eyduran et al., 2008b)*
Berry wt. (g)	*1.4-10.8*	*(Wu et al., 2012; Alisha L. Ruple et al., 2010)*	*1.0-3.6*	*(Vool et al., 2007)*
			6.6-9.2	*(Makus, 2011)*
Dry matter (%)	*10.0-15.1*	*(Tamer, 2012)*	*14.5-20.7*	*(Vool et al., 2007)*
TSS (%)	*8.2-21.3*	*(Vrhovsek et al., 2008)*	*8.9-16.8*	(Vool *et al.*, 2007)
pH	*2.6-3.5*	*(Fan-Chiang & Wrolstad, 2010; Alleyne & Clark, 1997)*	*2.3-4.3*	*(Reyes-Carmona et al., 2005)*
Total acidity	*1.1-2.6*	*(Fan-Chiang & Wrolstad, 2010)*	*0.3-0.6*	*(Vool et al., 2007)*
			1.1-4.2	*(Josefina et al., 2005)*
Total sugars (g/100g FW)	*3.2-10.5*	*(Fan-Chiang & Wrolstad, 2010)*	*5.4-6.8*	*(Fan-Chiang & Wrolstad, 2010)*

Table 6. Mean values (mg/100 g FW) of different phytonutrients in raspberries and blackberries with respect to genetics and pre-harvest cultural regime

Spp.	Compounds	Effect of genetic variation	Reference	Effect of climate and cultural regime	Reference
Raspberry					
	Vitamin C	*15.6-37.7*	*(Bobinaite et al., 2012; Pantelidisa et al., 2007)*	*22.2-54.2*	*(Vool et al., 2007)*
				19.4-28.6	*(Remberg et al., 2010)*
	Total phenolics	*278.6-714.7*	*(Cekic & Ozgen, 2010; Weber et al., 2008)*	*186.9-249.2*	*(Remberg et al., 2010)*
				189.3-275.8	*(Skupien et al., 2011)*
	Anthocyanins	*2.1-400.0*	*(Weber et al., 2008; Pantelidisa et al., 2007)*	*166.0-306.0*	*(Lugasi et al., 2011)*
	Ellagic acid	*83.9-246.3*	*(Vrhovsek et al., 2008)*	*79.0-103.5*	*(Vrhovsek et al., 2008)*
Blackberry					
	Vitamin C	*8.3-16.5*	*(Pantelidisa et al., 2007; Benvenuti et al., 2004)*	*8.6-17.5*	*(Vool et al., 2007)*
	Total phenolics	*143.1-566.1*	*(Lugasi et al., 2011; Perkins-Veazie & Kalt, 2002)*	*296.0-311.0*	*(Perkins-Veazie & Kalt, 2002)*
	Anthocyanins	*50.0-256.0*	*(Lugasi et al., 2011; Siriwoharn et al., 2004)*	*123.0-135.0*	*(Perkins-Veazie & Kalt, 2002)*
	Ellagic acid	*38.0-244.0*	*(Vrhovsek et al., 2008)*	*87.9-119.4*	*(Ali et al., 2012)*

4.3 Genetic variations and cultivar differences (Paper IV)

There were large variations in fruit size and yield, as well as in concentrations of nutrients and bioactive compounds such as vitamin C, total phenolics, anthocyanins and ellagic acid, with respect to fruit genetics (Paper IV). In raspberries, the average berry size of the cultivar ‘Autumn Bliss’ was lower than that of ‘Polka’ and ‘Autumn Treasure’. The cultivar ‘Polka’ produced the highest yield in 2009, and ‘Autumn Bliss’ the highest in 2010, but ‘Autumn Treasure’ produced the lowest yield in both years.

Principal component analysis was used to identify patterns in bioactive compounds as regards the influence of cultivar and nutrient regime (Figure 14).

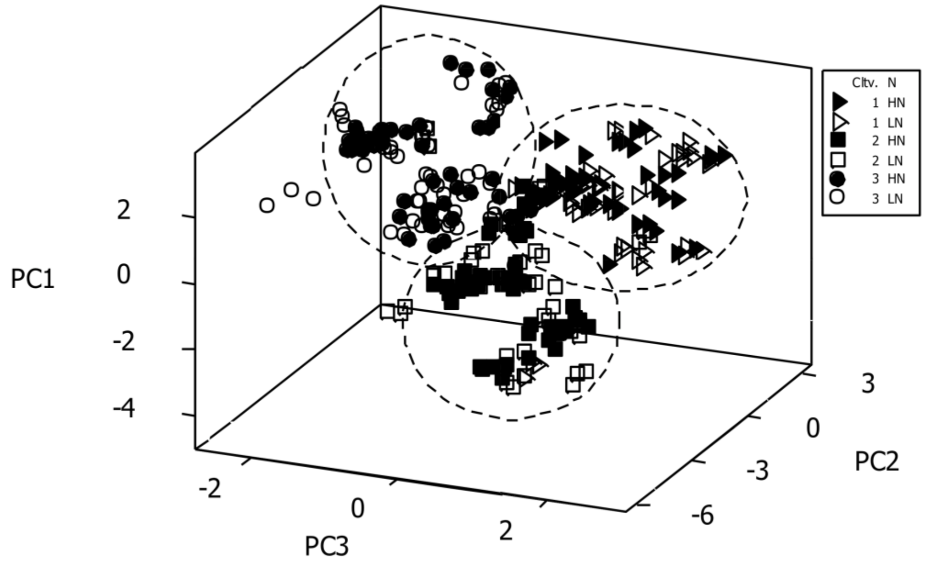

Figure 14. Principal component analysis for bioactive compound composition in three primocane cultivars (‘Autumn Bliss’, ‘Polka’ and ‘Autumn Treasure’) and two organic nitrogen levels, low (6 g/plant & year) and high (9 g/plant for 2009 and 12 g/plant for 2010). Open symbols represent low nitrogen (LN or N1) and filled symbols represent high nitrogen (HN or N2). The numbers 1, 2 and 3 represent ‘Autumn Bliss’, ‘Polka’ and ‘Autumn Treasure’, respectively. PC1, PC2 and PV3 explained 40, 20 and 14 % of the data, respectively.

Principal component analysis (PCA) is a multivariate programme for identifying patterns in data, and for graphically expressing the data on the basis of their similarities and differences (Figure 14). Here, PCA was applied to investigate the data obtained in Papers II and IV. In Paper II, conventional fertilisation gave significant pattern in the levels of bioactive compounds at harvest and during storage, while in Paper IV, with organic fertilisation, there was only a cultivar difference. Thus the reason for using PCA in the thesis was to identify cultivar differences in terms of the content of bioactive compounds in primocane raspberries. Primocane raspberry cultivars evaluated for two nitrogen levels were conclusively differentiated into three components, PC 1, PC 2 and PC3, explaining 40%, 20% and 14% of the variation (Figure 14). Table 6 shows the loading of the different phytochemicals on these three components. Comparisons of phytochemicals between different cultivars and nitrogen levels showed that PC 1 was mostly dominated by total phenolics, TSS, acidity and sucrose levels, whereas different sugars (fructose, glucose and total sugars) dominated PC 2. PC3 was dominated by vitamin C, anthocyanin and ellagic acid. The graphical representation of the PC showed discrimination between cultivars, but did not show any significant difference for nitrogen level (Figure 14). PC 1 discriminated the cultivars ‘Autumn Bliss’ and ‘Autumn Treasure’ from (Polka’, with low and high scores corresponding to total phenolics, TSS, acidity of the fruit and sucrose level (Table 7). The PC2 compounds were only dominant in cultivar ‘Polka’ and showed major changes for different sugars. The PC3 compounds were dominant in all three cultivars.

Table 7. *Loading of measured bioactive phytochemicals on principal components (PC) 1, 2 and 3 for the primocane raspberry cultivars 'Autumn Bliss', 'Polka' and 'Autumn Treasure' under two organic fertiliser levels low; (6g/plant for year 2009& and 12 g/plant for year 2010) and high (9 g/plant for the year 2009 and 17 g/ plant for the year 2010)*

Variable	*Comparison between 3 cultivars under two nitrogen level*		
	PC1	*PC2*	*PC3*
Vitamin C	*0.214*	*-0.050*	*0.408*
Tot. phenolics	*0.341*	*-0.261*	*-0.227*
Anthocyanins	*0.244*	*0.041*	*0.634*
Ellagic acid	*0.260*	*0.076*	*-0.600*
TSS	*-0.402*	*-0.293*	*0.006*
Total acidity	*0.258*	*0.071*	*0.010*
Fructose	*0.321*	*-0.470*	*0.107*
Glucose	*0.220*	*-0.596*	*-0.088*
Sucrose	*-0.472*	*-0.113*	*0.026*
Tot. sugars	*-0.330*	*-0.492*	*0.035*

There were even larger variations for the two different Rubus species studied i.e. raspberries and blackberries. In general, the raspberries produced sweeter berries with high vitamin C, whereas the blackberries had high concentrations of antioxidant compounds such as total phenolics, anthocyanins and ellagic acid (Papers I-IV). The conventional fertilisation based on plant requirement produced larger changes in blackberry phytochemicals (Paper II). Similar genetic differences and responses to pre-harvest conditions have also been found in other studies (Milivojevic et al., 2011; Yao & Rosen, 2011; Stephens et al., 2009). The influence of cultivar found in this thesis indicates that the yield components as well as the phenolic compounds are heritably controlled and respond differently to pre-harvest conditions. Moderately negative genotype correlations for yield components to the level of phytochemicals was found in primocane raspberries, suggesting that it is possible to develop high-yielding types high in phytochemicals.

4.4 Cultural regime and effects of fertilisation (Paper II and IV)

Variations in the organic fertiliser supplied, especially with respect to nitrogen level, gave significant variations in fruit size and yield, but no appreciable difference in bioactive compounds (Paper IV). The reason for the lack of response could be that the soil contained sufficient nitrogen at the lower nitrogen fertiliser applied and increasing the level was not capable of increasing the fruit generative and quality attributes in primocane raspberries. In addition, organic fertilisers release nutrients slowly, and it might be difficult to adjust plant nutrition to the right time of the season. (Vason Boonterm *et al.*, 2010; Unuk *et al.*, 2006; Alleyne & Clark, 1997).

However, when the effects of conventional fertilization supplied on plant demand were studied, a large variation in the content of bioactive compounds was found (Paper II). The blackberry plants treated with high nitrogen produced more total sugar in the berries (values not shown). This is not in agreement with previous theories of a dilution effect with increased plant growth, resulting in accumulation of more water in the berry fruit. This might be explained by the amount of fertiliser applied in Paper II, as the high nitrogen level applied may not have attained the level to affect plant growth and adversely the level of bioactive compounds. However, when high amounts of potassium fertiliser were supplied together with the high amount of nitrogen, the values were in a good range in average for the total phenolics, anthocyanins and vitamin C as compared with other treatments. In another investigation in grape berries, high contents of polyphenols were found when high nitrogen fertiliser levels were applied in combination with high levels of potassium (Dario Stefanelli *et al.*, 2010; Vason *et al.*, 2010). Individual high nitrogen application, with low potassium applied reduced the vitamin C content in blackberries in Paper II, in accordance with previous studies (Lee & Kader, 2000). These results suggest that balanced plant nutrition and other cultivation factors are very important in defining Rubus berry yield, content of phytochemicals and shelf-life after harvest.

4.5 Effects of annual climate variations and harvesting time differences (Paper I, III and IV)

4.5.1 Harvesting time

Compounds contributing to taste, such as sugars (fructose, glucose and sucrose), are consumer-driven traits that together with visible appearance play an important role for fruit marketability during the season. In this thesis floricane raspberries (Paper III) grown in the open field and in glasshouses produced increased sugars from early to late in the season, while the berries grown in high tunnels did not. Instead, berries cultivated in high tunnels produced high sugar levels early and late in the season. The trend of an increase in sugars from early to late in the season was also seen for primocane raspberries, even with reduced light and average temperature in late season (Paper IV). This might be explained by physiological factors such as plant age and increased fruit sink strength at the end of the harvest season (Watson *et al.*, 2002; Lee & Kader, 2000).

Harvesting time had a notable influence on vitamin C content in both floricane and primocane raspberries. The floricane raspberries (Paper III) contained more vitamin C at the beginning of the season with the expection of greenhouse cultivated berries, whereas in primocane raspberries (Paper IV) the content was highest in late harvests. The results in Papers IV support previous findings for raspberries and strawberries of increasing ascorbic acid (vitamin C) content with decreasing growing temperature from 24 to 12 °C (including ambient conditions) (Remberg *et al.*, 2010; Wang & Camp, 2000). Comparing the vitamin C content to average temperature during the season in Papers IV, the primocane raspberries produced more vitamin C at lower temperatures. Thus this thesis contributes some experimental evidence for the long-standing Nordic claim that fruit and vegetables grown in specific temperature and light conditions at high latitude may have a high vitamin C content (Remberg *et al.*, 2010).

The concentrations of bioactive compounds in the berries studied here, especially antioxidants such as anthocyanins, ellagic acid and total phenolics, also varied from early to late in the season. The total phenolics varied differently (Paper III), while the ellagic acid content was high in the early part of the season. No specific trend was found for anthocyanin content during the season. The results for total phenolics and anthocyanins were in agreement with those in previous studies but the ellagic acid content was higher in late harvested fruits (Anttonen & Karjalainen, 2009). In the beginning of the season, plants preferentially use the allocated resources for vegetative growth. This might lead to lower substrate availability for phenylalanine ammonium lyase enzyme, which stimulates phenolic compound synthesis, ultimately lowering the phenolic content. The effect might also be due to the dilution factor, the increased biomass and decreased fruit size at late harvesting due to plant physiological factors. High concentrations of phenolic compounds, including ellagic acid, have been reported in late harvested blackberries and strawberries (Anttonen *et al.*, 2006; Wang, 2006).

4.5.2 Annuals variations

Variations between years may be due to climate or other environmental factors if the cultural practices remain consistent. The variations between years shown in this thesis in floricane raspberries harvested from open field sites, high tunnels and greenhouses (Paper III) and in primocane raspberries harvested from high tunnels (Paper IV) were most likely due to climate factors. Details on actual mean temperature and relative humidity in the relevant growing seasons can be found in Papers III and IV.

In terms of variations in taste compounds such as different sugars (fructose, glucose and sucrose), the floricane raspberries grown in high tunnels and the greenhouse produced significantly higher sugars in 2008 than in 2009, although there was no significant change for field-grown berries (Paper III). The differences in total sugars could be explained by the average global radiation received and sunshine hours during the fruit season. The higher global radiation and sunshine hours in 2008 may have resulted in increased photosynthesis, thereby providing the plants with the capacity to accumulate more sugars. The minor differences in mean temperature and light intensity between 2009 and 2010 did not significantly influence the content of total sugars in primocane raspberries (Paper IV). These annual variations are in accordance with previous findings (Wang, 2006).

Total phenolics in floricane raspberries also showed high levels in 2008, and ellagic acid in 2009 (Paper III), but no considerable differences between years were found for primocane raspberries ((Paper IV)). The anthocyanins did not show annual variations (Papers III and IV). The contents of anthocyanins and ellagic acid have been reported to be elevated by high temperature and increased light intensity (Kassim *et al.*, 2009; Anttonen *et al.*, 2006; Wang, 2006), but this trend was not seen in the present study. Ultraviolet light stimulates the synthesis of individual anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) in plants. In particular, cyanidin-3-glucoside is a UV-absorbing anthocyanin (Kassim *et al.*, 2009; Grimplet *et al.*, 2007). The increased daylight hours during the season are reported to increase accumulation of anthocyanins (Lu & Yang, 2006). Dry matter

4.6 Dry matter (Paper I to IV)

The reasons for measuring dry weight were to investigate substrate accumulation during the season, and to limit the influence on the results of transpiration and thereby weight loss during storage. The data presented are based on both a fresh weight (Paper I) and dry weight basis (Papers II-IV; data not shown) depending on the research question. The percentage dry weight for raspberries studied in this thesis ranged from 8.5 to 16.14 %, and for blackberries from 9.5 to 12.5 % (Papers I-IV). Dry matter production and accumulation in raspberries varied during the season, with cultivar and also between different years (data not shown). The blackberries cultivated with different nutrient strategies also showed significant variations in dry matter level at harvest. The changes in dry matter between different years and harvest time could be related to climate conditions, especially as affecting the root zone environment, *i.e.* temperature, water content and nutrient availability, and to plant age. Plant age is negatively correlated with physiological functions such as water movement in the plant and photosynthesis. The results presented in this thesis confirm previous findings of increased content of dry matter from early to late in the season and variations between different years, cultivars and nutrient supplies (Vool *et al.*, 2007; Haffner *et al.*, 2002; Percival *et al.*, 1998). During post-harvest storage, there were also minor but significant changes in dry matter content (Papers I-IV). In general, irrespective of the pre-harvest conditions, dry matter content showed unchanged or small increase with increasing storage time (Papers I-IV; data not shown). Metabolic activity in fresh fruits continues after harvest. The use of sugars for respiration or water losses through transpiration may influence dry matter content during storage (Tano *et al.*, 2005; Becker *et al.*, 1996). However, previous studies reported better preservation during storage in controlled atmosphere conditions (Agar & Streif, 1996). The small increases or minor fluctuations during storage in Papers I-III probably reveal that respiration and transpiration in the berries continued even at low temperature (2 °C).

4.7 Effects of pre-harvest factors on changes during post-harvest storage (Paper I to III)

The fructose and glucose in raspberries increased during storage, while the sucrose content decreased (Papers I and III). Large fluctuations in the sugar content were found in fruit cultivated in the open field (Paper III) and in late season raspberries (Paper I). A possible reason could be the variable open field environment in terms of rainfall or changing humidity during the fruit growing season. The results presented in this thesis support previous findings suggesting that taste compounds, such as different sugars and acidity, are determined by genotypic variation as well as environmental factors, varying with time of the harvest and annual variation. Further, it has been shown that cell wall disassembly can lead to high fluctuations in fruit quality grown in the uncertain climate conditions that prevail in the open field. A high water content and low turgor in cell walls could lead to fruit susceptibility to mechanical damage, physiological disorders and decay (Cordenunsi *et al.*, 2003; Kader, 2002; Sams, 1999). The average light intensity and temperature during the fruit season has also been reported to affect the post-harvest quality of berries (Cordenunsi *et al.*, 2005). The level of changes reported in this thesis, in particular for fructose but also for sucrose and glucose, was also influenced by the time of harvest. The floricane raspberries from the latest harvest tended to show the smallest changes during storage. In general, a decrease in sucrose content and an increase in fructose and glucose indicate enzymatic hydrolytic activity during storage (Ayala-Zavala *et al.*, 2004; Perkiins-Veazie. *et al.*, 1999). The smaller changes found during storage at the end of the season could be attributable to lower enzymatic activity at that time.

In blackberries, the different nutrient strategies did not show any specific trend in the fruit during storage, and the levels of different sugars were maintained, though with fluctuations during storage, while no significant changes were found for sucrose (Paper II). These results are in agreement with previous findings (Perkiins-Veazie *et al.*, 1999; Plowman, 1991). The overall findings for the flavour compounds show that the total sugars and acidity were metabolised in both raspberries and blackberries, resulting in increased fructose and glucose and decreased sucrose and total acidity.

The changes reported in this thesis regarding the content of different sugars during storage were also affected by the time of harvest, *i.e.* seasonal variations (Paper III). For fructose in particular, but also for sucrose and glucose, the latest harvest time tended to show the smallest changes during storage. The concomitant decrease in sucrose and increase in fructose and glucose during storage for the majority of the harvest occasions indicate that enzymatic hydrolysis took place during storage.

Vitamin C in raspberries showed fluctuations during storage, but no specific trend for fruit harvested at different times during the season, or variations between different years (Papers I and III). The same trend was found for blackberries treated with different nutrient strategies (Paper II) or harvested late in the season (Paper I). The results confirm that low temperature and an acidic medium (low pH) during storage stabilise vitamin C (Haffner *et al.*, 2002; Agar *et al.*, 1997).

The concentrations of phenolic compounds such as anthocyanins and ellagic acid in raspberries and blackberries varied significantly during storage. The amount of anthocyanins and ellagic acid in floricane and primocane raspberries was well maintained or the anthocyanins slightly increased during storage, but no changes were found for total phenolics content (Papers I and III). The variations found are not likely to be due to pre-harvest (harvest time and annual variation) conditions but rather being the result of physiological process caused by the post-harvest conditions during storage The results confirm previous findings that low temperature storage (0.5 °C) positively influences the content of phenolic compounds including anthocyanins, total phenolics and ellagic acid (Shin *et al.*, 2007; Cordenunsi *et al.*, 2005; Nunes *et al.*, 2003; Kalt *et al.*, 1999).

However, in blackberries, conventional fertilisation (applied with plant demand with high nitrogen levels, significantly reduced the content of anthocyanins during storage (Paper II). The anthocyanins and in addition the total phenolics were also decreased during storage when fruit was harvested late in the season (Paper I). Excess nitrogen fertiliser has been reported to decrease the post-harvest quality in some berry fruits such as chokeberries (Jeppsson, 2000) and strawberries (Anttonen & Karjalainen, 2009). Concerning how blackberry phenolic compounds are maintained during storage, varying results have been presented in the literature (Pantelidisa *et al.*, 2007; Perkins-Veazie & Kalt, 2002; Perkiins-Veazie. *et al.*, 1999). In Paper II ellagic acid was quite stable during storage, except for minor changes, irrespective of the pre-harvest factors applied (late harvest and treatment with different nutrient strategies), supporting previous findings (Vrhovsek *et al.*, 2009). The overall results for raspberries and blackberries in this thesis show that pre-harvest factors are very important in defining the shelf-life quality after harvest.

5 Concluding remarks

Based on the research questions formulated at the start of this thesis, the following conclusions were drawn:

- Primocane raspberries are a very good source of bioactive compounds, especially when harvested late in the season.

- Chemical fertilisation significantly influences the level of different bioactive compounds in blackberries. Compounds contributing to taste, such as sugars and acidity, are particularly sensitive, so optimised fertilisation is very important for marketability after harvest.

- Organic fertilisation in the range 6 g nitrogen/plant (new plants) to 12 g/plant (after the first year) is sufficient to produce a high yield of good quality fruit.

- High tunnel farming extends the season and gives good quality regarding the content of taste compounds and bioactive compounds. Greenhouse cultivation gives fruit with good quality of post-harvest.

- Variations between years and harvest times can have a great impact on the content of bioactive compounds in Rubus berry fruit. Climate factors should be taken into consideration in addition with other cultivation parameters.

- Early harvesting is recommended in order to obtain a high ellagic acid content in raspberries.

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