



Sugars, Acids and
Phenolic Compounds
in Currants and
Sea Buckthorn in Relation
to the Effects of
Environmental Factors

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**Food Chemistry and Food Development
Department of Biochemistry**

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In memory of my father

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ABSTRACT

Currants (*Ribes* spp.) and sea buckthorn (*Hippophaë rhamnoides*) are cultivated and applied as raw materials in food, cosmetics and medicines in Europe and Asia. Sugars, sugar derivatives, fruit acids, vitamin C and phenolic compounds are important components contributing to the nutritional value and sensory properties of berries. A thorough understanding of the factors influencing the biosyntheses of these compounds is of crucial importance in order to obtain targeted quality of berries by proper cultivation, breeding and biotechnological methods.

Sugars, sugar alcohols, fruit acids, ascorbic acid and phenolic compounds were investigated quantitatively in berries of six currant cultivars and two sea buckthorn subspecies grown at different locations in Finland, Canada and China during 2003–2011. The effects of genetic background, growth latitude and altitude, and weather conditions on the concentration of these compounds in the berries were studied systematically and statistically.

Fructose (3.47–4.57 and 0.95–2.61 g/100 mL juice, respectively) and glucose (3.02–4.38 and 2.52–2.74 g/100 mL juice) were the two most abundant sugars in currant and sea buckthorn samples. Citric acid (1.61–3.34 g/100 mL juice) dominated in currants, while malic acid (2.22–4.60 g/100 mL juice) and quinic acid (1.41–1.51 g/100 mL juice) were the two most abundant acids in sea buckthorns. L-Quebrachitol (0.19–0.56 g/100 mL juice), methyl-*myo*-inositol (0.02–0.06 g/100 mL juice), *myo*-inositol (~ 0.02 g/100 mL juice), and ethyl β -D-glucopyranoside (0.02–0.09 g/100 mL juice) were detected only in sea buckthorns.

Significant differences were observed between different subspecies/varieties/cultivars of currants and sea buckthorn. Among the six currant cultivars, black currant cultivars Mortti and Ola showed similar compositional profiles of sugars, organic acids and phenolic compounds, and contained the highest amount of total organic acids (3.65–3.74 g/100 mL juice in Mortti and Ola vs. 2.70–3.11 g/100 mL juice in the other cultivars), ascorbic acid (179–190 vs. 22–107 mg/100 mL juice) and total phenolic compounds (340.21–350.71 vs. 2.65–240.72 mg/100 g fresh weight). The green currant cultivar Vertti had a higher total sugar content (9.92 vs. 6.99–8.61 g/100 mL juice) and sugar/acid ratio (3.34 vs. 2.17–2.80) than all the other currant cultivars. Anthocyanins were abundant in berries of black currant [in the form of delphinidin (108–193 mg/100 g berry) and cyanidin glycosides (117–141 mg/100 g berry)] and red currant [in the form of cyanidin glycosides (27 mg/100 g berry)], but were absent in green and white currants. Black and green currants (*R. nigrum*) contained significantly higher amounts of

hydroxycinnamic acids (5.00–11.35 vs. 0.23–1.25 mg/100 g berry) and flavonols (8.88–10.70 vs. 1.40–2.35 mg/100 g berry) than red and white currants (*R. rubrum*). Berries of *H. rhamnoides* ssp. *mongolica* showed clearly lower contents of sugars (3.80 vs. 6.05 g/100 mL juice), sugar alcohols (0.23 vs. 0.65 g/100 mL juice) and organic acids (3.87 vs. 6.93 g/100 mL juice) than *H. rhamnoides* ssp. *sinensis*.

Berries of different subspecies/cultivars showed differences in the compositional response to growth latitude and weather conditions. The black currant cultivar Melalahti was less influenced by weather conditions, and therefore exhibited stable qualities under varying growth sites and cropping years. In contrast, the quality components of the other currant cultivars showed a clear response to varying weather conditions. Temperature had a positive influence on the contents of major sugars and acids in black currants Mortti and Ola ($r = 0.53\text{--}0.87$, $p < 0.01$), but negative influences on the contents of these compounds in white currant ($r = -0.59$ to -0.77 , $p < 0.01$). In red currant, the concentration of sugars increased as temperature and radiation increased ($r = 0.34\text{--}0.83$, $p < 0.01$). Temperature and radiation displayed a negative impact on the accumulation of major phenolic group in red ($r = -0.23$ to -0.69 for cyanidin-3-*O*-sambubioside, $p < 0.05$), green ($r = -0.24$ to -0.74 for total hydroxycinnamic acid content, $p < 0.05$), and white currant ($r = -0.23$ to -0.67 for total hydroxycinnamic acid content, $p < 0.05$). In contrast, temperature ($r = 0.23\text{--}0.76$, $p < 0.05$) and radiation ($r = 0.25\text{--}0.67$, $p < 0.05$) positively affected the accumulation of anthocyanins in black currants. Dry weather with relative air humidity $< 50\%$ favored the accumulation of major sugars and acids in green currant ($r = 0.34\text{--}0.67$, $p < 0.01$), and of major acids in red ($r = 0.37\text{--}0.68$, $p < 0.01$) and white currants ($r = 0.31\text{--}0.63$, $p < 0.01$), whereas it suppressed the accumulation of major sugars and acids in black currants Mortti and Ola ($r = -0.47$ to -0.76 , $p < 0.01$), and of major sugars in red ($r = -0.74$ to -0.78 , $p < 0.01$) and white currants ($r = -0.67$ to -0.68 , $p < 0.01$). The accumulation of hydroxycinnamic acids in green ($r = 0.27\text{--}0.64$, $p < 0.01$) and white ($r = 0.21\text{--}0.56$, $p < 0.05$) currants increased under weather conditions with relative air humidity $< 70\%$, but that of anthocyanins in black and red currant was less affected by relative humidity variables. High radiation, high precipitation and low air humidity showed negative impacts on the total acid content ($r = -0.26$ to -0.75 , $p < 0.01$) but no impact on the total sugar content in the berries of sea buckthorn of *H. rhamnoides* ssp. *mongolica*. High temperature from January to March had a positive effect ($r = 0.43\text{--}0.46$, $p < 0.01$) on the accumulation of total acids, and high temperature in the other months showed a negative impact ($r = -0.37$ to -0.71 , $p < 0.01$).

LIST OF ABBREVIATIONS

AsA	ascorbic acid
cv.	cultivar
Cy-glc	cyanidin-3- <i>O</i> -glucoside
DHA	dehydroascorbate
DHAR	dehydroascorbate reductase
Dp-glc	delphinidin-3- <i>O</i> -glucoside
DW	dry weight
Fru	fructose
FW	fresh weight
Glc	glucose
GLDH	L-galactono-1,4-lactone dehydrogenase
HCA	hydroxycinnamic acid
Ka-glc	kaempferol-3- <i>O</i> -glucoside
Ka-rut	kaempferol-3- <i>O</i> -rutinoside
MDHAR	monodehydroascorbate reductase
MI	<i>myo</i> -inositol
MI-1-P	<i>myo</i> -inositol 1-phosphate
My-glc	myricetin-3- <i>O</i> -glucoside
PAL	phenylalanine ammonia lyase
Qu-glc	quercetin-3- <i>O</i> -glucoside
Qu-rut	quercetin-3- <i>O</i> -rutinoside
SPS	sucrose-phosphate synthase
Suc	sucrose
SuSy	sucrose synthase
Temp	temperature
var.	variety

LIST OF ORIGINAL PUBLICATIONS

- I. Zheng, J.; Yang, B.; Tuomasjukka, S.; Ou, S.; Kallio, H. Effects of latitude and weather conditions on contents of sugars, fruit acids, and ascorbic acid in black currant (*Ribes nigrum* L.) juice. *J. Agric. Food Chem.* **2009**, *57*, 2977–2987.
- II. Zheng, J.; Kallio, H.; Yang, B. Effects of latitude and weather conditions on sugars, fruit acids and ascorbic acid in currant (*Ribes* sp.) cultivars. *J. Sci. Food Agric.* **2009**, *89*, 2011–2023.
- III. Zheng, J.; Kallio, H.; Linderborg, K.; Yang, B. Sugars, sugar alcohols, fruit acids, and ascorbic acid in wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*) with special reference to influence of latitude and altitude. *Food Res. Int.* **2011**, *44*, 2018–2026.
- IV. Zheng, J.; Yang, B.; Trépanier, M.; Kallio, H. Effects of genotype, latitude and weather conditions on the composition of sugars, sugar alcohols, fruit acids and ascorbic acid in sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) berry juice. *J. Agric. Food Chem.* **2012**, *60*, 3180–3189.
- V. Zheng, J.; Yang, B.; Ruusunen, V.; Laaksonen, O.; Tahvonen, R.; Hellsten, J.; Kallio, H. Compositional differences of phenolic compounds between black currant (*Ribes nigrum* L.) cultivars and their response to latitude and weather conditions. *J. Agric. Food Chem.* **2012**, *60*, 6581–6593.
- VI. Yang, B.; Zheng, J.; Laaksonen, O.; Tahvonen, R.; Kallio, H. Effects of latitude and weather conditions on phenolic compounds in currant (*Ribes* spp.) cultivars. *J. Agric. Food Chem.* **2013**, *61*, 3517–3532.

1 INTRODUCTION

Sea buckthorn, belonging to the genus *Hippophaë*, family Elaeagnaceae L., consists of seven species and generally grows in cold and dry areas of Asia and Europe. It has also been introduced in America and Canada. The species *Hippophaë rhamnoides* is geographically the most widely distributed species and is further divided into eight subspecies¹, among which the two subspecies *sinensis* and *mongolica* were selected as the study targets of this thesis due to their commercial importance.

Sea buckthorn is resistant to drought, cold and soil salinity. Therefore, it is suitable for many situations that are simply too demanding for most other plants. It roots easily even in dry and infertile soil due to the presence of nitrogen-fixing bacteria, *Frankia*, in its roots and is a very important plant for soil and water conservation in China. More than 90 percent of the world's sea buckthorn resources, about two million hectares, are in China. The fruits of sea buckthorn have been used as a drug in traditional Tibetan and Mongolian medicines since ancient times for their pharmacological effects on the lungs, stomach, spleen, and blood circulation. In 1977, sea buckthorn was, officially for the first time, listed in the Chinese Pharmacopoeia by the Ministry of Public Health as a medicine to relieve cough, to aid digestion and to invigorate blood circulation². Recently, scientific research on sea buckthorn has been extensively conducted and high antioxidant activities^{3,4} and beneficial effects on the skin⁵⁻⁷, mucosa⁸, eyes⁹, cardiovascular system¹⁰⁻¹² and sugar metabolism¹³ have been reported for the berries or berry fractions. Based on its high nutritive value and potential health effects, sea buckthorn has become more and more popular and widely consumed as food and used as raw materials for food, food supplements and cosmetics in Europe and Asia. However, the sour and bitter taste, as well as the high astringency, decrease the consumer preference for sea buckthorn products and restrict the exploitation and utilization of sea buckthorn berries in foods.

Currants are fruit-bearing shrubs of the genus *Ribes*, family Grossulariaceae, and are native to central and northern Europe and northern Asia. They are noted for cold hardiness and grow in temperate climates. Most species can survive temperatures of -40°C or lower. The plants perform best on deep, organic, well-drained soils with good water-holding capacity¹⁴. The species of *R. nigrum* (black currants and green currants) and *R. rubrum* (red currants and white currants) studied in this thesis are economically important species cultivated widely in European countries^{14,15}. Compared to sea buckthorn, currant berries are considered to have better flavor and taste, and are widely consumed as fresh table fruits and processed into jams, jellies, juices, liquors, and extracts for nutritional supplements. The berries have high nutritive value,

and have shown positive effects on human health, such as a reduction in the risk of atopic dermatitis¹⁶, cardiovascular diseases^{17,18}, cancers¹⁹⁻²¹, and type 2 diabetes²².

The contents of sugars and acids as well as the sugar/acid ratio are important compositional parameters influencing the sensory properties and consumer acceptance of berries and berry products²³⁻²⁵. Secondary metabolites, such as vitamin C, phenolic acids, flavonols, anthocyanins and inositols, play an important role in the nutritional value and potential health benefits of the berry fruits. In addition, phenolic compounds, with an astringent and bitter taste, often have a negative impact on the sensory quality and consumer preference of berries²⁶⁻²⁸. A thorough understanding of the factors influencing the biosynthesis of these metabolites and their concentrations in fruits is of crucial importance for further enrichment of bioactive components and for improving the sensory quality of berries by proper cultivation, breeding, and biotechnological methods.

Genetic differences, growth locations, environmental factors, cultivation techniques, and harvesting time are all important factors regulating the biosynthesis and accumulation of metabolites in fruits and berries²⁹⁻³⁸. The growth conditions at different latitudes and altitudes vary markedly in terms of growth season, day length, light quality, radiation intensity and temperature. The differences in composition and biological activities observed among the natural populations represent both the short term effects of the growth conditions and the genetic evolution as a result of long term adaptation to the environment. For a better understanding of acclimation and adaptation of plants to different latitudes, altitudes, and climates, detailed studies that focus on a comparison of genetically identical plants at different growth latitudes, altitudes, and climates are essential.

In this research, six currant (*Ribes nigrum* and *Ribes rubrum*) cultivars were planted in southern and northern Finland. Sea buckthorn (*Hippophaë rhamnoides*) bushes of nine varieties were grown in southern and northern Finland, and/or in Québec, Canada. Wild berries of sea buckthorn were collected from nine natural growth sites in China. The berries were harvested in three to seven years. The contents of sugars, sugar derivatives, fruit acids, ascorbic acid, hydroxycinnamic acid conjugates, flavonol glycosides and anthocyanins were thoroughly analyzed and compared between samples of different species/varieties/cultivars and from different origins. This study focused on the effects of natural variation in weather variables, including temperature, radiation, humidity, and precipitation, on the composition of these primary and secondary metabolites in the berries. The correlations between the compounds were also investigated to provide information for basic biochemical

and physiological studies of the biosynthetic pathways of the corresponding metabolites in currants and sea buckthorn.

The thesis starts with a literature review on the regulation of primary and secondary metabolites in fruits and berries in response to the varying environmental factors, with a special focus on the metabolites studied in this thesis.

2 REVIEW OF THE LITERATURE

2.1 Regulation of metabolites in fruits and berries by environmental variation

Their sessile nature makes plants, unlike animals, unable to take refuge from adverse conditions³¹. They must tolerate changes in temperature, irradiation, water supply, nutrient supply, and attack by herbivory³⁰. The wide range of environmental variations has driven the evolution of a complex network of acclimation mechanisms in plants that attempt to optimize resource use and survive under various stresses³¹. The main focus of this review is on the regulation of primary and secondary metabolites in fruits and berries in response to varying environmental factors. The environmental factors of interest include temperature, radiation, water supply and air humidity, although other factors such as soil composition, air CO₂ concentration, nitrogen supply, phosphorus supply, iron supply, aluminum toxicity, wounding, pathogen infection and fungal elicitors all exhibit regulatory impacts on the accumulation of metabolites. The compounds of interest are the corresponding compounds investigated in berries of currants and sea buckthorn in this thesis, including sugars, sugar derivatives, organic acids, ascorbic acid, phenolic acids, flavonols, and anthocyanins.

2.1.1 Primary metabolites

2.1.1.1 Sugars

Soluble sugars, especially sucrose, glucose and fructose, play a central role in plant structure and metabolism at the cellular and whole organism levels³⁹. Their presence in fruits is important for the dissemination of seeds. Many plants that bear edible fruits have propagated with the movements of humans and animals in a symbiotic relationship as a means for seed dispersal and nutrition, respectively. In fact, humans and many animals have become dependent on fruits as a source of food. Sugars, contributing to the sweetness of fruits and berries, are important components determining the sensory properties and influencing preference for fruits/berries. Sugars also contribute as important energy resources for animals and humans.

Therefore, the sugar concentration and its regulation in fruits and berries under various environmental conditions are of great interest in view of plant propagation as well as their consumption and utilization as food materials. Various investigations have shown complex responses of sugar contents to varying environmental factors in fruits and berries (**Table 1**).

The concentration of sugars in fruits is a result of the complex contribution of various regulation steps in the process from photoassimilate synthesis in leaves to sugar accumulation in fruit, including photosynthesis, synthesis of translocation sugars, loading of translocation sugars, translocation, unloading, membrane transport, metabolic conversion, and compartmentalization in vacuoles⁴⁰.

Table 1 shows that there is a general increasing trend in sugar accumulation in fruits and berries under elevated radiation⁴¹⁻⁴³ regardless of the plant species. When comparing shaded plants to light-exposed ones, the contents of fructose and glucose both increased from 50–60 mg/g fresh weight (FW) to 70–80 mg/g FW⁴³ in grapes, and the content of reducing sugars increased from 4.61 to 6.18 g glucose equivalent/100 g FW in strawberry⁴¹. The concentration of photoassimilates in fruits depends mainly on the photosynthetic supply from leaves, although some fruits in early stages of development can supply it by their own photosynthesis⁴⁰. The photosynthetic rate increases proportionally as incident light increases until the saturation point is reached, after which photoinhibition may occur when leaves are exposed to more light than they can utilize. Because the photosynthetic response of the intact plant is the sum of the photosynthetic activity of all the leaves, photosynthesis is rarely saturated with light at the level of the whole plant²⁹, which might explain the general increasing trends of sugar contents in response to increased light intensity (**Table 1**).

Translocation sugars (sucrose, verbascose, raffinose, stachyose, sorbitol, or mannitol^{29,40}), after being transported from leaves to fruits, can be ultimately degraded into hexose sugars or their derivatives, and metabolized for energy production or for C-skeletons for growth or storage product accumulation⁴⁴. Therefore, the metabolic conversion of translocation sugars in fruit is another important factor influencing the final concentration of the sugars in fruits. Sucrose-phosphate synthase (SPS), invertase, and sucrose synthase (SuSy)⁴⁰ are important enzymes involved in sucrose/hexose interchange and influence the sugar content and composition in fruit^{40,45}. Their availability and activity depend on the developmental status of fruits⁴⁶⁻⁵⁰ and on environmental factors. Light modulation of SPS activity is thought to be one mechanism to adjust the capacity for sucrose biosynthesis in relation to the rate of photosynthesis⁴⁴.

Other enzymes, such as fructokinase, ADP-glucose pyrophosphorylase, starch synthase, and amylase are also important to regulate the accumulation of sugars in fruits⁴⁰. During fruit development, starch degradation may contribute to sugar accumulation in some fruits. Hancock et al.⁵¹ speculated that approximately two-thirds of the sugars found in black currants (*Ribes nigrum* L.) at harvest result from starch mobilization, assuming that all of the starch lost from other plant tissues was translocated to the fruit.

In contrast to radiation, the influence of temperature on the accumulation of sugars in fruits and berries seems more complex (**Table 1**). The photosynthetic rate increases with temperature until reaching an optimal temperature point. When the temperature exceeds the optimal values required for plant growth, the inhibition of photosynthesis and stimulation of photorespiration may cause a decrease in the sugar content. An optimal temperature has been suggested for the maximum accumulation of sugars in fruits in some investigations. The sucrose content in fruits of strawberry cultivars Earliglow and Kent was highest in plants grown at 25/12°C and 25/22°C (day/night) [39.0–49.9 mg/g dry weight (DW), respectively] than those grown at 18/12°C and 30/22°C (11.3–21.5 mg/g DW, respectively)⁵². However, the total content of sugars in strawberries decreased gradually from values over 750 mg/g DW to values below 550 mg/g DW as the temperature increased, mostly because of the decreasing trends in major sugars (fructose and glucose)⁵². In purple passionfruit⁵³, the total sugar content was higher in fruits grown at 28/23°C (around 14%) than in those grown at 23/18°C (around 12.5%) and 33/28°C (around 11%). The fructose (from about 3.5% to 4.5%) and glucose (from 3.3% to 4.3%) content increased but sucrose content decreased (from 5.7% to 2%) under increased temperature in purple passionfruit⁵³.

Different responses of sugar contents to variations in temperature were reported in various plant species/cultivars (**Table 1**). Higher temperatures showed negative impacts on the accumulation of fructose and glucose in fruits of bilberry⁵⁵, grape⁵⁶, and strawberry⁶², but positive impacts in fruits of apple⁵⁴ and purple passionfruit⁵³, and no impact on satsuma mandarins⁶¹. The content of sucrose increased in fruits of melon^{58,59} and watermelon⁶⁴ but decreased in fruits of bilberry⁵⁵ and purple passionfruit⁵³ as temperature increased. In apple fruits, an optimal temperature around 20°C for sucrose accumulation in the cultivar Himekami, and a simple decreasing trend of sucrose content with increased temperature in the cultivar Fuji were reported⁵⁴. In bilberry, the content of fructose, glucose, and sucrose all decreased by as much as 17%, 20%, and 66% when the temperature increased from 12°C to 18°C. The total sugar content showed a decreasing trends as temperature increased in strawberry^{52,62} and watermelon⁶³, while this did not vary in apple fruit⁵⁴. In satsuma mandarins^{60,61}, the variations in sucrose content and total sugar content in response to temperature changes depended on the growing period when heat treatment was applied.

Table 1 Concentration response of sugars in fruits and berries in relation to the variation of environmental factors.

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
Temperature apple (flesh)	<i>Malus domestica</i> Borkh. cv. Himekami	Fru & Glc	Temp ↑		↑	54
		Suc total sugars	Temp Temp		optimal around 20°C -	
bilberry grape (flesh)	<i>Malus domestica</i> Borkh. cv. Fuji	Fru & Glc	Temp ↑		↑	55
		Suc	Temp ↑		↓	
		total sugars	Temp		-	
		Fru, Glc & Suc	Temp ↑		↓	
bilberry	<i>Vaccinium myrtillus</i> L.	Fru, Glc & Suc	Temp ↑		↓	56
		Fru & Glc	Temp ↑		↓	
kiwifruit	<i>Vitis ficifolia</i> var. <i>ganebu</i> × <i>V. vinifera</i> cv. Muscat of Alexandria <i>Actinidia deliciosa</i> (A. Chev.) C. F. Liang et A.R. Ferguson var. <i>deliciosa</i> Hayward	Fru, Glc & Suc	Temp ↑ (cell division stage of fruit)		-	57
		Fru, Glc & Suc	Temp ↑ (starch accumulation & maturation stage of fruit)		↓	
		Fru, Glc & Suc	Temp ↑		↑	
melon (juice)	<i>Cucumis melo</i> L. cv. Earl's Knight Soshumbanshu	Glc	Temp ↑		↑	58
		Fru Suc	Temp ↑ Temp ↑		- ↑	
melon (juice)	<i>Cucumis melo</i> L. cv. Earl's Knight Natsukei No. 2	Glc	Temp ↑		-	59
		Fru Suc	Temp ↑ Temp ↑		- ↑	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
purple passion fruit (juice)	<i>Passiflora edulis</i> Sims var. <i>edulis</i>	Fru & Glc sucrose total sugars	Temp ↑ Temp ↑ Temp		↑ ↓ 28/23°C > 23/18°C > 33/28°C	⁵³
satsuma mandarins (juice sac)	<i>Citrus unshiu</i> Marc. cv. MihoWase	Suc total sugars	Temp ↑ (anthesis to harvest) Temp ↑ (anthesis to harvest)		↑ ↑	⁶⁰
satsuma mandarins (juice sac)	<i>Citrus unshiu</i> Marc. cv. MihoWase	Fru & Glc Suc total sugars	Temp ↑ (0–10 weeks after anthesis) Temp ↑ (0–10 weeks after anthesis) Temp ↑ (0–10 weeks after anthesis)		– ↑ ↑	⁶¹
		Fru, Glc & Suc total sugars	Temp ↑ (10–20 weeks after anthesis)		–	
		total sugars	Temp ↑ (10–20 weeks after anthesis)		–	
strawberry	<i>Fragaria</i> × <i>ananassa</i> Duch. cv. Earliglow & cv. Kent	Fru & Glc Suc total sugars total sugars	Temp ↑ Temp Temp ↑ Temp ↑		↓ 25/12°C & 25/22°C > 18/12°C > 30/22°C	⁵²
strawberry	<i>Fragaria</i> × <i>ananassa</i> Duch.	total sugars total sugars	Temp ↑ Temp ↑ (day before harvest)	√	↓ ↓	⁶²

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
watermelon (juice)	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai cv. Kansen	total sugars	Temp ↑	(Light)	↓	63
watermelon (juice)	<i>Citrullus lanatus</i> Matsumu. et Nakai cv. Matsuribayashi NK	Glc Fru & Suc	Temp ↑ Temp ↑		- ↑	64
<u>Light conditions</u>						
bilberry	<i>Vaccinium myrtillus</i> L.	Fru, Glc & Suc	Light		-	55
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	hexose sugar	Light ↑		↑	43
grape (pulp)	<i>Vitis vinifera</i> L. cv. Pinot noir <i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	hexose sugar total sugars	Light ↑ Light ↑	Temp ↑	↑ ↑	42
strawberry	<i>Fragaria</i> × <i>ananassa</i> L. cv. Rapella	reducing sugar	Light		↑	41
<u>Water supply</u>						
apple	<i>Malus domestica</i> Borkh. cv. Braeburn	Fru Fru total sugars total sugars total sugars total sugars	Irrigation ↓ (whole season) Irrigation ↓ (late in season) Irrigation ↓ (whole season) Irrigation ↓ (late in season) Irrigation ↓ (early in season) Irrigation ↓ (late in season)		↑ - ↑ - ↓ -	65
apple	<i>Malus domestica</i> Borkh. cv. Braeburn	total sugars	Irrigation ↓ (late in season)		↓	66
black currant	<i>Ribes nigrum</i> cv. Magnus	total sugars	Irrigation		-	67

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
kiwifruit	<i>Actinidia deliciosa</i> (A. Chev.) C. F. Liang et A. R. Ferguson var. <i>deliciosa</i> cv. Hayward	Fru & Glc Suc	Water stress Water stress (early summer)		- -	⁶⁸
mango fruit	<i>Mangifera indica</i> cv. Lirfa	Suc Glc	Water stress (late summer) Irrigation		↑ response varied with leaf:fruit ratio treatment	⁶⁹
nectarine (pulp)	<i>Prunus persica</i> Batsch var. Nectarina cv. Spring Bright	Fru & Suc Glc Fru Suc total sugars Glc	Irrigation Irrigation ↓ Irrigation ↓ Irrigation ↓ Irrigation ↓ Irrigation ↓ Irrigation ↓		- ↓ ↓ ↑ - ↑ -	⁷⁰
satsuma (juice sac)	<i>Prunus persica</i> Batsch var. Nectarina cv. Summer Bright	Fru Suc total sugars	Irrigation ↓ Irrigation ↓ Irrigation ↓		- ↑ -	
mandarins	<i>Citrus unshiu</i> Marc. cv. Okitsu-Wase	Fru, Glc & Suc total sugars	Irrigation ↓ Irrigation ↓ Irrigation ↓		↑ ↑ ↑	⁷¹
strawberry	<i>Fragaria</i> × <i>ananassa</i> L. cv. Elsanta	Glc Fru Suc total sugars	Irrigation ↓ Irrigation ↓ Irrigation ↓ Irrigation ↓		↑ ↑ ↑ -	^{72,73}

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
Complex factors pomegranate (aril juice)	<i>Fragaria</i> × <i>ananassa</i> L. cv. Sonata	Glc	Irrigation		-	
		Fru	Irrigation ↓		↑	
		Suc	Irrigation		-	
		total sugars	Irrigation ↓		↑	
	<i>Fragaria</i> × <i>ananassa</i> L. cv. Florence	Fru, Glc & Suc	Irrigation		-	
		total sugars	Irrigation ↓		↓	
		Fru, Glc & Suc	Irrigation		-	
	<i>Fragaria</i> × <i>ananassa</i> L. cvs. Christine & Symphony	total sugars	Irrigation		-	
		Fru & Glc	Temp ↑, light ↑, relative humidity ↓		√	
	<i>Punica granatum</i> L.					

√, experiments were carried out in open fields over years or at different growth sites, or multi-interference between different factors may exist. Abbreviations: Temp, temperature; Fru, fructose; Glc, glucose; Suc, sucrose.

Matsumoto et al.⁵⁸ reported an increase of 100% in sucrose content in melon fruit (*Cucumis melo* L.) in associated with an increase in SPS activity when the shoots near fruits were heated at night to a minimum of 30°C. The authors inferred that heating bearing shoots near fruits, which heats the fruit, can accelerate the rate at which cells enlarge and mature, and in turn, causes an increase in SPS activity and an increase in the sucrose content in the fruit⁵⁸.

The activity of SPS and invertase in plants of different genetic backgrounds was reported to be influenced differently by temperature changes. In both spinach leaves⁷⁵ and potato tubers⁷⁶, increased SPS activity as a result of cold exposure was paralleled by an increased steady-state level of SPS enzyme protein. But, in contrast, in maturing tomato pollen⁷⁷, expression of a SPS was up-regulated by heat, which may increase the production of sucrose to play a role as osmoprotectant in maintaining cell membrane integrity and cellular function under stress. Down-regulation of the genes coding for invertases and the diminished activity were reported in the ovaries of maize and in the anthers of rice under water deficit conditions⁷⁸⁻⁸⁰. In contrast, a strong enhancement of vacuolar invertase activity was observed in mature leaves of maize subjected to water deprivation⁸¹. These results indicated the expression of gene and the enzyme activity in response to environmental conditions can differ markedly with tissue or family/species.

Sugars are also essential to the synthesis of numerous compounds that are involved in anti-oxidative protection. Glucose feeding of primary metabolism can result in enhanced reducing power in the form of NADH or NADPH. It is also the main carbon initial precursor for carotenoid and ascorbate synthesis, for the carbon skeletons of amino acids, and for glutathione synthesis. All of these compounds have been involved in defenses against oxidative stress. Therefore, different stress situations which cause the accumulation of reactive oxygen species (ROS), such as pathogen challenge, drought, salt stress, abscisic acid (ABA) treatment, low temperature, or excess excitation energy (excess light), are associated with soluble sugar (sucrose, glucose, and fructose) accumulation⁸². For example, the sucrose concentration in leaves of barley (*Hordeum vulgare*) increased under cold treatment (10°C/5°C, 16 h light/8 h dark). This might be attributed to its role in fructan biosynthesis, which is involved in cold temperature tolerance⁸³.

Water deficit is another factor influencing photosynthesis, via decreased CO₂ diffusion to the chloroplast and metabolic constraints⁸⁴. Inhibition of photosynthesis by water deficit depletes the daily production of photosynthetic products (sucrose)⁸⁵. Factors such as the intensity of water stress, the occurrence of superimposed stresses, and the plant species⁸⁴ all affect the response of photosynthesis to water stress.

In contrast to the speculation of inhibited photosynthetic rates under water deficit, most studies have reported that the content of total sugar either increased or did not change in mature fruits under drought stress (**Table 1**). Different cultivars of strawberry^{72,73} and nectarine⁷⁰ displayed different responses to variations in the water supply regarding the sugar content. Giné Bordonaba et al.⁷³ reported that the total content of sugars decreased (from 70.8 to 67.1 mg/g FW) in strawberry cv. Florence, increased in cvs. Elsanta (from 58.6 to 82.3 mg/g FW) and Sonata (from 68.8 to 81.6 mg/g FW), and did not change significantly in cvs. Christine and Symphony under low irrigation. In the case of fructose and glucose, a study on satsuma mandarins⁷¹ indicated negative correlations between irrigation and the contents of these two sugars, while a study on kiwifruits⁶⁸ showed no impact of irrigation on their concentration. The sucrose content increased in fruits of nectarine⁷⁰ and satsuma mandarins⁷¹, but did not vary in mango fruit and strawberry^{72,73} under deficit irrigation. The contradictory results reported in these studies against the speculation of reduced photosynthetic rates under a limited water supply might be explained by the regulation of sugar translocation.

Yakushiji et al.⁷¹ reported a significant increase trend in the sugar concentration, varying from 9.0 to 17.9 in fructose content, 8.5 to 17.8 in glucose content, 19.8 to 40.1 in sucrose content, and 37.8 to 75.8 mg/g in total sugar content, in response to drought stress in satsuma mandarin fruits. At the same time, the photosynthetic rates and stomatal conductance of drought-stressed trees were significantly lower than those of the well-watered plants. Thus, other mechanisms might have contributed to the increase in the sugar content of drought-stressed fruits. A ¹³C labeling experiment showed that the ¹³C distribution in fruit grown under both moderate and severe drought stress was higher than in well-watered plants. Compared with well-watered fruits, the fruit mass of moderately drought-stressed trees was similar, while that of severely drought-stressed trees was significantly lower. Thus, the authors suggested that the higher sugar content in moderately drought-stressed fruits is caused by an increase in the translocation of photosynthates into fruit under drought stress in spite of a presumed decrease in biochemical photosynthesis⁷¹. The hypothesis was that higher sugar content under water stress might be associated with restricted vegetative growth and a shift of photoassimilates to fruits, due to an attempt by the plant to reduce osmotic potential by the accumulation of solutes⁷³. In the case of severe drought stress, dehydration may function as an additional cause of solute accumulation in fruit^{71,73}. Although, in most cases, the increases in fruit soluble solids can be attributed to decreased fruit water content (solute concentration), some studies have reported increased active synthesis, and the increase in soluble solids cannot be explained solely by a concentration effect⁸⁶.

Temperature and irrigation have both been shown to affect sugar accumulation differently at different fruit developmental periods (**Table 1**). In a study on kiwifruit⁵⁷, the expression of SUS1, a sucrose synthase with the greatest homology (83–88% amino acid identity) to sucrose synthases postulated to play a role in sucrose unloading in storage organs, increased as the vines were exposed to elevated temperatures during the starch accumulation stage [41–161 DAA (days after 50% anthesis)] and maintained increased levels of mRNA during fruit maturation. No response of SUS1 to heat treatment was evident during the stage of fruit cell division (0–40 DAA). In accordance, the sugar content in mature fruits decreased in vines heated during the starch accumulation stage (18.9, 16.4 and 13.7 mg/g FW of treated fruits vs. 40.6, 35.7 and 32.9 mg/g FW of control fruits for fructose, glucose and sucrose, respectively, $p < 0.05$), but did not change in those heated during fruit cell division ($p > 0.05$). Mills et al.^{65,66} reported a different response in the sugar concentration of Braeburn apple fruit to deficit irrigation applied at different growing periods. The total sugar content was higher in mature fruit deficit-irrigated in the whole season from 55 days after full bloom (DAFB) until final harvest than in those of the control (fully irrigated)⁶⁵. In contrast, the sugar concentration was lower in mature fruit deficit-irrigated from 55 DAFB until rewatering at 100 DAFB, although a sharp increase in the sugar content was detected at the end of the stress period (100 DAFB)⁶⁶. The authors suggested this might have occurred because of the dilution of the sugar concentration in the previously stressed fruit following rewatering⁶⁶. They also reported that fruits deficit-irrigated in the late season from 105 DAFB until harvest showed no change in sugar content compared with control plants, which suggested that fruit water relations and sugar concentration are modified if a water deficit is imposed from early in the season, and are less influenced by a water deficit imposed later in the season^{65,66}.

Overall, the accumulation of sugars is the outcome of a complex result of numerous primary and secondary metabolic pathways, regulated by a combination of various factors. In grapevines, the climatic requirements for optimum photosynthetic activity were defined: temperature between 25 to 30°C, relative humidity between 60 to 70%, and wind speed below 4 m/s⁸⁷. The presence of a co-occurrence factor may cause a large difference in the response of fruit composition to certain environmental changes. Factors other than climatic parameters may also play a role. For example, the difference in the leaf:fruit ratio causes a different compositional response of glucose to irrigation treatment in mango fruit⁶⁹.

However, it should be mentioned that the variation of experimental conditions between different investigations should be considered. For example, different varying trends of glucose in response to increases in temperature were

reported in melon fruit of different cultivars^{58,59}, which might be due to either genetic differences in the plants or the different temperature treatments applied on the plants in two investigations. In one of these investigations, the shoots near melon fruits were heated at night to a minimum of 30°C from the fifth day after anthesis (DAA) until harvest, while in the other investigation, the heat treatment was applied directly to the fruit during the early stage of the growing period from 3 to 17 DAA.

2.1.1.2 Organic acids

Organic acid metabolism is of fundamental importance at the cellular level for several biochemical pathways, including energy production, the formation of precursors for amino acid biosynthesis, and at the whole plant level in modulating adaptation to the environment⁸⁸. Organic acids, such as malic acid and citric acid, are mainly produced in mitochondria through the tricarboxylic acid or Krebs cycle³⁰ and to a lesser extent in the glyoxysome as part of the glyoxylate cycle^{88,89}. Quinic acid is a byproduct of the shikimic acid pathway (see **section 2.1.2.1.3**)^{90,91}. The high accumulation of organic acids in plant tissues is most probably due to their important role as photosynthetic intermediates⁸⁸. However, organic acids also have a potential role as metabolically active solutes for osmotic adjustment and participation in the balance of charges formed during the extensive metabolism of anions such as nitrate (NO₃)⁸⁸.

Table 2 shows that the contents of organic acid in fruits and berries varied differently in response to changes of temperature, depending on individual acids, plant species/cultivars, and growth stages. The synthesis of malate involves an exothermic reaction that may occur more favorably at lower temperatures. In contrast to this speculation, the content of malate in bilberry⁵⁵ and strawberry⁵² were reported to increase as temperature increased. The content of malic acid in bilberry increased from 380.5 to 484.9 mg/100 g FW, and that of quinic acid decreased from 2321.4 to 1811.4 mg/100 g FW as the temperature increased from 12°C to 18°C⁵⁵. But, for the citrate content, there was an interaction between origin and temperature, where northern and southern clones produced equal amount of citrate at 12°C, but the production of northern clones was higher than that of southern clones at 18°C⁵⁵. In strawberry⁵², the malic acid content increased from 10.5–11.4 to 24.5–26.6 mg/g DW as day/night temperature varied from 18/12 to 30/22°C, while the contents of citrate and total acid decreased, from 70.5–76.8 to 46.3–47.3 and from 81.0–88.2 to 71.8–72.9 mg/g DW, respectively. Different peach cultivars also showed different responses in the citric acid content to varying temperature⁹² (**Table 2**).

Fruit developmental stage is an important factor determining the accumulation of organic acids in relation to varying environmental conditions. Increasing the canopy temperature 10–20 weeks after anthesis caused a reduction in malate and citrate accumulation as well as total acid content in mature fruit of satsuma mandarins, whereas a temperature increase 0–10 weeks after anthesis did not affect the concentration of these acids. However, the accumulation of quinate in satsuma mandarin fruit was not influenced by variations in temperature at any developmental stage⁶¹. In kiwifruit⁵⁷, the content of malate was reported to decrease by 37% and 34% ($p < 0.05$), respectively, as temperature increased during the stages of starch accumulation and fruit maturation. However, increasing temperature during the cell division stage of fruit did not cause a change in the malate content in the fruit. Heating vines during different developmental stages showed no impact on the content of citrate and quinate in mature kiwifruit ($p > 0.05$). Nevertheless, the mature fruit produced under heat treatment during fruit maturation contained significantly (57%) higher quinate than those produced under heat treatment during starch accumulation, although no significant differences were found when comparing these two treated samples to control fruits⁵⁷. Or et al.⁹³ suggested that the control of malate metabolism throughout berry growth involves developmental regulation of phosphoenolpyruvate carboxylase (PEPC), malate dehydrogenase, and malic enzyme transcript availability. In a study in grape berries (*Vitis vinifera* L.), enzymes involved in malate metabolism were reported to respond differently to temperature in berries at different development stages^{94,95}. However, the genetic differences between different cultivars investigated in these studies should also be considered. A study on tomato fruits of *Solanum lycopersicum* L. cv. Micro-Tom indicated that the transcript level of genes encoding enzymes involved in organic acid metabolism responded to salt stress differently at different developmental stages. Salt stress led to a down-regulation of citrate synthase during the stages of immature green and mature green, but no significant changes were seen during the other developmental stages⁹⁶.

Light intensity also showed an impact on the concentration of organic acids in mature fruits. The content of malic acid was reported to decrease as photoperiod or light intensity increased in bilberry⁵⁵ and grapes^{42,43}, while the citric acid content in bilberry was not influenced by the change of light conditions. Grape (*Vitis berlandieri* × *Vitis vinifera*) berries of cultivar Merlot exposed to light were reported to contain a lower amount of organic acids, especially malic acid (42% lower), and a higher content of flavonols (79% higher), than those grown under shade. The authors suggested that the decrease in organic acids may be mainly explained by the higher temperature caused by light exposure⁴². Sweetman et al.⁹⁷ suggested that the formation of secondary

compounds requires a source of carbon that could have been provided, at least in part, by the increased usage of malate in the light-treated fruit. This indicated the possibility of an impact of light on the concentration of organic acids in plants and fruits. In a study on strawberry⁶², there appeared to be a positive response of the total acid content to both global radiation and sunshine hours in most of the genotypes, although some genotypes showed different varying trends.

The interaction between temperature and radiation and their influence on plant metabolism in some investigations should be considered. In bilberries, the response of quinic acid content to light intensity depended on the temperature applied⁵⁵. There was no difference in quinic acid content in berries between the light treatments at 12°C, but long day light exposure induced a higher content of quinic acid at 18°C⁵⁵ (**Table 2**).

Different investigations on various grape cultivars showed an analogous decreasing trend of malate content, by as high as 50% in grape berries in response to a decrease in irrigation⁹⁸⁻¹⁰². In contrast to the findings on grapes, a study conducted on five cultivars of strawberry plants⁷³ showed differences between genotypes on the compositional response of harvested fruits to irrigation treatments. Two cultivars, Symphony and Florence, showed significant increases by 11% in acid content under deficit irrigation-treatment, while the rest of the cultivars (Sonata, Christine, and Elsanta) showed no effect with changes in irrigation. The authors suggested that deficit irrigation had a genotype-dependent effect on acid metabolism, resulting from variable respiratory metabolism among cultivars and hence different utilization of respiratory substrates such as organic acids. The irrigation level did not influence the acid content in mango, nectarine, and peach^{69,70,103}.

Other than biosynthesis, the catabolism of organic acids, which may include oxidation by the Krebs cycle, gluconeogenesis, ethanol fermentation, synthesis of flavonols and anthocyanins, and amino acid interconversions, also influence the final concentration of citrate and malate in fruits^{97,104}. Bulky fruit such as apples and pears subjected to high temperatures will have lower internal O₂ and higher internal CO₂ concentrations, which could lead to an induction of anaerobic respiration. Therefore, enzymes (pyruvate decarboxylase and alcohol dehydrogenase) involved in the ethanol fermentation pathway, which utilize pyruvate as a substrate, may also play a role in the temperature response of malate degradation⁹⁷.

Abiotic stresses, such as salt or cold¹⁰⁵⁻¹⁰⁷, nitrogen deficiency¹⁰⁸, phosphorus deficiency^{109,110}, iron deficiency¹¹¹⁻¹¹³, aluminum toxicity^{114,115}, and air CO₂ concentration¹¹⁶, have also been reported to have impact on organic acid accumulation and the expression and activity of the corresponding enzymes.

Table 2 Concentration response of organic acids in fruits and berries in relation to the variation of environmental factors.

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
Temperature bilberry	<i>Vaccinium myrtillus</i> L.	citrate malate quinate	Temp Temp ↑ Temp ↑		depending on clone ↑ ↓	55
kiwifruit	<i>Actinidia deliciosa</i> (A. Chev.) C. F. Liang et A.R. Ferguson var. <i>deliciosa</i> Hayward	citrate, quinate malate malate	Temp ↑ (cell division stage of fruit) Temp ↑ (starch accumulation & maturation stage of fruit)		– – ↓	57
peach (pulp)	<i>Prunus persica</i> L. cv. Suncrest	citrate	Temp ↑	√	↓	92
satsuma mandarins (juice sac)	<i>Prunus persica</i> L. cv. Fidelia	citrate	Temp ↑	√	↑	60
	<i>Citrus unshiu</i> Marc. cv. MihoWase	citrate	Temp ↑ (anthesis to harvest)		↓	
satsuma mandarins (juice sac)	<i>Citrus unshiu</i> Marc. cv. MihoWase	citrate, malate & quinate total acids citrate & malate quinate total acids	Temp ↑ (0–10 weeks after anthesis)		–	61
			Temp ↑ (0–10 weeks after anthesis)		–	
			Temp ↑ (10–20 weeks after anthesis)		↓	
			Temp ↑ (10–20 weeks after anthesis)		–	
			Temp ↑ (10–20 weeks after anthesis)		↓	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
strawberry	<i>Fragaria</i> × <i>ananassa</i> Duch. Earlsglow & Kent	citrate malate total acids	Temp ↑ Temp ↑ Temp ↑		↓ ↑ ↓	52
<u>Light conditions</u> bilberry	<i>Vaccinium myrtillus</i> L.	citrate malate quininate	Light Light ↑ Light		- ↓ depending on temperature	55
grape (pulp)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> , cv. Merlot	malate	Light ↑	Temp ↑	↓	42
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon & Pinot Noir	malate	Light ↑		↓	43
strawberry	<i>Fragaria</i> × <i>ananassa</i> Duch.	total acids	Light	√	depending on genotype	62
<u>Water supply</u> grape	<i>Vitis vinifera</i> L. cv. Monastrell	malate	Irrigation ↓		↓	102
grape (must)	<i>Vitis vinifera</i> L. cv. Cabernet Franc	malate	Irrigation ↓		↓	101
grape (must)	<i>Vitis vinifera</i> L. cv. Tempranillo	malate	Irrigation ↓	√	↓	99,100

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (must)	<i>Vitis vinifera</i> cv. Baladi, Airén, Montepila, Muscat Blanc à Petits Grains & Pedro Ximénez	malate	Irrigation ↓	√	↓	98
mango fruit	<i>Mangifera indica</i> cv. Lirfa	malate & citrate	Irrigation		–	69
nectarine (pulp)	<i>Prunus persica</i> Batsch var. Nectarina cv. Spring Bright & Summer Bright	malate & citrate total acids	Irrigation ↓ Irrigation ↓		– –	70
peach (flesh)	<i>Prunus persica</i> L. cv. Suncrest	Malate, citrate & quinate	Irrigation ↓		–	103
strawberry	<i>Fragaria</i> × <i>ananassa</i> L. cv. Elsanta	malate & citrate	Irrigation		–	72,73
	<i>Fragaria</i> × <i>ananassa</i> L. cvs. Elsanta, Sonata & Christine	total acids	Irrigation		–	
	<i>Fragaria</i> × <i>ananassa</i> L. cvs. Symphony & Florence	total acids	Irrigation ↓		↑	
Complex factors						
pomegranate (aril juice)	<i>Punica granatum</i> L.	citrate malate	Temp ↑, light ↑, relative humidity ↓ Temp ↑, light ↑, relative humidity ↓	√ √	↓ (most accessions) ↓ (most accessions)	74

√, experiments were carried out in open fields over years or at different growth sites, or multi-interference between different factors may exist. Abbreviations: Temp, temperature.

2.1.2 Secondary metabolites

In addition to primary metabolites, plants produce a diverse array of organic compounds, known as secondary metabolites, to defend against herbivory and microbial infection. These compounds also function as attractants for pollinators and seed/dispersing animals and as allelopathic agents, and provide adaptive protection against various environmental stresses, etc.^{29,30}. Extensive attention has been paid to these compounds because of their great utility as dyes, perfumes, spices, food ingredients and components of drugs and medicines. The synthetic pathways and the factors influencing the metabolism and contents of the corresponding compounds in plants are of special importance for both scientific and practical reasons.

2.1.2.1 Phenolic compounds

Phenolic compounds are a large group of secondary metabolites playing diverse and significant roles in plants. Many of them serve as defense compounds against herbivores and pathogens (e.g. lignin and furanocoumarins). Others function in mechanical support (e.g. lignin), in attracting pollinators and fruit dispersers (e.g. anthocyanins), in absorbing harmful ultraviolet radiation (e.g. flavones and flavonols), or in reducing the growth of nearby competing plants (e.g. caffeic acid and ferulic acid). They are biosynthesized by several different routes in plants and constitute a heterogeneous group of compounds²⁹. The phenolic compounds of interest in this review include hydroxycinnamic acid conjugates, flavonol glycosides, and anthocyanins.

2.1.2.1.1 Hydroxycinnamic acids

Hydroxycinnamic acids constitute the so-called “hydroxycinnamic pool”, which provides the principal precursors of lignins and a diverse range of other phenolic compounds. They seldom occur in the free form, and usually exist in conjugated forms, mostly as esters of glucose or various organic acids or amides, or, less often, as glycosides³¹.

Very few studies have been conducted on the compositional response of hydroxycinnamic acids to environmental conditions in fruits and berries (**Table 3**). The contents of hydroxycinnamic acid conjugates showed varying trends in bilberry and strawberry in response to the increased temperature. The content of the major hydroxycinnamic acid, *p*-coumaroyl glucose, showed an increasing trend in both strawberry cultivars studied, with increased values from 28.7–30.8 to 65.9–73.4 mg *p*-coumaric acid equivalents/g FW, as the temperature increased from 18/12°C to 30/22°C (day/night temperature)¹¹⁷. In

contrast, the total content of hydroxycinnamic acid derivatives in bilberry decreased from 107.5 to 69.9 mg chlorogenic acid equivalent/100 g FW as the temperature increased from 12°C to 18°C⁵⁵. In the studies on currant berries included in this thesis, most of the currant cultivars showed negative correlations between total hydroxycinnamic acid content and temperature variables, which is in accordance with the findings in bilberry^{118,119}.

Biosynthesis of phenolic compounds is known to be sensitive to light, which reflects the possible role of these compounds for photoprotection in plants¹²⁰. Although the total content of hydroxycinnamic acid conjugates increased as the light intensity increased in bilberry (varying from 77.5 to 90.5 mg chlorogenic acid equivalent/100 g FW)⁵⁵ and in young berries of the Cabernet Sauvignon grape cultivar (increasing five-fold as the value of the control berries)¹²⁰, this did not vary in accordance with the variation in UV light or UV-B radiation in Cabernet Sauvignon¹²⁰ and Malbec grapes¹²¹. According to these findings, UV radiation might contribute little to variation in the hydroxycinnamic acid content. However, the conditions investigated in the two studies differed considerably. In grapes of the cultivar Cabernet Sauvignon¹²⁰, the shading treatment was applied for only 49 days after the flowering stage, and the samples were collected for analysis as immature berries one week before véraison. In the study of the grape cultivar Malbec¹²¹, UV-B irradiation treatment was applied from 15 days before flowering until the ripe berries were harvested for analysis. In this thesis, no clear correlation between the total content of hydroxycinnamic acids and radiation were found in berries of black and red currants, while the total hydroxycinnamic acid content seemed to increase as radiation decreased in green and white currants^{118,119}. However, the concurrent decrease in temperature with a decrease in radiation should be considered in these studies.

Low relative air humidity was reported, in this thesis, to associate with the accumulation of hydroxycinnamic acids in the black currants Mortti and Ola, the green currant Vertti and the white currant White Dutch. Precipitation and air humidity exhibited a positive impact on the concentration of total hydroxycinnamic acid during the winter season (January–March), but a negative impact was seen during subsequent seasons until harvest (May–September)^{118,119}.

Table 3 Compositional response of hydroxycinnamic acids in fruits and berries to the variation of environmental factors.

<i>Fruit/berry</i>	<i>Species/cultivar</i>	<i>Investigated component</i>	<i>Variation of environmental factors</i>	<i>Co-factor</i>	<i>Variation of component content</i>	<i>Ref.</i>
<u>Temperature</u>						
bilberry	<i>Vaccinium myrtillus</i> L.	total HCA	Temp ↑		↓	55
strawberry (juice)	<i>Fragaria × ananassa</i> Duch. cv. Earlsglow & Kent	<i>p</i> -coumaroylglucose	Temp ↑		↑	117
<u>Light conditions</u>						
bilberry	<i>Vaccinium myrtillus</i> L.	total HCA	Light ↑		↑	55
grape (skin of young berry)	<i>Vitis vinifera</i> cv. Cabernet Sauvignon	total HCA	Light ↑ UV radiation		↑	120
grape (skin)	<i>Vitis vinifera</i> L. cv. Malbec	caffeic acid total HCA	UV-B radiation UV-B radiation		– –	121

Abbreviations: Temp, temperature; HCA, hydroxycinnamic acid.

Hydroxycinnamic acids are derived from the corresponding carboxylic acids and ultimately from L-phenylalanine and/or L-tyrosine (mainly limited to members of the grass family)^{29,31,122}. Phenylalanine ammonia lyase (PAL) is situated at a branch point between primary and secondary metabolism and plays important regulatory role in the formation of many phenolic compounds²⁹. Several copies of the PAL genes are found in all plant species¹²³, which may respond differentially to biotic and abiotic stressors. Their expression is developmentally and spatially controlled^{124,125}.

The activity of PAL is increased by environmental factors, such as low nutrient levels, light, and fungal infection. The point of control appears to be the initiation of transcription. In a study of cell suspension cultures of parsley (*Petroselinum hortense*)¹²⁶, the mRNAs encoding phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) and 4-coumarate:CoA ligase (4CL; EC 6.2.1.12) were induced both by irradiation with UV light and by treatment with a fungal elicitor. However, the regulation of PAL activity in many plant species is complex because of the existence of multiple PAL-encoding genes, some of which are expressed only in specific tissues or only under certain environmental conditions²⁹.

4-Coumaroyl CoA is the direct precursor for flavonoid or lignin biosynthesis¹²³. As a result, the regulation of the hydroxycinnamic pool may also influence the concentration of flavonoids by providing substrates for the biosynthesis of flavonoids in plant tissues. *Vice versa*, demand for the substrates of flavonoid biosynthesis may, in turn, affect the synthesis of the hydroxycinnamic pool.

2.1.2.1.2 Flavonoids

Flavonoids, including flavanones, dihydroflavonols, flavan-3,4,-diols, flavones, flavonols, flavan-3-ols, isoflavones, anthocyanidins, and proanthocyanidins, form one of the largest classes of plant phenolics¹²⁷. The accumulation of specific flavonoids is dependent both on the enzymes involved in the flavonoid biosynthetic pathway and on the competition between enzymes for common substrates (**Figure 1**). The flavanones and dihydroflavonols may represent the most important branching points in flavonoid metabolism, yielding a range of end products with distinct physiological functions, e.g. isoflavonoids, flavones, flavonols and anthocyanins^{30,128}. **Figure 1** illustrates the biosynthetic pathways of flavonols and anthocyanins, the major target compounds of this thesis.

In addition to the structural genes encoding anthocyanin biosynthetic enzymes (**Figure 1**), regulatory genes that control the expression of these structural genes are also of crucial importance and have been identified in many plants. These regulatory genes generally control the expression of many

different structural genes by transcriptional activation or repression of genes and, as a result, influence the intensity and pattern of anthocyanin biosynthesis¹²⁸.

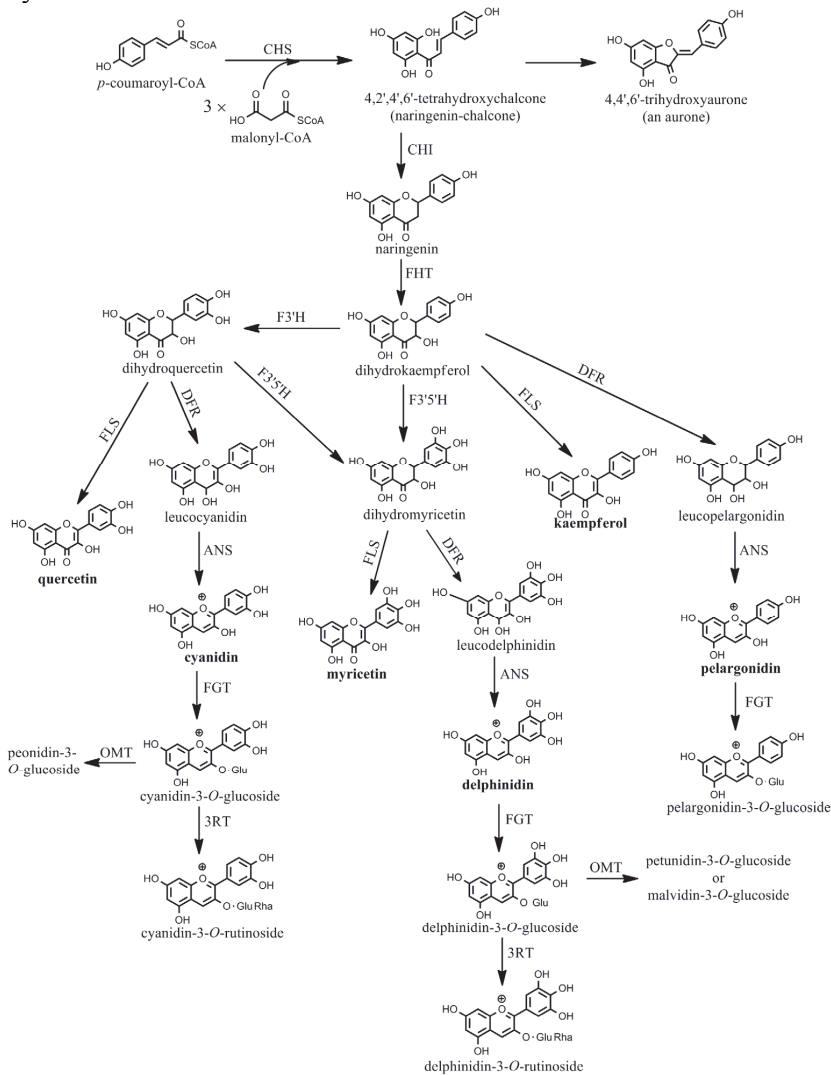


Fig. 1 Selected major enzymatic reactions in the flavonoid biosynthetic pathway^{30,128,129}. CHS, chalcone synthase; CHI, chalcone isomerase; FHT flavanone 3 β -hydroxylase; FLS, flavonol synthase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3',5'-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase (LDOX, leucoanthocyanidin dioxygenase); FGT, UDP-glucose:flavonoid 3-*O*-glucosyltransferase; OMT, *O*-methyltransferase; 3RT, UDP rhamnose:anthocyanidin-3-glucoside rhamnosyltransferase.

The compositional response of flavonols and anthocyanins to environmental factors has been studied extensively in fruits and berries (**Table 4 and 5**). The most studied fruits are grapes, which are of great interest to scientists and industry because of their wide utilization as raw materials in wine making. Temperature and radiation are among the most important factors influencing the phenolic composition of fruits. In addition, irrigation also influences grape quality for wine production.

2.1.2.1.2.1 Flavonols

Flavones and flavonols generally absorb light at shorter wavelengths, and function to protect cells from excessive UV-B radiation (280–320 nm). Exposure of plants to increased UV-B light has been demonstrated to increase the synthesis of flavones and flavonols²⁹. In accordance with this, all investigations conducted on grapes have reported increasing trends in the contents of all flavonols in berry skin in response to increases in either light intensity or UV radiation (**Table 4**). Downey et al.³⁷ investigated both the concentration of flavonol glycosides and the expression of the gene encoding flavonol synthase (*VvFLS1*), the first enzyme in the pathway of flavonol biosynthesis; this gene was studied in developing inflorescences, leaves, and bunches early in berry development and in ripe grapes (*Vitis vinifera* L. cv. Shiraz). The authors found that shading caused a concomitant reduction in the expression of *VvFLS1* and the concentration of flavonols in all these tissues. The greatest change in the flavonol content in grapes (0.12 mg/g FW skin in sun-exposed fruits vs. absent in shaded fruits) was detected in the 2000–2001 season when opaque boxes were applied to developing inflorescences five weeks before flowering. By separating the effects of UV-A and UV-B on the accumulation of phenolic compounds in grapes, Gregan et al.¹³⁰ suggested that the major increase in flavonols and total phenolic compounds was caused by an increase in UV-B.

Temperature and other environmental factors have also shown an obvious impact on flavonol accumulation. The strawberry cultivars Earliglow and Kent showed an increase in the contents of all flavonols under elevated temperature. The Kent cultivar even showed a ten-fold increase (2.2 vs. 21.4 µg quercetin 3-glucoside equivalents/g FW) in the summed content of quercetin 3-glucoside and quercetin 3-glucuronide as the temperature increased from 18/12 to 30/22°C (day/night)¹¹⁷. In the case of grape berries, the two investigations showed a different response in flavonol accumulation to an increase in temperature. An optimal temperature of 25°C, versus 20°C and 30°C, was suggested for the maximum accumulation of flavonols in the wine grape cultivar *Vitis ficifolia* var. *ganebu* × *V. vinifera* Muscat of Alexandria⁵⁶. In

contrast, in the grape cultivar *Vitis berlandieri* × *Vitis vinifera* cv. Merlot, the contents of flavonols were not influenced by variations in temperature, although they were highly influenced by light intensity¹³¹. The contradictory findings in these two investigations might be explained by the genetic differences between the cultivars or by the different conditions and different development stages of the plants in the two studies. The impact of growing season on the compositional response of flavonols was also reported in a study on cranberry¹³² (**Table 4**).

The co-variation in light and temperature was present in many investigations and made it difficult to conclude which of the variables was more important in terms of affecting the metabolite concentration in samples. Pereira et al.⁴² suggested a 36% increase in the optical density of the extract of grape skin in the near-UV range (360 nm), attributed to the content of flavonols; this was explained by the photochemical effect of light. However, an increase in kaempferol-3-glucoside and quercetin-3-glucoside in the pulp of sun-exposed berries was suggested to have been caused by a temperature effect. Myricetin-3-glucoside was conversely influenced in skin and pulp, i.e. the content was greater in the pulp and lower in the skin of shaded berries compared to sun-exposed ones (**Table 4**). Therefore, it can be suggested that the synthesis of flavonols is regulated by both light and temperature with a specific pattern for each compound⁴².

The effect of water supply on the accumulation of flavonols has not been well-studied (**Table 4**). Nevertheless, three studies on grape berries showed a variable influence of irrigation levels on the flavonol content in berries of different cultivars. The concentration of flavonols in the grape cultivars Merlot¹³³ and Cabernet Sauvignon¹³⁴ were not significantly influenced by irrigation treatments, while the total content of flavonols decreased up to 31% as irrigation levels decreased in the grape cultivar Aragonez¹³⁵.

Table 4 Compositional response of flavonols in fruits and berries to the variation of environmental factors.

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
<u>Temperature</u> cranberry	<i>Vaccinium macrocarpon</i> Ait. var. Early Black	total flavonols	Temp ↑ (bloom → fruit growth) Temp (pre-harvest) Temp difference ↑ (bloom → fruit set) Temp difference (fruit growth → harvest)	√ √ √ √	↑ - ↓ -	132
grape (skin)	<i>Vitis ficifolia</i> var. <i>ganebu</i> × <i>V. vinifera</i> Muscat of Alexandria	total flavonols	Temp		25 °C > 20 °C > 30 °C	56
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> , cv. Merlot	Qu-glc, My-glc & Ka-glc total flavonols	Temp Temp	√ √	- -	131
strawberry (juice)	<i>Fragaria</i> × <i>ananassa</i> Duch. cv. Earlsglow & Kent	Qu-glc & Ka-glc total flavonols	Temp ↑ Temp ↑		↑ ↑	117
<u>Light conditions</u> grape (pulp)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	Qu-glc & Ka-glc My-glc	Light ↑ Light ↑	Temp ↑ Temp ↑	↑ ↓	42
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	Qu-glc, Ka-glc & My-glc total flavonols	Light ↑ Light ↑	Temp ↑ Temp ↑	↑ ↑	42

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> , cv. Merlot	Qu-glc, My-glc & Ka-glc total flavonols	Light ↑	√	↑	131
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon & Merlot	quercetin	Light ↑	√	↑	136
grape (skin)	<i>Vitis vinifera</i> L. cv. Shiraz	total flavonols	Light ↑		↑	37,137
grape (skin of young berry)	<i>Vitis vinifera</i> cv. Cabernet Sauvignon	total flavonols	UV radiation ↑		↑	120
grape (skin)	<i>Vitis vinifera</i> L. cv. Malbec	Qu-glc, My-glc & Ka-glc total flavonols	UV-B radiation ↑		↑	121
grape (skin)	<i>Vitis vinifera</i> cv. Sauvignon Blanc	Qu-glc, Ka-glc, Qu-rut & Ka-rut	UV-B radiation ↑		↑	130
grape (skin)	<i>Vitis vinifera</i> L. cv. Malbec planted at 1500, 1000 and 500 m	quercetin	UV-B radiation ↑		–	138
<u>Water supply</u>						
grape (skin)	<i>Vitis vinifera</i> L. cv. Merlot	Qu-glc	Irrigation	√	–	133
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total flavonols	Irrigation		–	134
grape (skin)	<i>Vitis vinifera</i> L. cv. Aragonez	total flavonols	Irrigation ↓	√	↓	135

√, experiments were carried out in open fields over years or at different growth sites, or multi-interference between different factors may exist. Abbreviations: Temp, temperature; Qu-glc, quercetin-3-*O*-glucoside; Qu-rut, quercetin-3-*O*-rutinoside; Ka-glc, kaempferol-3-*O*-glucoside; Ka-rut, kaempferol-3-*O*-rutinoside; My-glc, myricetin-3-*O*-glucoside.

2.1.2.1.2.2 Anthocyanins

Anthocyanins, the most widespread group of pigments in flowers, fruits, and leaves³¹, are vitally important in attracting animals for pollination and seed dispersal²⁹. They are also important as feeding deterrents and as protection against damage from UV irradiation¹²⁸. Plant tissues are capable of accumulating anthocyanins under stress conditions¹²⁸, by the regulation of transcription level of genes and the activity of the enzymes involved in their metabolism¹²⁷.

Both low temperature and high radiation have been reported to enhance anthocyanin accumulation^{139,140}. Most studies (**Table 5**), especially those on apples and grapes, have shown a decrease in anthocyanin content in response to an increase in temperature. The variation of total anthocyanin content in apple peels was found to be as high as 92% as the growth temperature increased^{54,141,142}. In contrast, studies on raspberry¹⁴³ and strawberry¹¹⁷ have reported increasing trends in anthocyanins with an increase in temperature. The total anthocyanin content increased from 32.7 to 38.4 and to 37.5 mg/100 g FW in raspberry as the temperature increased from 12 to 18°C and 24°C, respectively¹⁴³. The major anthocyanins in strawberry fruits, pelargonidin 3-glucoside and pelargonidin 3-glucoside-succinate, increased from 291.3–363.6 to 782.7–945.1 and from 60.8–62.2 to 224.5–244.0 µg/g cyanidin 3-glucoside equivalents/g FW, respectively¹¹⁷. Genetic differences in the compositional response of anthocyanins to temperature variation were reported in black currants¹⁴⁴ and bilberries⁵⁵. Moreover, fruit developmental stages also had an impact on the response of anthocyanin accumulation to temperature changes in cranberry¹³².

In contrast to the negative effect of temperature, an increase in radiation has been found to exert a positive effect on the accumulation of anthocyanins in apples^{142,145}, some grape cultivars^{43,121,138,146} and strawberries¹⁴⁷. The contradictory findings reported regarding the anthocyanin content in grape berries^{42,43,131,148-150} in response to varying light conditions might have been caused either by genetic differences between the grape cultivars as in the case of bilberry⁵⁵, and/or by the co-influences of the other factors. For example, Nicholas et al.¹⁵⁰ reported a different response in terms of the anthocyanin content to elevated radiation compared with the findings by Dokoozlian et al.⁴³ on the grape cultivar Pinot Noir. Dokoozlian et al.⁴³ reported that clusters exposed to light produced berries with a higher anthocyanin (by up to 78%) content than those grown without light. In their study, all the other conditions were kept the same in the light-exposure and light-exclusion treatments, whereas co-influences of other environmental factors on the accumulation of

anthocyanins existed in the study by Nicholas et al.¹⁵⁰, who reported a negative correlation between irradiation and anthocyanin content in berries.

In a natural environment, temperature and radiation usually change simultaneously. Therefore, the final concentration of anthocyanins in fruits is a combination of these two parameters and depends on which of these factors plays the dominating role. This is supported by the findings of Bergqvist et al.¹⁴⁸. For grape clusters on the north side of the canopy, skin anthocyanins increased linearly as sunlight exposure increased (mid-day photosynthetically active radiation (PAR) 0–100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$), but for the clusters on the south side of canopy, the anthocyanins increased as sunlight exposure increased in range of PAR 0–100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$, then decreased when the exposure exceeded PAR 100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$. In addition, they also reported that clusters on the north side (afternoon shaded) of the canopy generally produced fruits with a higher anthocyanin content than those grown on the south side (afternoon exposed). At the same exposure level or PAR, the mid-day berry temperature was generally 3 to 4°C greater for clusters on the south side of the canopy compared to clusters on the north. Thus, the results suggest that the effects of light on fruit composition are heavily dependent on the extent to which berry temperature is elevated as a result of increased sunlight exposure. Spayd et al.¹³¹ suggested that temperature and light both contribute as the main factors regulating anthocyanin accumulation in grape. Shade treatment decreased the total anthocyanin content by 22–34%, while increases in temperature reduced the accumulation of anthocyanins by 5–29% in the skin of the grape cultivar Merlot.

Table 5 Contents of anthocyanins and total phenolic compounds in fruits and berries in response to the variation of environmental factors.

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
Temperature						
<u><i>Anthocyanins</i></u>						
apple (peel)	<i>Malus domestica</i> Borkh. cv. Himekami & Fuji	total anthocyanins	Temp ↑		↓	54
apple (peel)	<i>Malus × domestica</i> cv. Iwai, Sansa, Tsugaru, & Akane	total anthocyanins	Temp ↑		↓	142
apple (peel)	<i>Malus × domestica</i> Borkh. cv. Mondial Gala	total anthocyanins	Temp ↑	(cooling irrigation)	↓	141
black currant	12 cultivars of <i>Ribes nigrum</i>	total anthocyanins	Temp ↑	√	↑	144
	2 cultivars of <i>Ribes nigrum</i>	total anthocyanins	Temp ↑	√	↓	
	6 cultivars of <i>Ribes nigrum</i>	total anthocyanins	Temp	√	-	
bilberry	<i>Vaccinium myrtillus</i> L. northern clone	Cy-glc	Temp ↑		↑	55
		Dp-glc	Temp		-	
		total anthocyanins	Temp ↑		↑	
	<i>Vaccinium myrtillus</i> L. southern clone	Cy-glc	Temp		-	
		Dp-glc	Temp ↑		↓	
	total anthocyanins	total anthocyanins	Temp		-	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.	
cranberry	<i>Vaccinium myrtilloides</i> L. (statistical analysis based on two clones) <i>Vaccinium macrocarpon</i> Ait. var. Early Black	Cy-glc	Temp ↑		↑		
		Dp-glc	Temp		-		
		total anthocyanins	Temp ↑		↑		
grape (skin)	<i>Vitis ficifolia</i> var. <i>ganebu</i> × <i>V. vinifera</i> Muscat of Alexandria <i>V. labrusca</i> × <i>V. vinifera</i> cv. Aki Queen <i>Vitis vinifera</i> L. cv. Pinot noir	total anthocyanins	Temp ↑ (bloom → fruit set) Temp (fruit growth → harvest) Temp difference ↑ (bloom → fruit set)	✓ ✓ ✓	↑ - ↓	132	
		total anthocyanins	Temp difference (fruit growth → harvest)	✓	-		
		total anthocyanins	Temp		25 °C > 30 °C > 20 °C		56
		total anthocyanins	Temp ↑		↓		151,152
grape (skin)	<i>Vitis vinifera</i> L. cv. Pinot noir	total anthocyanins	Temp ↑ (budburst → bloom) Temp ↑ (previous fall & bloom → véraison)	✓ ✓	↑ ↓	150	
		Cy-glc & Dp-glc	Temp ↑		↓	153	
grape (skin)	<i>Vitis vinifera</i> L. cv. Shiraz & Cabernet Franc	total anthocyanins	Temp ↑		↓		
		total anthocyanins	Temp ↑		↓	154	
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total anthocyanins	Temp ↑	✓	↑	155	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	Cy-glc & Dp-glc total anthocyanins	Temp ↑		↓	156
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	Cy-glc & Dp-glc total anthocyanins	Temp ↑	√	↓	131
pomegranate (aril)	<i>Punica granatum</i> L.	Cy-glc & Dp-glc total anthocyanins	Temp ↑	√	↓	157
raspberry	<i>R. idaeus</i> L. cv. Glen Ample	cyanidin-3-sophoroside	Temp ↑	√	↓	143
		Cy-glc & Cy-rut	Temp ↑		↑	
		cyanidin-3-(2 ^G -glucosylrutinoside)	Temp ↑		↑	
strawberry (juice)	<i>Fragaria</i> × <i>ananassa</i> Duch. cv. Earliglow & Kent	total anthocyanins	Temp ↑		↑	117
		Cy-glc	Temp ↑		↑	
		total anthocyanins	Temp ↑		↑	
Total phenolics						
black currant	10 cultivars of <i>Ribes nigrum</i>	total phenolics	Temp ↑	√	↑	144
	4 cultivars of <i>Ribes nigrum</i>	total phenolics	Temp ↑	√	↓	
	9 cultivars of <i>Ribes nigrum</i>	total phenolics	Temp	√	-	
bilberry	<i>Vaccinium myrtillus</i> L.	total phenolics	Temp		-	55
cranberry	<i>Vaccinium macrocarpon</i> Ait. var. Early Black	total phenolics	Temp (bloom)	√	-	132
			Temp ↑ (fruit set → harvest)	√	↑	
			Temp difference	√	-	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (skin)	<i>Vitis ficifolia</i> var. <i>ganebu</i> × <i>V. vinifera</i> Muscat of Alexandria	total phenolics	Temp		25 °C > 30 °C > 20 °C	56
pomegranate (aril)	<i>Punica granatum</i> L.	total phenolics	Temp ↑	√	↓	157,158
raspberry	<i>R. idaeus</i> L. cv. Glen Ample	total phenolics	Temp ↑		↑	143
strawberry (juice)	<i>Fragaria</i> × <i>ananassa</i> Duch. cv. Earliglow & Kent	total phenolics	Temp ↑		↑	117
<u>Light conditions</u>						
<u>Anthocyanins</u>						
apple (peel)	<i>Malus</i> × <i>domestica</i> cv. Iwai, Sansa, Tsugaru, & Akane	total anthocyanins	Light ↑		↑	142
apple (peel)	<i>Malus</i> × <i>domestica</i> cv. Sansa, Tsugaru, and Akane	total anthocyanins	UV-B irradiation ↑		↑	142
apple (peel)	<i>Malus domestica</i> Borkh cv. Fuji	total anthocyanins	Light ↑	(Temp)	↑	145
bilberry	<i>Vaccinium myrtillius</i> L. northern clone	total anthocyanins	Red light ↑		↑	55
	<i>Vaccinium myrtillius</i> L. southern clone	total anthocyanins	Red light ↑		↓	
	statistical analysis based on two clones	Cy-glc Dp-glc total anthocyanins	Light Light ↑ Light		- ↑ -	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	Cy-glc & Dp-glc total anthocyanins	Light ↑		↑	146
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon & Pinot noir	total anthocyanins	Light ↑		↑	43
grape (skin)	<i>Vitis vinifera</i> L. cv. Pinot noir	total anthocyanins	Light ↑	√	↓	150
grape (skin)	<i>Vitis vinifera</i> L. cv. Pinot noir	delphinidin	Light ↑	√	↑	149
		cyanidin	Light ↑	√	-	
		anthocyanin monomers	Light ↑	√	↓	
		total anthocyanins	Light ↑	√	↑	
	<i>Vitis vinifera</i> L. cv. Croatina	delphinidin	Light ↑	√	-	
		cyanidin	Light ↑	√	-	
		anthocyanin monomers	Light ↑	√	↓	
		total anthocyanins	Light ↑	√	-	
grape (skin)	<i>Vitis vinifera</i> cv. Cabernet Sauvignon & Grenache	total anthocyanins	Light ↑ (0–100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$)	Temp ↑	↑	148
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	Cy-glc & Dp-glc total anthocyanins	Light ↑ (> 100 $\mu\text{mol m}^{-2} \text{sec}^{-1}$)	Temp ↑	↓	42
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	total anthocyanins	Light ↑	Temp ↑	↓	
grape (skin)	<i>Vitis berlandieri</i> × <i>Vitis vinifera</i> cv. Merlot	Cy-glc & Dp-glc total anthocyanins	Light ↑	√	-	131
grape (skin)	<i>Vitis vinifera</i> L. cv. Shiraz	total anthocyanins	Light ↑	√	↑	37,137
		total anthocyanins	Light		-	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (skin)	<i>Vitis vinifera</i> L. cv. Malbec	total delphinidins total cyanidins	UV-B radiation ↑ UV-B radiation ↑		↑ ↑	121
grape (skin)	<i>Vitis vinifera</i> L. cv. Malbec planted at 1500 m	total anthocyanins total anthocyanins	UV-B radiation ↑ UV-B radiation ↑		↑ ↑	138
strawberry	<i>Vitis vinifera</i> L. cv. Malbec planted at 1000 m & 500 m <i>Fragaria</i> × <i>ananassa</i> Duch.	total anthocyanins total anthocyanins	UV-B radiation ↑ Light ↑	(Temp)	– ↑	147
<u>Total phenolics</u>						
apple (peel)	<i>Malus domestica</i> Borkh cv. Fuji	total flavonoids	Light	(Temp)	–	145
bilberry	<i>Vaccinium myrtillus</i> L.	total phenolics	Light		–	55
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total phenolics	Light ↑		↑	146
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon & Pinot noir	total phenolics	Light ↑		↑	43
grape (skin)	<i>Vitis vinifera</i> cv. Cabernet Sauvignon & Grenache	total phenolics	Light ↑	Temp ↑	↑	148
grape (skin)	<i>Vitis vinifera</i> cv. Sauvignon Blanc	total phenolics	UV-B radiation ↑		↑	130

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (skin)	<i>Vitis vinifera</i> L. cv. Malbec planted at 1500 m	total phenolics	UV-B radiation ↑		↑	138
strawberry	<i>Vitis vinifera</i> L. cv. Malbec planted at 1000 m & 500 m	total phenolics	UV-B radiation ↑		–	147
	<i>Fragaria</i> × <i>ananassa</i> Duch.	total phenolics	Light	(Temp)	–	
<u>Water supply</u>						
<u>Anthocyanins</u>						
grape	<i>Vitis vinifera</i> L. cv. Monastrell	total anthocyanins	Irrigation ↓		↑	102
grape	<i>Vitis vinifera</i> L. cv. Tempranillo	total anthocyanins	Irrigation ↓	√	↑	159
grape	<i>Vitis vinifera</i> L. cv. Tempranillo	total anthocyanins	Irrigation ↓ (pre-véraison)		↓	160
			Irrigation ↓ (post-véraison, light–mid water stress)		↑	
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total anthocyanins	Irrigation ↓ (post-véraison, severe water stress)		↓	161
			Irrigation	√	–	
grape (must)	<i>Vitis vinifera</i> cv. Tempranillo	total anthocyanins	Irrigation ↓	√	↑	99
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Franc	total anthocyanins	Irrigation ↓		↑	101

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape (skin)	<i>Vitis vinifera</i> L. cv. Merlot	Dp-glc & Cy-glc total anthocyanins	Irrigation ↓ Irrigation ↓	√ √	↑ ↑	133 129,162
grape (skin)	<i>Vitis vinifera</i> L. cv. Merlot	total anthocyanins	Irrigation ↓		↑	134,163
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total anthocyanins	Irrigation ↓		↑	
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	Dp-glc total anthocyanins	Irrigation Irrigation ↓	√ √	- ↑	164 165
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon & Merlot	total anthocyanins	Irrigation ↓		-	166
grape (skin)	<i>Vitis vinifera</i> L. cv. Tempranillo	total anthocyanins	Irrigation ↓		-	
grape (skin)	<i>Vitis vinifera</i> L. cv. Aragonez	total anthocyanins	Irrigation ↓	√	↓	135
strawberry	<i>Fragaria × ananassa</i> L. cv. Elsanta	Cy-glc	Irrigation ↓		↓	72
<u>Total phenolics</u>						
grape	<i>Vitis vinifera</i> L. cv. Tempranillo	total phenolics	Irrigation ↓ (pre-véraison) Irrigation ↓ (post-véraison, light-mid water stress)		↓ ↑	160
grape	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total phenolics	Irrigation ↓ (post-véraison, severe water stress) Irrigation	√	↓ -	161

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
grape	<i>Vitis vinifera</i> L. var. Moscatel de Setúbal syn. Muscat of Alexandria	total phenolics	Irrigation ↓		↑	167
grape (must)	<i>Vitis vinifera</i> cv. Tempranillo	total phenolics	Irrigation ↓	√	↑	99
grape (must)	<i>Vitis vinifera</i> L. cv. Cabernet Franc	total phenolics	Irrigation ↓		↑	101
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Franc	total phenolics	Irrigation ↓		↑	101
grape (skin)	<i>Vitis vinifera</i> L. cv. Cabernet Sauvignon	total phenolics	Irrigation ↓	√	depending on rootstocks	164
grape (skin)	<i>Vitis vinifera</i> L. cv. Tempranillo	total phenolics	Irrigation ↓		–	166
strawberry	<i>Fragaria × ananassa</i> L. cv. Elsanta	total phenolics	Irrigation ↓		↑	72
<u>Complex factors</u>						
apple (peel)	<i>Malus × domestica</i> Borkh. cv. Mondial Gala	total anthocyanins	Temp ↑, light ↑	√	↓	168
pomegranate (aril juice)	<i>Punica granatum</i> L.	total anthocyanins	Temp ↑, light ↑, relative humidity ↓	√	↓	74
		total phenolics	Temp ↑, light ↑, relative humidity ↓	√	↓ (most accessions)	

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
pomegranate (peel)	<i>Punica granatum</i> L.	total anthocyanins total phenolics	Temp ↑, light ↑, relative humidity ↓ Temp ↑, light ↑, relative humidity ↓	√ √	↓ ↑ (most accessions)	⁷⁴

√, experiments were carried out in open fields over years or at different growth sites, or multi-interference between different factors may exist. Abbreviations: Temp, temperature; Cy-glc, cyanidin-3-O-glucoside; Dp-glc, delphinidin-3-O-glucoside.

In accordance with the variation in anthocyanin concentrations, the expression of anthocyanin biosynthetic genes has been shown to be induced by low temperature and repressed by high temperature in many plants^{74,139}. For instance, Ubi et al.¹⁴² reported that the expression of the five anthocyanin biosynthetic genes, i.e. chalcone synthase (*CHS*), anthocyanidin synthase (*ANS*), UDP-glucose: flavonoid 3-*O*-glucosyltransferase (*UGluT*), flavanone 3-hydroxylase (*F3H*), and dihydroflavonol 4-reductase (*DFR*), especially the first three were up-regulated by low temperature and UV-B irradiation, with an increase in anthocyanins in apple skin. The authors suggested that UV-B, among the wavelengths of ambient light, is a major contributor to the accumulation of anthocyanins in apple skin. In addition to biosynthesis, chemical or enzymatic degradation also plays a role in the anthocyanin content in response to temperature^{74,139}. Therefore, a decrease in the anthocyanin content in fruits in response to an increase in temperature could be caused by a combination of reduced synthesis, increased degradation, and increased photo-oxidation of the phenylpropanoid intermediates produced by an otherwise active pathway¹⁶⁸. Mori et al.¹⁵⁶ reported a 51% decrease in the total anthocyanin content in the skin of grape berries grown under high temperature (maximum 35°C) compared to control berries (maximum 25°C). Using GeneChip microarray analysis, quantitative real-time PCR analysis, and ¹³C stable isotope tracer experiments, the authors suggested that the reduction in anthocyanin content could be attributed to enhanced anthocyanin degradation and inhibition of transcription of anthocyanin biosynthesis genes. The GeneChip microarray analysis suggested that grape berries grown under high temperature were under greater oxidative stress since genes encoding peroxidase and some oxidoreduction enzymes were induced. Oxidative stress may promote the degradation of anthocyanins in the skin of berries. In addition to temperature, degradation can also be affected by pH, light, oxygen, and anthocyanin structure¹⁵⁶.

Although most plants have shown a decrease in the amount of anthocyanins with an increase in temperature, a study on two strawberry (*Fragaria × ananassa* Duch.) cultivars¹¹⁷ and raspberry (*R. idaeus* L.)¹⁴³ showed increases in the anthocyanin content in response to elevated temperature, which might implicate a genetic difference in the regulation of anthocyanin biosynthesis in relation to temperature in plants. Wang et al.¹¹⁷ suggested that the increase in the phenolic acid, flavonol, and anthocyanin content in response to an increase in environmental temperature might have contributed to the antioxidant activity against reactive oxygen species in strawberry fruit juice. Conversely, Remberg et al.¹⁴³ explained the increased anthocyanin content in response to the increase in growing temperature to be a function of a decrease in berry weight. When analyzed on a dry weight basis, the temperature did not show a significant

influence on the anthocyanin content. Therefore, the final anthocyanin content in the berries depends not only on the net accumulation of anthocyanins but also on the dilution effects related to berry size under variable temperature.

Genetic differences in the compositional response of anthocyanins to light conditions may exist as well in grapes of different cultivars, although the influences of co-existing environmental factors in the investigations should be considered. Low light intensity restricted the accumulation of anthocyanins in grape (*Vitis vinifera* L.) cultivars like Cabernet Sauvignon and Malbec^{43,121,146}. In contrast, Downey et al.³⁷ did not find significant differences between shaded and exposed bunches in terms of the total anthocyanin content in fruits of the grape cultivar Shiraz (*Vitis vinifera* L.). In agreement, they observed that the expression level of the genes encoding UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT), one of the key enzymes in anthocyanin biosynthesis, did not differ between exposed and shaded fruits. In a study on bilberry (*Vaccinium myrtillus* L.)⁵⁵, the plants from northern and southern clones were studied in a controlled phytotrone in Tromsø, Norway under different temperature or light conditions. The results showed that the northern and southern clones responded differently to the varying conditions in terms of the anthocyanin concentrations in berries (**Table 5**) and indicated variable anthocyanin metabolic regulation between clones with environmental changes.

The growing period is another factor that may contribute to the impact of environmental conditions on berry composition. Justine et al.¹³² found that anthocyanins and flavonols in harvested cranberry fruits were increased by elevated temperature in the early stages of fruit development starting from bloom, but were not influenced by increased temperature in the late stage before harvest. The level of total phenolic compounds was positively associated with temperature at stages after the fruits were formed (**Table 5**). It was not clear how higher temperatures during blooming positively influenced content of anthocyanins and flavonols in harvested fruit. The authors hypothesized that higher temperatures through bloom accelerated plant development, resulting in advanced fruit maturity at the corresponding locations. However, their investigation was based on a study of samples grown in seven different locations, and the results might have been influenced by other naturally occurring environmental parameters. Similar findings were reported in a three-year field study conducted on the grape berry Pinot Noir¹⁵⁰, in which several different statistical approaches showed that heat accumulation in the postharvest period of the previous year, and heat from bloom to véraison, were negatively correlated with the concentration of anthocyanins, tannins, and iron-reactive phenolics. In contrast, warm temperatures from budburst to bloom showed a trend for a positive correlation with the concentrations of all these classes of phenolics. Moreover, Nicholas et al.¹⁵⁰ found that anthocyanins in

grape skin were positively and significantly correlated with temperatures between 16 and 22°C from véraison to harvest, and suggested that this temperature interval was particularly favorable for enzymatic activity involved in anthocyanin biosynthesis. However, the study was, again, conducted in open fields with natural environmental variations. Other than temperature, vine light interception in the fruiting zone was reported to explain the variation in total phenolics and tannins more than vineyard temperature, and the intensity of light in this study correlated negatively with the concentrations of anthocyanins, tannins, and total phenolics¹⁵⁰. Therefore, the concentrations of phenolic compounds in berries should be considered as a complex combination of the influence of temperature and light, as well as other factors which might have an impact on metabolism.

Furthermore, different anthocyanins may even respond to environmental changes differently^{37,146,157}. Although no significant difference in total anthocyanins was found between shaded and exposed grape fruits, Downey et al.³⁷ reported that shaded fruits consistently showed up to a 20% lower proportion of anthocyanins with three oxygen substituents on ring B (glucosides of delphinidin, petunidin and malvidin) and up to a 97% increase in those with two oxygen substituents (glucosides of cyanidin and peonidin) compared to exposed fruits. Nevertheless, in both shaded fruits and sun-exposed fruits, trioxxygenated anthocyanins accounted for the majority (65–89%) of anthocyanins. Ristic et al.¹³⁷ reported that bunch shading of Shiraz grape vines did not influence the total anthocyanin content in the skin of grapes, but significantly increased both the concentration (from 0.06 to 0.09 mg and from 0.35 to 0.58 mg malvidin-3-glucoside equivalent/g berry weight for cyanidin and peonidin derivatives, respectively) and proportion (from 2.67 to 3.48% and from 15.67 to 23.57%, respectively) of dioxygenated anthocyanins and decreased the proportion (from 13.07 to 12.02% and from 55.78 to 48.84% for petunidin and malvidin derivatives, respectively) of trioxxygenated anthocyanins compared to control berries. Mori et al.¹⁶⁹ reported that high temperatures decreased the levels of delphinidin 3-glucosides, petunidin 3-glucosides, and malvidin 3-glucosides in the skin of Pinot Noir grapes. At the same time, grape berries grown under high temperature had a low abundance of mRNA for flavonoid 3',5'-hydroxylase (F3'5'H) in the skin. These results suggest that changes in the accumulation of individual anthocyanins in the skins of Pinot Noir grape berries, due to high temperature, are regulated at the level of transcription of the flavonoid 3',5'-hydroxylase gene. In the studies on currants in this thesis work, delphinidin glycosides in black currants of three cultivars (Mortti, Ola, and Melalahti)¹¹⁸ all displayed positive correlations with temperature and radiation variables, while in the red current cultivar Red Dutch¹¹⁹, cyanidin glycosides appeared as an exclusive anthocyanin group in

berries and showed negative correlations with temperature and radiation variables. The results of this thesis further support the notion of a differential response of individual anthocyanins to varying environmental factors, although it is difficult to distinguish the effects of temperature from those of radiation.

In a study on pomegranate¹⁵⁷, although all the anthocyanins were decreased in the arils of two deciduous ('PG 128-29' and 'PG 130-31') and one evergreen ('EG 2') accessions of pomegranate as the temperature increased, the decrease in the diglucosylated anthocyanins (delphinidin 3,5-diglucoside and cyanidin 3,5-diglucoside) was less than that of the monoglucosylated anthocyanins (delphinidin 3-glucoside and cyanidin 3-glucoside). In pomegranate arils of evergreen accession 'EG 1', the monoglucosylated anthocyanins showed a clear decreasing trend in the arils of fruits from early winter to later summer as the temperature rose from approximately 7 to 40°C. However, the concentration of the diglucosylated anthocyanins, especially that of delphinidin 3,5-diglucoside, increased with season advancement toward and during spring and then decreased during summer. As a result, diglucosylated anthocyanins contributed as the major anthocyanin group in the arils of pomegranate fruits in hot environments, while monoglucosylated anthocyanins were the major group in cool environments¹⁵⁷. In terms of anthocyanidins, the decreasing range of cyanidin glycosides was greater than that of delphinidin glycosides as the temperature increased¹⁵⁷. However, the opposite findings were found in this thesis. In black currants¹¹⁸, the contents of delphinidin glycosides were highly and positively correlated with temperature and radiation, but those of cyanidin glycosides were less affected by these variables.

Although Schwartz et al.⁷⁴ reported that the total anthocyanin content decreased in both the arils and peels of pomegranate fruits as temperature and radiation rose, the total phenolic content responded oppositely in the arils and peels of pomegranate to variations in growth conditions. The content of total phenolic compounds was higher in peels of pomegranate fruits but lower in the aril juice of the fruits grown in the south with higher temperature and radiation than those grown in the north. This difference might be explained by the fact that anthocyanins are the main contributors to antioxidant activity and total phenolic compounds of aril juice, whereas hydrolyzable tannins may be the main contributors to those in the peels. In southern areas, high temperature leads most probably to a decrease in anthocyanin levels in aril juice and peels, whereas, together with radiation, it stimulated the synthesis of hydrolyzable tannins in the peels, which resulted in the different response of arils and peels of pomegranate fruits to different growth sites⁷⁴.

Osmotic or drought stress may also induce the accumulation of anthocyanins¹⁴⁰. The flavonoid biosynthetic pathway appears to be stimulated by water deficiency, particularly in wine or table grapes⁸⁶. The majority of

studies in grapes have reported an increase in berry anthocyanin and phenolic concentration by water deficits, although exceptions exist (**Table 5**). The increase in anthocyanins might have been ascribed to either the reduction of berry size/moisture content or the induction anthocyanin biosynthesis, or a combination of both⁸⁶. In support of this, Castellarin et al.^{129,134} found that the integral of the expression of the anthocyanin biosynthetic genes UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT), chalcone synthase (*CHS2*, *CHS3*), and flavanone 3-hydroxylase (F3H) of the flavonoid pathway showed a high correlation with the anthocyanin content and explained a large part of the increase in anthocyanin accumulation in water stressed grape fruits of Merlot or/and Cabernet Sauvignon (*Vitis vinifera* L.).

Different responses of individual anthocyanins to variations in the water supply exist. Castellarin et al.^{129,134} reported that genes coding for flavonoid 3',5'-hydroxylase (F3'5'H) were up-regulated in Merlot and Cabernet Sauvignon grape berries under dehydrated conditions, resulting in higher proportions of monoglucosides of 3'4'5'-hydroxylated anthocyanidins (delphinidin, petunidin, malvidin). The expression of the genes dihydroflavonol 4-reductase (DFR) and leucoanthocyanidin dioxygenase (LDOX) showed differences in response to water stress between these two cultivars^{129,134}. In contrast to the studies by Castellarin et al.^{129,134}, the ratio between tri-hydroxylated anthocyanins and di-hydroxylated anthocyanins in grape skins of the cultivar Tempranillo remained relatively constant, regardless of the water supply¹⁶⁶, which indicated a genetic difference in the regulation of the biosynthesis of anthocyanin groups. Furthermore, Koundouras et al.¹⁶⁴ reported that malvidin-3-*O*-glucoside was the most highly affected by water supply among all the individual anthocyanins in grape (*Vitis vinifera* L. cv. Cabernet Sauvignon). The differences in growth conditions between different investigations should also be considered. Bowen et al.¹⁶⁵ suggested that the different effects of soil texture on moisture release and the resulting degree and duration of water stress might be an explanation for the differences between their findings and the reports of others on the response of anthocyanin accumulation to irrigation treatments in the same species of grapes.

Optimal water status has been suggested for anthocyanin and phenolic accumulation in grape. Girona et al.¹⁶⁰ reported that berry quality (including anthocyanins and phenolic content) at harvest increased linearly with light to mild levels of water stress during the post-véraison period, whereas the berry quality decreased above a certain water stress threshold. The positive effect of applying light to mild water stress after véraison on berry quality may be related to an increased concentration of cell sap because of berry dehydration. The reduction in berry quality induced by severe post-véraison water stress could be partially explained by the delay in berry ripening and partially by

carbon limitations¹⁶⁰. Moreover, a different response of the anthocyanin concentration to water stress applied at different growth periods (pre-véraison vs. post-véraison) were also reported in this study¹⁶⁰.

Other environmental factors, such as deficiencies in nitrogen or phosphorus, exposure to lower pH, wounding, pathogen infection and fungal elicitors, have also been linked to anthocyanin induction, accumulation or inhibition¹⁴⁰, although these aspects are not included in this review.

2.1.2.1.3 Possible regulation by the shikimic acid pathway

The plant shikimic acid pathway is the entry to the biosynthesis of phenylpropanoids¹²³. All the phenolic compounds discussed above are ultimately derived from L-phenylalanine via the shikimic acid pathway. Therefore, the regulation of the shikimic acid pathway may play a considerable role in the accumulation and regulation of phenolic compounds in fruits and berries. The pathway is affected by various factors, such as light, pathogens, wounding, and nitrogen deficiency^{125,170,171}.

McCue et al.¹⁷² examined in a suspension cultured cells of parsley (*Petroselinum crispum*) the effects of light on the activities of two enzymes involved in the production of phenylalanine: 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHPS) and chorismate mutase (CM). Light treatment was shown to increase the activity of the plastidic isoform of DAHP synthase, but did not significantly affect either the plastidic or cytosolic isoforms of chorismate mutase.

The shikimic acid pathway is localized in the chloroplast, and regulation coupled to photosynthesis is anticipated. DAHPS is a crucial control point linking the synthesis of aromatic amino acids to photosynthesis, possibly by redox regulation¹²⁵. Heterologously expressed *DHS1*, for instance, one of the three genes coding for DAHPS in *Arabidopsis*, requires reduced thioredoxin for activity, thereby suggesting a link between carbon flow into the shikimate pathway and electron flow from photosynthesis¹⁷³. Thus, synthesis of phenylalanine (Phe), and subsequently flavonoids, may be redox activated by light through the thioredoxin system¹²⁵.

Moreover, responses to other factors, e.g. a positive response of *DHS1* and *DHS3* to sucrose¹⁷⁴ and of *DHS3* to phosphorus depletion^{175,176}, have been reported as well. In contrast, the genes encoding 3-dehydroquinate synthase and 3-dehydroquinate dehydratase/shikimate dehydrogenase apparently do not represent important regulatory steps¹²⁵.

2.1.2.2 Ascorbic acid (vitamin C)

Ascorbic acid (AsA) is one of the most abundant antioxidants in plants. Several alternative ascorbic acid biosynthetic pathways have been proposed (**Figure 2**), including the L-galactose pathway¹⁷⁷, the *myo*-inositol pathway¹⁷⁸, the D-galacturonic acid pathway¹⁷⁹, and a branch of the L-galactose pathway that utilizes L-gulonic intermediates (L-gulose pathway)¹⁸⁰. Among them, the L-galactose pathway has been suggested to be the primary ascorbic acid biosynthetic route in plants¹⁷⁷.

Climatic conditions have a strong influence on the vitamin C content of horticultural crops. Walker et al.¹⁸¹ investigated environmental influences on the ascorbic acid concentration in berries of different black currant cultivars grown at the same site over 35 years, and found that different cultivars showed similar responses to prevailing environmental conditions. Among the environmental factors studied, total solar radiation showed the strongest correlation with the fruit ascorbic acid content, particularly during the period of flowering and fruit development, followed by average temperature, while total precipitation had little influence on the fruit ascorbic acid content.

Although light is not essential for the biosynthesis of ascorbic acid in plants, good exposure or high light intensity is, generally, a positive factor for the accumulation of ascorbic acid as observed in many plants¹⁸⁷ and fruits¹⁸⁸. This is shown in **Table 6**, as light intensity has a common positive contribution to the accumulation of either ascorbic acid or total vitamin C (AsA + DHA) in fruits. This could be explained by the fact that ascorbic acid is synthesized from sugars supplied by photosynthesis in plants. Yabuta et al.¹⁸⁹ also suggested that photosynthetic electron transport of chloroplasts is closely related to the regulation of the ascorbate pool in leaves. Ma et al.¹⁹⁰ reported that sun exposure increased the ascorbate pool by as high as seven-fold in apple peel, with increased activities of ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and a higher reduction state of the pool. In a study on grape berries¹⁹¹, the expression of the GalUR gene, encoding for D-galacturonate reductase, showed up-regulation with high light and, simultaneously, the content of ascorbic acid in fruits increased by two-fold compared to the fruits grown under low light. Therefore, both the increased availability of carbohydrates in leaves grown at high light intensity and a direct effect of light on the expression of ascorbate biosynthesis genes could contribute to the regulation of the biosynthesis and pool size of ascorbate¹⁹².

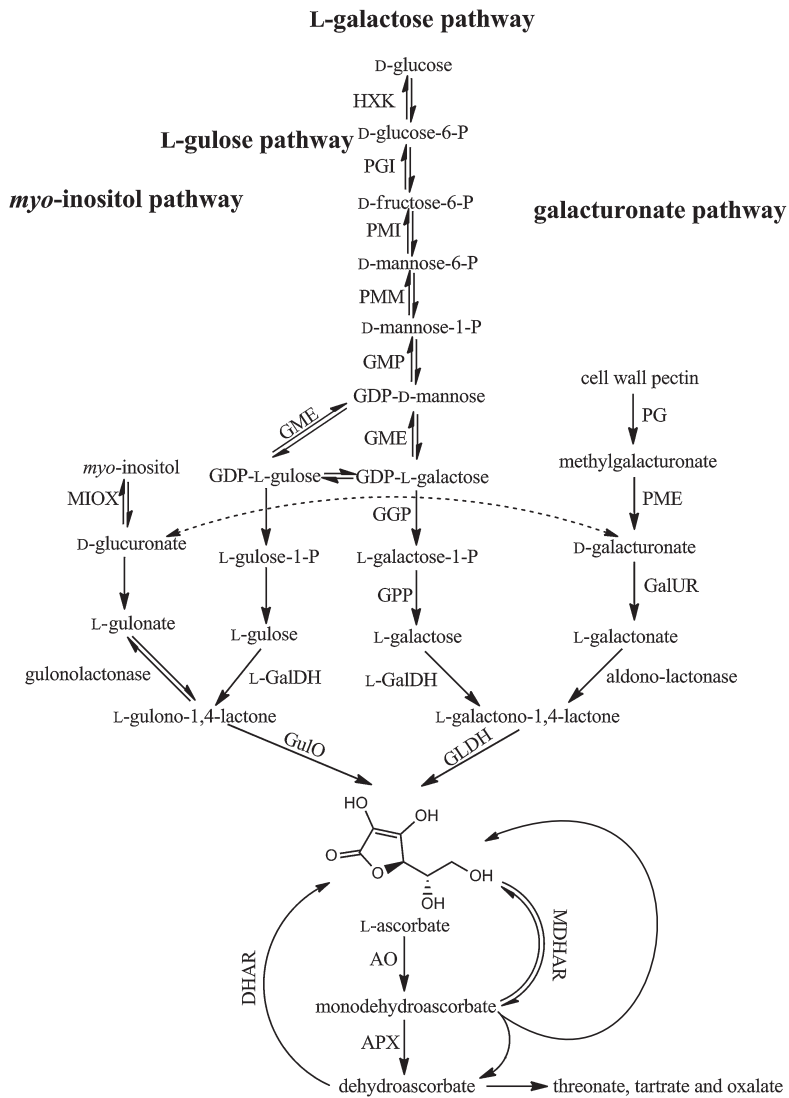


Fig. 2 Ascorbic acid biosynthetic and recycling pathway in plants. GDP, guanosine diphosphate; HXK, hexokinase; PGI, phosphoglucose isomerase; PMI, phosphomannose isomerase; PMM, phosphomannomutase; GMP, GDP-D-mannose pyrophosphorylase; GME, GDP-D-mannose 3',5'-epimerase; GGP, GDP-L-galactose phosphorylase (GDP-L-galactose-hexose-1-phosphate guanyltransferase)¹⁸²; GPP, L-galactose-1-P phosphatase; L-GalDH, L-galactose dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; MIOX, *myo*-inositol oxygenase; PG, polygalacturonase; PME, pectin methylesterase; GalUR, D-galacturonate reductase; GulO, L-gulonolactone oxidase; AO, ascorbate oxidase; APX, ascorbate peroxidase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase^{177-180,183-186}

However, light regulation of ascorbic acid synthesis in fruits may be more complex than in leaves. As confirmed by biochemical analysis, heterotrophic tissues are capable of ascorbic acid synthesis. Thus, the accumulation of ascorbic acid in fruits is either synthesized *in situ* or translocated from source to sink tissues *via* the phloem, or a combination of both⁵¹. As a result, changes in the ascorbic acid content in fruits could be due to light regulation of ascorbate-related gene expression or enzyme activities in fruits, and also to light regulation of the intake of ascorbate (or its precursor) from leaves to the fruit¹⁷⁷. Hancock et al.⁵¹ suggested that ascorbic acid accumulation in black currant fruit occurs primarily as a result of *in situ* biosynthesis *via* the L-galactose pathway.

Light regulation of ascorbate-related gene expression has been studied primarily in leaves. The ascorbate level in leaves of *Arabidopsis* plants previously grown under a 16 h daily photoperiod decreased by 91% in 72 hours when moved into the dark, but increased by 171% when exposed to continuous light. Among the numerous enzymes of the ascorbic acid biosynthetic pathway, the transcript levels of GDP-D-mannose pyrophosphorylase (GMP), L-galactose 1-P phosphatase (GPP), L-galactono-1,4-lactone dehydrogenase (GLDH), and the *VTC2* gene (coding for GDP-L-galactose phosphorylase) were down-regulated in the dark¹⁸⁹. Dowdel et al.¹⁹⁷ speculated that *VTC2* is a key gene regulated by light, such that the GDP-L-galactose phosphorylase step may play an important role in controlling ascorbate biosynthesis, due to findings that *VTC2* expression and GDP-L-galactose phosphorylase activity rapidly increased upon transfer to high light while the activity of other enzymes in the GDP-mannose pathway were barely affected. In a study on *Arabidopsis thaliana* seedlings, Tamaoki et al.¹⁹⁸ suggested that the ascorbic acid pool size was, at least partly, determined by the transcription rate of a gene (AtGLDH) encoding L-galactono-1,4-lactone dehydrogenase (GLDH), and the diurnal change in the expression of AtGLDH was regulated by light instead of a circadian rhythm. Down-regulation of GLDH by shade or dark was also detected in mature leaves of Chinese cabbage¹⁹⁹ and in the leaves and peel of apple (*Malus domestica* Borkh)¹⁹³. But, in contrast to these findings, the expression of GLDH in tobacco leaves was not influenced by dark/light treatment, and the authors²⁰⁰ suggested that the light-dependent ascorbic acid accumulation observed over the day/night cycle in leaves was the result of other mechanisms, such as the varying supply of carbon skeletons from photosynthesis. Other than GLDH, phosphomannose isomerase 1 (PMI1), which catalyzes reversible isomerization between D-fructose 6-phosphate and D-mannose 6-phosphate, responded to continuous light treatment with enhanced expression in leaves of *Arabidopsis thaliana*, together with an increase in the ascorbate level²⁰¹.

Table 6 Concentration response of vitamin C in fruits and berries in relation to the variation of environmental factors.

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
<u>Temperature</u>						
bilberry	<i>Vaccinium myrtillus</i> L.	AsA	Temp		–	55
black currant	<i>Ribes nigrum</i> L. cv. Ben Tirran, Ben Alder, & Ben Lomond <i>Ribes nigrum</i> L. cv. Baldwin	AsA AsA	Temp ↑ Temp ↑ (late winter → early spring)	√ √	↑ ↓	181
black currant	<i>Ribes nigrum</i> L. 23 cultivars	AsA	Temp ↑ (spring → winter)	√	↑	144
raspberry	<i>R. idaeus</i> L. cv. Glen Ample	AsA	Temp	√	–	143
strawberry	<i>Fragaria</i> × <i>ananassa</i> Duch. Earliglow & Kent	AsA	Temp ↑		↓	52
<u>Light conditions</u>						
apple (flesh)	<i>Malus domestica</i> Borkh. cv. Gala	AsA, DHA & AsA + DHA AsA/DHA AsA, DHA & AsA + DHA AsA/DHA	Light ↑ (exposure/shade-treatment, whole tree) Light ↑ (fruits naturally grown in sun-exposed/shaded side of canopy)		↑ – ↑ –	193

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
apple (peel)	<i>Malus domestica</i> Borkh. cv. Gala	AsA, DHA & AsA + DHA AsA/DHA AsA DHA & AsA + DHA DHA AsA/DHA	Light ↑ (exposure/shade-treatment, fruit only) Light ↑ (exposure/shade-treatment, whole tree)		- - - ↑ ↓ ↑	193
apple (peel)	<i>Malus domestica</i> Borkh. cv. Gala & Smoothie	AsA, DHA & AsA + DHA AsA/DHA AsA, DHA & AsA + DHA AsA/DHA	Light ↑ (fruits naturally grown in sun-exposed/shaded side of canopy) Light ↑ (exposure/shade-treatment, fruits only)		↓ ↑ ↓ ↑	190
black currant	<i>Ribes nigrum</i> L. cv. Ben Tiran, Baldwin, & Ben Lomond	AsA AsA + DHA AsA	Light ↑ Light ↑ Light ↑	✓ ✓ ✓	↑ ↑ ↑	181
grape	<i>Vitis vinifera</i> L. cv Palomino	ASA	Light ↑		↑	191
meiwa kumquats (flesh)	<i>Fortunella crassifolia</i> Swingle	AsA + DHA	Light ↑	✓	↑	194
meiwa kumquats (skin)	<i>Fortunella crassifolia</i> Swingle	AsA + DHA	Light ↑	✓	↑	194

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
strawberry	<i>Fragaria</i> × <i>ananassa</i> L. cv. Elsanta	ASA + DHA	Light ↑	(Temp)	↑	195
	<i>Fragaria</i> × <i>ananassa</i> L. cv. Flamenco	ASA + DHA	Light	(Temp)	–	
Water supply						
pear-jujube	<i>Zizyphus jujuba</i> Mill. cv. Lizzao	AsA	Irrigation ↓ (bud burst → fruit set) Irrigation ↓ (fruit growth → fruit maturation)	√ √	– low & moderate water stress > severe water stress & full irrigation	196
strawberry	<i>Fragaria</i> × <i>ananassa</i> L. cv. Elsanta	AsA	Irrigation		–	72
strawberry	<i>Fragaria</i> × <i>ananassa</i> L. cv. Elsanta	AsA	Irrigation ↓		↓	73
	<i>Fragaria</i> × <i>ananassa</i> L. cv. Florence	AsA	Irrigation ↓		↑	
	<i>Fragaria</i> × <i>ananassa</i> L. cvs. Symphony, Sonata & Christine	AsA	Irrigation		–	
Complex factors						
pomegranate (aril juice)	<i>Punica granatum</i> L.	AsA	Temp ↑, light ↑, relative humidity ↓	√	–	74

√, experiments were carried out in open fields over years or at different growth sites, or multi-interference between different factors may exist. Abbreviations: Temp, temperature; AsA, ascorbic acid; DHA, dehydroascorbic acid.

Ascorbic acid is not a stable metabolic product and is utilized as both a cellular reductant and a substrate for the synthesis of organic acids⁵¹. Therefore, other than the biosynthesis of ascorbic acid, its oxidation and recycling (**Figure 2**) also contribute to the final concentration of ascorbic acid or vitamin C in plant tissues. The regulation of environmental factors on the reduction of DHA and the enzyme activities involved should be considered when analyzing the ascorbic acid content in plants and regulation under various conditions. For example, in a study on tobacco (*Nicotiana tabacum*), a diurnal increase in MDHAR and DHAR was observed²⁰². Up-regulation of MDHAR and DHAR by light treatment was also reported in study on wheat leaves²⁰³. It was even found, under the same light treatment, that plants exposed to lower temperature exhibited higher activity of MDHAR, but no difference in DHAR activity²⁰³. Similar findings were reported in spinach (*Spinacia oleracea* L.) leaves²⁰⁴, with significantly increased activities of MDHAR in the chloroplasts of leaves exposed to cold, but no change in the level of DHAR.

Temperature and water supply also markedly influence the ascorbic acid contents in plant tissues during growth and development. Different responses in the ascorbic acid content to temperature and irrigation changes have been reported in various fruits (**Table 6**). Only fruits of pear-jujube and strawberry were investigated regarding the effects of irrigation on the fruit ascorbate concentration. Plant genotype, growing period, and stress level all had an impact on the response of ascorbate accumulation to irrigation. In terms of the temperature effect, the content of ascorbic acid in bilberry⁵⁵ and raspberry¹⁴³ was not influenced by a variation in temperature, while that in strawberry⁵² decreased as temperature increased. Black currant of different cultivars^{144,181} displayed different responses in terms of the ascorbic acid concentration to varying temperature. Krüger et al.¹⁴⁴ reported that the ascorbic acid content in black currants was not influenced by temperature. Walker et al.¹⁸¹ reported that the content of ascorbic acid increased in most of the cultivars as the average temperature increased, with the exception of the cultivar Baldwin, which showed a weak negative correlation with average temperature during the late winter and early spring period and a positive correlation afterwards during spring to winter.

The changes in ascorbic acid content and its metabolism in relation to temperature might be light-dependent. A positive effect of low temperature on ascorbic acid seems to be enhanced in broccoli²⁰⁵ by the presence of high light. In cucumber leaf discs, low temperature and high light treatment reduced the total cellular ascorbate level by about 50%, while dark chilling of cucumber caused no such loss of ascorbate²⁰⁶. Chilling treatment of pea either in dark or under light did not affect the ascorbate pool in leaves²⁰⁶. Moreover, increases in the ascorbate contents of wheat (*Triticum aestivum* L.)²⁰³ and spinach (*Spinacia*

oleracea L.)²⁰⁴ leaves were observed following light treatment at lower temperatures in comparison with that at higher temperature. These findings further suggest the influence of genetic effects on the compositional response of ascorbic acid in plants and fruits (**Table 6**) to varying temperature.

2.1.2.3 Sugar alcohols

Myo-inositol (MI) plays a central role in the growth and development of plants. The pathway from glucose 6-phosphate (Glc-6-P) to *myo*-inositol 1-phosphate (MI-1-P) and *myo*-inositol (MI) is essential for the synthesis of various metabolites²⁰⁷. Metabolic processing of MI beyond biosynthesis produces other stereo-forms of inositol with a host of functional roles (**Figure 3**)²⁰⁸. Free MI is generally regarded as a ubiquitous constituent of plant tissues and, in some species, notably *Actinidia arguta*, MI is the major sugar constituent (60–65%) during early fruit development²⁰⁸. *Actinidia arguta* is a cold tolerant and heavily cropping species that produces a small fruit resembling kiwifruit (*Actinidia deliciosa* var. *deliciosa*) containing glucose as the major sugar. Although different in their content of *myo*-inositol in the fruit²⁰⁹, both *A. arguta* and *A. deliciosa* responded to salt stress with an increase in the accumulation of *myo*-inositol in leaf tissue²¹⁰. Sucrose was found to be the predominant sugar (> 95%) in the phloem of both species, suggesting that the accumulation of *myo*-inositol during the early stages of fruit development might be due to MI synthesis in the fruit²⁰⁹. However, in the roots of *Mesembryanthemum crystallinum*, the level of MI-1-P synthase was suppressed after salinity stress, while the level in leaves was enhanced. The proportion of inositols in phloem exudates also increased under salt stress. Therefore, it has been suggested that the absence of *myo*-inositol synthesis in roots is compensated for by inositol/ononitol transport in the phloem²¹¹.

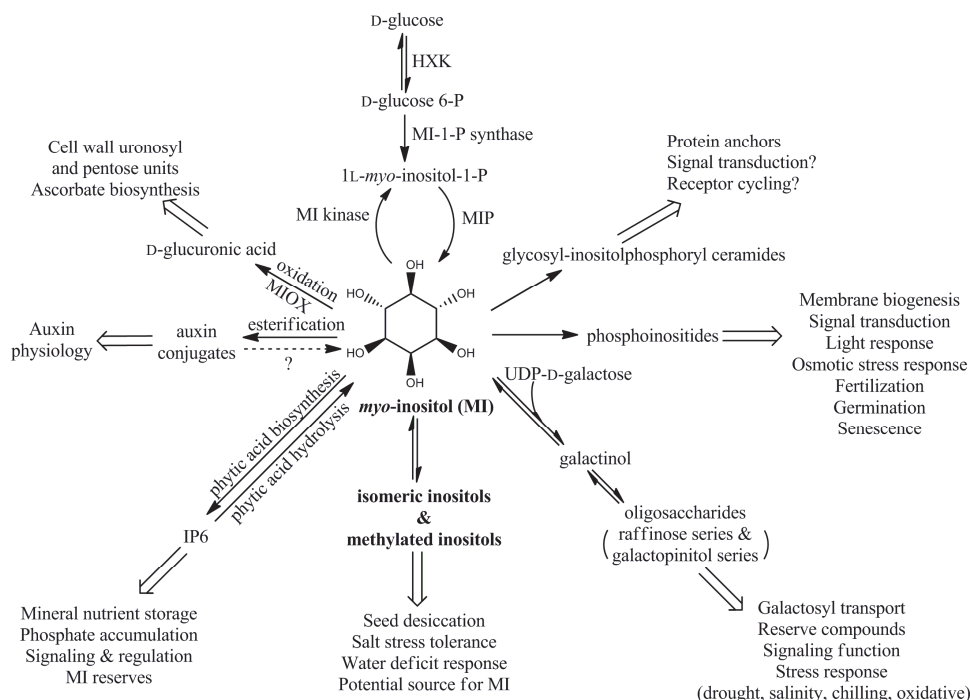


Fig. 3 Metabolism and functional roles of *myo*-inositol in plants. HKX, hexokinase; MI-1-P, *myo*-inositol 1-phosphate; MIP, *myo*-inositol monophosphatase; MI, *myo*-inositol; MIOX, *myo*-inositol oxygenase; IP6, *myo*-inositol-1,2,3,4,5,6-hexakisphosphate (phytic acid)^{178,208,212-215}.

One of the most important roles of MI in plant metabolism is the formation of various isomeric inositols and methylated inositols (**Figure 7**), which participate in stress-related responses, storage of seed products, and production of inositol-glycosides such as pinitol-galactosides²⁰⁸. In response to cellular dehydration, many plants and microorganisms accumulate compatible solutes, of which sugar alcohols (mannitol, sorbitol, and pinitol) are one group^{216,217}. Pinitol is abundant in a number of salt- and drought-tolerant plants. When irrigated with 400 mM sodium chloride, the halophytic ice plant *Mesembryanthemum crystallinum* accumulated pinitol, which eventually constituted over two-thirds of the soluble carbohydrate fraction and approximately 10% of the dry weight²¹⁸. In the ice plant, *myo*-inositol is methylated to D-ononitol (1-D-4-*O*-methyl-*myo*-inositol) and subsequently epimerized to D-pinitol (3-*O*-methyl-D-*chiro*-inositol) via *myo*-inositol *O*-methyltransferase (IMT1) and ononitol epimerase (OEP1), respectively²⁰⁷. The gene encoding *myo*-inositol *O*-methyltransferase for the methylation reaction, *Imt1*, is transcriptionally induced under water stress^{219,220}. This provides strong support for the importance of sugar alcohols in establishing tolerance to

osmotic stress in higher plants²¹⁹. This is supported by findings in transgenic tobacco (*Nicotiana tabacum* L. cv. SRI) plants and soybean (*Glycine max* L. Merr. cv. Jack) embryogenic tissue bearing *myo*-inositol *O*-methyl transferase from the ice plant, which were found to accumulate ononitol and provide better protection under drought and salt stress conditions than wild-type plants²²¹. An increase of 10- to 80-fold in ononitol levels in transgenic embryogenic soybean tissue led to 3- to 7-fold higher pinitol levels in transgenic embryos compared to non-transformed tissues, illustrating the positive relationship between ononitol and pinitol production in transgenic tissues²²².

Ishitani et al. also reported up-regulated expression of the gene encoding *myo*-inositol 1-phosphate synthase (MI-1-P synthase), *Inps1*, in the ice plant under salinity stress. Meanwhile, free *myo*-inositol accumulated by approximately 10-fold during salinity stress²⁰⁷. In contrast, *Arabidopsis thaliana* under salt stress did not show an up-regulation of *Inps1* or increased amounts of *myo*-inositol. The lack of *Inps1* induction in *Arabidopsis* exemplifies differences in the regulation of gene expression in glycophytic and halophytic plants at the point of entry into a pathway that leads to osmoprotection²⁰⁷. Up-regulation of MI-1-P synthase was also detected in salt-tolerant varieties of rice (*Oryza sativa* L.), but not in salt-sensitive varieties²²³. Wang et al.²²⁴ reported that the transcription of the MI-1-P synthase encoding gene (JcMIPS) in leaves of *Jatropha curcas* was up-regulated by abscisic acid (ABA), drought, NaCl, and low temperature (4°C) treatments. Interestingly, they found that the enzyme activity of MI-1-P synthase also increased in response to treatment with drought, NaCl, ABA and low temperature, among which the low temperature treatment showed the least impact.

Gillaspy et al.²¹⁴ reported that *myo*-inositol monophosphatase (MIP) in tomato accumulated at higher levels in light-grown seedlings compared with dark-grown seedlings. Khurana et al.²²⁵ identified a heat-inducible, *myo*-inositol-1-phosphate synthase gene, *TaMIPS*, from wheat (*Triticum aestivum* cv. CPAN 1676) and reported an increase in the transcript levels under heat stress (37°C/40°C). Meanwhile, an increase in the levels of *myo*-inositol was detected in shoots, but not in roots²²⁵.

Other studies have indicated a complex response of inositol accumulation in plants with respect to changes in environmental factors. In leaves of passion fruit plants under both dark and continuous light, enhanced transcription of the *myo*-inositol-1-phosphate synthase gene (*PeMIPS1*) was observed after 16 h at a temperature of 27°C but not at 37°C, which showed the lowest amounts of transcripts compared with the control. In a cold stress study, *PeMIPS1* transcripts were up-regulated during a short period (8 h) of cold stress (5°C) under both dark and continuous light conditions. However, under dark conditions, after an increase at 8 h, *PeMIPS* transcription showed a decrease in

leaves exposed to cold stress for 16 h²²⁶. In barley (*Hordeum vulgare*) leaves, the expression of genes encoding sucrose: fructan 6-fructosyltransferase (6-SFT) and *myo*-inositol 1-phosphate synthase (MIPS) were enhanced by cool temperature (10°C/5°C, 16 h light/8 h dark) treatment within two days and then declined after four days⁸³.

Although the metabolism of inositols and its regulation by environmental conditions have been studied in many plants, information on the concentration of these metabolites in fruits and berries and their response to a varying environment is limited. Only a few reports have been published, as listed below (**Table 7**).

In a study on bilberries (*Vaccinium myrtillus* L.), the content of *myo*-inositol was significantly (19%) higher at 12°C than at 18°C; however, the effect of light on the concentration of *myo*-inositol in bilberries was complex and depended on the temperature applied. At 12°C, *myo*-inositol content was highest with a 12 h photoperiod treatment, whereas at 18°C, the level was highest with a 24 h photoperiod with additional red light⁵⁵. Yang et al.³³ reported a negative correlation between the content of L-quebrachitol and temperature variables in sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*). The content of *myo*-inositol correlated negatively with the number of frost-free days. In contrast, the study in this thesis showed that the contents of *myo*-inositol and L-quebrachitol in sea buckthorn of ssp. *mongolica* correlated positively with temperature parameters²²⁷. Co-influences of radiation, precipitation and humidity on the composition of inositols in berries should be considered.

The inositol content in kiwifruits has been reported to be affected differently by increased vine temperatures according to stage of fruit development⁵⁷. Inositol concentrations in fruit were elevated to approximately double after vines were heated during starch accumulation [41–161 DAA (days after 50% anthesis)], and this difference was maintained thereafter until fruit maturation (194 DAA). However, the concentration of inositol did not vary in the fruits from vines heated during cell division (0–40 DAA) and maturation (162–184 DAA).

Table 7 Environmental effects on contents of sugar alcohols in fruits and berries.

Fruit/berry	Species/cultivar	Investigated component	Variation of environmental factors	Co-factor	Variation of component content	Ref.
bilberry	<i>Vaccinium myrtillus</i> L.	myo-inositol	Temp ↑ Light		↓	55
kiwifruit	<i>Actinidia deliciosa</i> (A. Chev.) C. F. Liang et A.R. Ferguson var. <i>deliciosa</i> Hayward	inositol	Temp ↑ (cell division & maturation stage of fruit)		-	57
kiwifruit	<i>Actinidia deliciosa</i> (A. Chev.) C. F. Liang et A. R. Ferguson var. <i>deliciosa</i> cv. Hayward	inositol	Temp ↑ (starch accumulation stage of fruit)		↑	
kiwifruit	<i>Actinidia deliciosa</i> (A. Chev.) C. F. Liang et A. R. Ferguson var. <i>deliciosa</i> cv. Hayward	myo-inositol	Water stress (21 days in early summer/late summer)		-	68
sea buckthorn	<i>Hippophaë rhamnoides</i> ssp. <i>sinensis</i>	L-quebrachitol myo-inositol methyl-myo-inositol total inositol	Temp ↑ Frost-free days ↑ Precipitation ↑ Temp ↑	√ √ √ √	↓ ↓ ↑ ↓	33

Abbreviations: Temp, temperature.

2.2 Latitudinal and altitudinal effects on the composition of fruits and berries

Many investigations have shown that growth location affects the chemical composition of fruits of strawberry²²⁸, black mulberry²²⁹, black currant¹⁸¹, raspberry²³⁰, sea buckthorn³³, and quince fruits²³¹. Changes in latitude/altitude always cause concomitant changes in environmental conditions, such as temperature, light intensity, and photoperiod. These factors determine the metabolism of plants and the concentration of various compounds in fruits/berries, which have a final impact on the quality and commercial value of fruits. Therefore, the proper selection of cultivars and cultivation regions according to the environmental conditions are important. The improvement of sensory quality or the enhancement of certain bioactive compounds in fruits might also be achieved by the selection of growth sites, together with cultivar breeding, and perhaps some agricultural practices.

Many studies have investigated the impact of growth location on the sugar content by determining the total soluble solids in harvested fruits, although the value indicates not only the sugar content but also other soluble compounds. Only a few studies have conducted quantitative analyses of individual sugars. Pomegranate fruits grown at a lower latitude (southern Israel, latitude 29°53'N, longitude 35°03'E) were found to contain lower levels of glucose (up to 50%), fructose (up to 50%), total soluble solids (up to 30%), and lower levels of citric acid (up to 40%) and malic acid (up to 75%) when compared to fruits grown at a higher latitude (northern Israel, 32°42'N, 35°11'E). The content of ascorbic acid did not vary significantly in fruits grown at different locations. The northern location (Newe Ya'ar) is characterized by a Mediterranean temperate to subtropical climate, whereas the southern location (southern Arava) is characterized by a hot and dry desert climate. Therefore, the authors⁷⁴ suggested that the relatively cooler temperatures in the north most likely promoted an increase in glucose and fructose, as well as enhanced the production of citrate and malate. In agreement, Hårdh et al.²³² reported that strawberries grown at a lower latitude (latitude 60°11' N) had a lower sugar content (by 45%) than those grown at a higher latitude (69°04' N) in Finland.

In a study on three cape gooseberry (*Physalis peruviana* L.) ecotypes, i.e. 'Kenya', 'South Africa' and 'Colombia'²³³, the sugar contents in fruits were affected differently by the altitude between plant origins. The 'Kenyan' fruits significantly accumulated about two-fold more fructose and glucose at a high altitude of 2690 m than at the lower site (2300 m), whereas the fructose and glucose concentration in 'Colombian' fruits showed a decreasing trend (by about 17% and 12% for fructose and glucose, respectively) as altitude

increased. The 'South Africa' fruit was not affected by growth altitude regarding its fructose and glucose contents ($p > 0.05$). The sucrose content showed a decreasing trend (decreased by up to 25%) as growth latitude increased in all these ecotypes. The small range of altitudinal difference (less than 400 m) in this study should be considered.

The dependence of compositional response to altitudinal changes on genetic backgrounds has also been detected in other fruits. Crespo et al.²³⁴ investigated the concentration of both healthy and taste-related compounds in strawberry fruits of four cultivars grown at two different altitudes (1060 and 480 above sea level, with the latitude/longitude being 46°04'N/7°18'E vs. 46°12'N/7°18'E). They found that the compositional variations of strawberry fruits in response to different production sites were genotype specific. Fruits of cv. Antea showed a significantly lower glucose (10.1 vs. 16.0 mg/g FW) and fructose (11.6 vs. 18.3 mg/g FW) content when plants were grown at a higher altitude compared with those grown at a lower altitude. For cvs. Asia, Clery, and Matis, no significant differences in the hexose contents were found between the two altitudes. The sucrose content did not vary between fruits grown at the two studied altitudes. However, the organic acid content of the fruits was generally affected by production site and no genotype \times production site interaction was found. Lower values of citric acid (up to 25% lower) and ascorbic acid (up to 33%) were observed in fruits grown at higher altitude than those at lower altitude, but no significant difference was observed in the malic acid content. The vitamin C (AsA + DHA) content was lower (up to 29%) in the fruit of plants grown in the mountain region with higher altitude compared to those grown in a region of lower altitude. The differences in total vitamin C and ascorbic acid between production sites leveled off when calculated on a dry matter basis, suggesting the dilution of vitamin C in the fruits grown at higher altitude. The content of total anthocyanins in each cultivar of strawberry did not vary between different production sites.

Many plant species have adapted well to distinct environments at different latitudes through different strategies, one of which is the production of secondary metabolites. Flavonoids are a widely spread group of secondary metabolites that have many crucial functions in plants²³⁵. In a study conducted by Carbone et al.²³⁶, the influence of internal (genetic and developmental) and external (environmental) factors on the content of flavonoids was studied in fruits of six cultivated strawberry (*Fragaria* \times *ananassa* Duch.) genotypes grown at two locations (southern location: 40°23'40''N, 16°46'55''E vs. northern location: 44°09'55''N, 12°16'00''E) in Italy. The authors found that the variation in anthocyanins in ripe strawberry fruits was largely due to the genetic background rather than environmental factors. However, the flavonol levels were about two-fold higher in fruits grown at the northern location than

those grown at the southern location, with a large inter-genotype variation. In a study in bog bilberry (*Vaccinium uliginosum* L.) in Finland, flavonols also showed an increasing trend (from 104–151 mg/100g FW to 169–197 mg/100g FW) as the growth latitude increased from 60–61°N to 66–69°N²³⁷. However, in the currant berries assessed in this study^{118,119}, the content of total flavonols either showed a decreasing trend (by 10–12%, $p < 0.05$) in black currant Mortti, Ola, and Melalahti or remained constant ($p > 0.05$ in the green currant Vertti, the white currant White Dutch and the red currant Red Dutch) as the growth latitude increased. It should be mentioned that the same latitudinal difference does not result in a large climate difference (e.g. photoperiod and solar radiation) in regions of low latitude such as in Italy compared to high latitudes such as in Finland.

Anthocyanins in bilberries have been reported to accumulate more in fruits grown at higher latitude than at lower latitude, probably because of the lower temperature. Lätti et al.^{237,238} analyzed anthocyanins in two species of bilberries (*Vaccinium myrtillus* L. and *Vaccinium uliginosum* L.) collected from different populations on a south-north axis (latitude 60°21'N–68°34'N) in Finland and found significantly higher levels in berries from the northern and central regions (175–245 mg/100 g FW) than in those from the southern region (153–175 mg/100 g FW). A similar trend with increasing anthocyanidin levels toward the north was detected in bilberries (*Vaccinium myrtillus* L.)²³⁹ in Sweden and Denmark (the latitude varied from 56°50'N to 66°37'N). Moreover, studies on bilberry^{55,239} clones of different geographical origins grown at the same location showed that the northern clones had higher yields of anthocyanidins even when growing at the same site as the southern clones. These results suggest the existence of latitude related genetic adaptation in anthocyanin production in berries⁵⁵.

In *Vaccinium myrtillus* L., delphinidin and cyanidin are the two major anthocyanidins, and delphinidin glycosides have been reported to dominate in northern berries whereas the cyanidin glycosides were most common in southern ones²³⁸. This trend was also reported in black currant, with the varieties from Scandinavia containing more delphinidin glycosides while British varieties were dominated by cyanidin glycosides²⁴⁰. This might indicate a positive effect of low temperature on levels of delphinidin glycosides in bilberries, which was detected in a study conducted by Uleberg et al.⁵⁵. In addition, the results of Uleberg et al., showing that long days (24 h light and/or 24 h light with additional red light) significantly increased levels of all measured anthocyanin derivatives except cyanidin glycosides, may also provide an explanation as the photoperiod is longer at higher latitudes. However, contradictory results have been reported by Martinelli et al.²⁴¹, who found that delphinidin glycosides were relatively more abundant in berries

from Italy, Romania and Poland, while cyanidin glycosides were more abundant constituents in Swedish and Norwegian berries. In this thesis, the proportion of delphinidin glycosides was greater and cyanidin glycosides were lower in black currants grown in southern Finland than those grown in northern Finland¹¹⁸.

In studies by Schwartz et al.⁷⁴ and Borochoy-Neori et al.¹⁵⁷ on pomegranate fruits, the level of total anthocyanins both in aril juice and in peels was significantly higher in fruits obtained from northern areas (latitude 32°42'N, longitude 35°11'E) compared to those from southern areas (29°53'N, 35°03'E). The two major anthocyanidins, cyanidin and delphinidin, were both higher (by 4.7- and 1.4-fold, respectively) in the arils of fruits from the north than those from the south, but the increase in the concentration was greater in cyanidin than in delphinidin in response to an increase in latitude¹⁵⁷. Thus, the concentration and abundance of cyanidin in the arils of northern fruits were much higher compared to delphinidins, in contrast to the practically equal concentration of the two anthocyanin types in southern fruit¹⁵⁷. The content of total phenolic compounds was higher in peels but lower in aril juice from pomegranate fruits grown in the south with higher temperature and radiation than those grown in the north⁷⁴.

In the study included in this thesis, red currants grown at higher latitudes contained significantly higher total anthocyanin contents (12% higher) than those grown at lower latitudes¹¹⁹. However, black currants showed clear decreasing trends in the total content of anthocyanins (by 15–27%) as latitude increased from 60°23'N to 66°34'N in Finland¹¹⁸.

Higher levels of anthocyanins in apple peels were detected in fruits grown in Hawke's Bay, New Zealand (latitude 39°39'S, longitude 176°53'E, altitude 260 m) than those grown in Lleida, Spain (46°09' N, 3°22' E, 260 m)¹⁶⁸. It should be mentioned that the two growth sites are located on opposite sides of the equator. Hawke's Bay, New Zealand is characterized by moderate summer temperatures and lower radiation, while Lleida, Spain has high summer temperatures (>30°C) and higher radiation.

The effects of growth altitude on anthocyanin accumulation in fruits/berries have also been reported. In bilberry (*Vaccinium myrtillus* L.)²⁴² grown in Austria at different altitudes (800, 1200, and 1500 m, coordinates: 47°20'–47°27'N, 13°56'–14°02'E), the contents of all the anthocyanins in air-dried berries decreased as the growth altitude increased. But, in a study on elderberries (*Sambucus nigra* L.)²⁴² grown at two altitudes (670 and 1000 m), the content of cyanidin-3-*O*-glucoside was lower and that of quercetin-3-*O*-rutinoside was higher in the samples collected at higher altitudes, where greater solar radiation can be assumed. These results were contradictory to the hypothesis that plants from higher altitudes contain higher amounts of

anthocyanins as a result of their exposure to more severe climatic conditions, including increased solar radiation. In a study on grape (*Vitis vinifera* L. cv. Malbec)¹³⁸, fruits grown at 1500 m were most sensitive to variations in UV-B radiation and contained the highest amount of total anthocyanins and total polyphenols in the skin, followed by fruits grown at 1000 m and 500 m.

However, few studies have been conducted on black currant and sea buckthorn berries in terms of the compositional response to latitudinal and altitudinal variation. Lian et al.²⁴³ reported correlations between total sugar, total acids, and the sugar/acid ratio of *H. rhamnoides* ssp. *sinensis* with concurrent changes in latitude and altitude. Although the correlations were not quite apparent, latitude and altitude showed opposite effects on these compounds in the berries. They reported that the content of total sugar increased as latitude increased and as altitude decreased, while the total acid content showed the opposite trend. The values of total sugars varied from 2.63% to 7.08%, and those of total acids from 4.34% to 6.77% in berries from different growth sites. The sugar/acid ratio appeared to correlate positively with altitude and negatively with latitude. However, there was no detailed information on individual sugars and acids, or on other sea buckthorn species/varieties.

Genetic differentiation between sea buckthorn populations along latitudinal and altitudinal gradients have been reported^{244,245}. In a study on *H. rhamnoides* ssp. *sinensis* using random amplified polymorphic DNA (RAPD) markers²⁴⁴, the results of the multiple regression analysis indicated that genetic distance significantly correlated ($p < 0.05$) with altitudinal and latitudinal distances among the populations. However, the Mantel test in this study showed that genetic distance had a significant correlation only with altitudinal distance ($p < 0.05$). Chen et al.²⁴⁵ investigated the genetic diversity of sea buckthorn samples at varying altitudes from the same area, and revealed substantial genetic divergence among populations and significant correlations between altitudinal distance and genetic distance. This indicates that altitudinal gradients may be the prime cause affecting the genetic variation pattern of different populations in *H. rhamnoides*. Such variation according to the growth altitude may be caused by the complex topography, featuring tall, zig-zag positioned mountains, which effectively restrict gene flow. The altitudinal variation found in the genetic background may also have been the result of long adaptation to the different climatic conditions at different altitudes, which produces varying selective pressure, e.g. delayed flower development. However, in contrast to these studies, Tian et al.^{246,247}, Sun et al.²⁴⁸, and Bartish et al.²⁴⁹ reported no significant association between geographic and genetic distances among sea buckthorn (*H. rhamnoides*) populations. Sun et al.²⁴⁸ suggested that the low genetic differentiation among populations in ssp. *sinensis* may be attributed to

the long-distance dispersal of seeds facilitated by birds and small animals. Another study by Bartish et al.²⁵⁰ showed that the association between genetic and geographic distances can change considerably along a range of geographic distances. In their study based on RAPD polymorphic bands, spatial autocorrelation analyses performed on 12 populations of *H. rhamnoides* revealed a positive autocorrelation of allele frequencies when geographic distances ranged from 0 to 700 km, but no or negative autocorrelation with greater distances. Nevertheless, no significant correlation between genetic and geographic distances was found when populations from different species were included in the same analysis. The results of these studies suggest that geographic distance does not have a clear effect on genetic differentiation, and it is very important to understand a population's response to changing environmental conditions (or stress).

2.3 Conclusion

The composition and quality of fruits/berries varies remarkably depending on genetic background, growth sites and environmental factors. Different metabolic pathways or different regulation systems of these pathways exist in plants of different genetic backgrounds. The metabolic regulation in fruits may even vary between different plant developmental stages.

It has been observed that increases in light intensity induce increases in the content of sugars (by 7–35%) and ascorbic acid/total vitamin C (AsA + DHA) (by up to seven-fold) in fruits. Fruit components may have common responses to varying growth conditions within a specific plant genus/species. The content of malic acid in grapes decreases, by up to two-fold, as light intensity increases and the water supply decreases. The total content of anthocyanins in apple peels has been reported to increase by up to ten-fold as light intensity increases but as temperature decreases. Most grapes also show increases in anthocyanin content as temperature and the water supply decrease but as radiation increases. The content of flavonols in grape skins increases as light intensity increases, possibly caused by an increase in UV-B radiation, but no common trends have been detected with temperature and irrigation changes.

Different responses of the anthocyanin groups to environmental factors have been reported, depending on the genotype. The level/proportion of dioxygenated anthocyanins (cyanidin and peonidin derivatives) increases and that of trioxxygenated anthocyanins (delphinidin, petunidin and malvidin derivatives) decreases in grape skins with low radiation or high temperature. In pomegranate, the decrease in the level of diglucosylated anthocyanins (diglucosides of delphinidin and cyanidin)/trioxxygenated anthocyanins

(delphinidin glycosides) is less than that of monoglucosylated anthocyanins (glucosides of delphinidin and cyanidin)/dioxygenated anthocyanins (cyanidin glycosides) as the temperature increases. In the studies on currants included in this thesis, delphinidin glycosides in black currants correlated positively with temperature and radiation variables, whereas cyanidin glycosides in red currant correlated negatively with these parameters. Moreover, different responses of dioxygenated anthocyanins and trioxxygenated anthocyanins to different growth latitudes have been reported in studies on bilberries.

It is of crucial importance to understand the regulation of components and quality of fruits/berries in correlation to variations in environmental conditions and growth locations. Such information could provide useful guidelines for cultivar breeding, plantation development (growth area selection and cultivating practices), and commercial utilization for the purposes of enriching bioactive components and improving the sensory qualities of fruit/berry products.

3 AIMS OF THE STUDY

Currants and sea buckthorn berries have high nutritive value and are consumed as food or used as food raw materials in Europe and Asia. Thus, the sensory and nutritional properties are of great interest from the point of view of consumers and industry. Sugars, acids, phenolic compounds, vitamin C, and inositols are key components determining the bioactivities and sensory properties of berries. As reviewed from the literature, the content and composition of these components in fruits and berries vary greatly with genotype, growth site, growth environment, and cultivation practice.

Therefore, the aim of this study was to investigate the effects of genotype, growth latitude and altitude, and weather conditions on the composition and contents of sugars, organic acids, ascorbic acid, sugar alcohols, and phenolic compounds in currants and sea buckthorn berries. The study aimed to provide important guidelines for berry breeding and cultivation for the improvement of sensory qualities and the enrichment of nutrients.

The objectives of the current studies were to:

1. Compare the composition of berries between six currant cultivars (papers I, II, V and VI) and between sea buckthorn berries of various varieties/origins and two subspecies (papers III and IV).
2. Investigate the effects of growth latitude and altitude on the composition of sea buckthorn berries (papers III and IV).
3. Investigate the effects of growth latitude on the composition of the berries of various currant cultivars (papers I, II, V and VI).
4. Investigate the influence of weather variables on the composition of berries of different currant cultivars (papers I, II, V and VI) and sea buckthorn (paper IV).

4 MATERIALS AND METHODS

4.1 Samples

The berry samples used in the present investigations are listed in **Table 8**. Overall, six cultivars of currants (*Ribes* spp.) from two locations in Finland, nine varieties of sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) from three locations in Finland and Canada, and wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*) from nine locations in China were investigated. The berries were picked optimally ripe as defined by experienced horticulturists based on the color, flavor, and structure of the berries. The berries were frozen and stored at -20°C immediately after harvesting until analysis.

4.1.1 Currants

The study included three black currant (*Ribes nigrum* L.) cultivars, Mortti, Ola and Melalahti, a green currant (*Ribes nigrum* L.) cultivar Vertti, a red currant (*Ribes rubrum* L.) cultivar Red Dutch and a white currant (*Ribes rubrum* L.) cultivar White Dutch (**Table 8**). All bushes were planted in four field blocks (each block consisted of three bushes) in an identical way in the research fields of MTT Agrifood Research Finland in both Piikkiö (southern Finland) and Apukka (northern Finland) in May 2002. A ditch, 10 cm deep and 20 cm wide, was plowed through every block. The ditches were filled with white Sphagnum peat (pH 6) mixed with 8 kg dolomite lime and 1.5 kg/m^3 of NPK basic fertilizer. The seedlings were planted and the peat was covered with the local fine sand soil. All the bushes in both Piikkiö and Apukka were regularly fertilized by MTT. No irrigation was applied during the study period. The berries were harvested in quadruplicate from the four field blocks in each location, in order to minimize plant-to-plant variation, in three consecutive years for papers I and II, six years in paper V and seven years in paper VI.

Table 8 Sample collection for the study

Berry	Species	Subspecies	Variety/cultivar	Location	Country	Longitude	Latitude	Altitude (m)	Crop years
black currant	<i>Ribes nigrum</i> L.			Prikkiö	Finland	22°33'E	66°23'N	10	Aug 15, 2005; Aug 18, 2006; Aug 14, 2008; Aug 21, 2009; Aug 17, 2010
				Mortti	Finland	26°00'E	66°34'N	102	Aug 31, 2005; Aug 14, 2006; Aug 23, 2007; Sep 5, 2008; Aug 26, 2009; Aug 18, 2010
green currant				Prikkiö	Finland	22°33'E	66°23'N	10	Aug 19, 2005; Aug 11, 2006; Aug 7, 2007; Aug 21, 2009; Aug 17, 2010
				Ola	Finland	26°00'E	66°34'N	102	Aug 31, 2005; Aug 16, 2006; Aug 23, 2007; Sep 5, 2008; Aug 26, 2009; Aug 18, 2010
red currant	<i>Ribes rubrum</i> L.			Prikkiö	Finland	22°33'E	66°23'N	10	Aug 2, 2005; Aug 11, 2006; Aug 2, 2007; Aug 4, 2008; Aug 4, 2009; Jul 29, 2010
				Melalahiti	Finland	26°00'E	66°34'N	102	Aug 22, 2005; Aug 10, 2006; Aug 23, 2007; Sep 5, 2008; Aug 26, 2009; Aug 18, 2010
white currant				Vertti	Finland	22°33'E	66°23'N	10	Aug 9, 2005; Jul 31, 2006; Aug 1, 2007; Jul 29, 2008; Aug 4, 2009; Aug 10, 2010; Aug 8, 2011
				Red Dutch	Finland	26°00'E	66°34'N	102	Aug 29, 2005; Aug 16, 2006; Aug 23, 2007; Sep 5, 2008; Aug 26, 2009; Aug 18, 2010; Aug 30, 2011
white currant				Prikkiö	Finland	22°33'E	66°23'N	10	Aug 2, 2005; Jul 31, 2006; Jul 25, 2007; Jul 29, 2008; Aug 4, 2009; Jul 29, 2010; Aug 3, 2011
				Apukka	Finland	26°00'E	66°34'N	102	Aug 22, 2005; Aug 9, 2006; Aug 23, 2007; Sep 5, 2008; Aug 26, 2009; Aug 18, 2010; Aug 29, 2011
white currant				Apukka	Finland	22°33'E	66°23'N	10	Jul 27, 2005; Jul 25, 2006; Jul 16, 2007; Jul 29, 2008; Aug 4, 2009; Jul 29, 2010; Jul 22, 2011
				Apukka	Finland	26°00'E	66°34'N	102	Aug 22, 2005; Aug 8, 2006; Aug 23, 2007; Aug 21, 2008; Aug 26, 2009; Aug 29, 2011
white currant				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 5, 2003
				Kittilä	Finland	24°37'E	68°02'N	210	Sep 21, 2003
white currant				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 5, 2003; Aug 28, 2008; Aug 26, 2010
				Kittilä	Finland	24°37'E	68°02'N	210	Sep 22, 2003
white currant				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 5, 2003; Aug 28, 2008; Aug 31, 2009
				Kittilä	Finland	24°37'E	68°02'N	210	Sep 21, 2003
white currant				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 9, 2003
				Kittilä	Finland	24°37'E	68°02'N	210	Sep 22, 2003
white currant				Prevosodnaya	Finland	24°37'E	68°02'N	210	Sep 8, 2003
				Prevosodnaya	Finland	24°37'E	68°02'N	210	Sep 8, 2003
white currant				Québec	Canada	71°17'W	46°47'N	100	Aug 28, 2007; Sep 3, 2008; Aug 24, 2009; Aug 16, 2010
				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 4, 2003; Aug 28, 2008
white currant				Québec	Canada	71°17'W	46°47'N	100	Sep 22, 2003
				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 4, 2003; Aug 28, 2008
white currant				Québec	Canada	71°17'W	46°47'N	100	Aug 28, 2007; Sep 3, 2008; Aug 24, 2009; Aug 23, 2010
				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 4, 2003; Aug 28, 2008
white currant				Québec	Canada	71°17'W	46°47'N	100	Aug 28, 2007; Sep 3, 2008; Aug 24, 2009; Aug 16, 2010
				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 4, 2003; Aug 28, 2008
white currant				Québec	Canada	71°17'W	46°47'N	100	Aug 28, 2007; Sep 3, 2008; Aug 24, 2009; Aug 23, 2010
				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 4, 2003; Aug 28, 2008
white currant				Québec	Canada	71°17'W	46°47'N	100	Aug 28, 2007; Sep 3, 2008; Aug 24, 2009; Aug 23, 2010
				Sammalmäki	Finland	22°09'E	66°23'N	1	Sep 4, 2003; Aug 28, 2008
white currant				Hebei	China	116°34'E	41°17'N	818	Oct 29, 2006; Nov 25, 2007; Oct 16, 2008
				Inner Mongolia	China	109°48'E	39°47'N	1480	Nov 24, 2006; Nov 30, 2007; Oct 25, 2008
white currant				Heilongjiang	China	127°06'E	47°14'N	210	Nov 28, 2006; Nov 28, 2007; Dec 15, 2008
				Qinghai	China	101°23'E	36°45'N	3115	Oct 29, 2006; Oct 29, 2007; Oct 25, 2008
white currant				Shanxi	China	113°52'E	37°05'N	1512	Oct 21, 2006; Oct 23, 2007; Oct 16, 2008
				Shanxi	China	113°52'E	37°05'N	1512	Oct 21, 2006; Oct 23, 2007; Oct 16, 2008
white currant				Sichuan	China	106°54'E	31°01'N	2000	Oct 20, 2006; Oct 20, 2007; Oct 15, 2008
				Sichuan	China	106°54'E	31°01'N	2500	Oct 20, 2006; Oct 20, 2007; Oct 15, 2008
white currant				Sichuan	China	106°54'E	31°01'N	3000	Oct 20, 2006; Oct 20, 2007; Oct 15, 2008
				Sichuan	China	106°54'E	31°01'N	3000	Oct 20, 2006; Oct 20, 2007; Oct 15, 2008

4.1.2 Sea buckthorn

In paper III, wild sea buckthorn berries of *H. rhamnoides* ssp. *sinensis* were collected from nine locations in six provinces in China from 2006 to 2008 (**Table 8**). Two to four lots of samples were harvested in different field blocks at each growth site.

For paper IV, sea buckthorn bushes of six varieties (**Table 8**) were planted in Sammalmäki, Finland in 1998–1999. In 2003, some of the bushes were transported with soil after pollination from Sammalmäki, Finland to Kittilä, Finland. However, these bushes did not survive after 2003, which suggested that they were not good choices for sea buckthorn cultivation at such high latitudes with extreme environmental stresses. In Canada, 1–2 years old sea buckthorn bushes of five varieties (**Table 8**) were received bare-rooted and were planted in Québec, Canada in June, 2004. During the study, no fertilization was applied on any of the bushes in these three growth locations.

4.2 Analytical methods

4.2.1 Sugars, sugar alcohols, organic acids, and ascorbic acid

4.2.1.1 Sample preparation

Quadruplicate extractions of sugars, sugar alcohols, acids and ascorbic acid were performed using a method applied earlier in our laboratory²⁵¹, with the exception of black currant berries collected in 2005 and 2006 (paper I). The berries were weighed accurately in duplicate, thawed, and pressed manually. After centrifugation, a portion of 0.25 mL of the juice was taken in duplicate, and internal standards, sorbitol (Fluka, Buchs, Switzerland) and tartaric acid (Merck, Darmstadt, Germany) were added. The juice was then diluted to a final volume of 5 mL and filtered (0.45 µm). An aliquot of 300 µL of the filtrate was evaporated to dryness under a nitrogen stream at 40°C and kept in a desiccator over P₂O₅ overnight. Trimethylsilyl (TMS) derivatives of the juice samples were prepared by adding 600 µL of Tri-Sil reagent (Pierce, Rockford, IL), shaking vigorously with a Vortex for 5 min, and incubating at 60°C for 30 min.

For black currant samples collected in 2005 and 2006 (paper I), a fractionation procedure was applied on the diluted juice samples to separate the sugars and acids. The diluted juice sample was fractionated using a dual solid-phase extraction procedure, consisting of a non-polar cyclohexyl Isolute CH (EC) column and an anion-exchange Isolute SAX column (International Sorbent Technology, Hengoed, U.K.). Sugars and fruit acids were eluted from the SAX column with water and 15 M formic acid, respectively. Both fractions

were diluted to a final volume of 3 mL. A sample of 1 mL was evaporated to dryness and prepared as a TMS derivative.

4.2.1.2 Gas chromatographic (GC) analysis

For black currant samples collected in 2005 and 2006 (paper I), the TMS derivatives of the fractionated samples were analyzed with a Perkin Elmer Auto System gas chromatograph (GC) equipped with a flame ionization detector (FID). For all of the rest currant samples (papers I and II) and sea buckthorn samples (papers III and IV), the TMS derivatives of the dried juice samples were analyzed with a Hewlett Packard 5890 Series II GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a FID. The analyses were carried out with a methyl silicone Supelco Simplicity-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm d_f) (Bellefonte, PA). A sample of 1 μL was injected for analysis. The temperature of the injector was 210°C and that of the detector 290°C. Different temperature programs were used for different currant samples and sea buckthorn analysis (refer to original publications^{227,252-254}).

Quantification was carried out using sorbitol as an internal standard for sugars and sugar alcohols, and tartaric acid for fruit acids and ascorbic acid. The ethyl β-D-glucopyranoside (henceforth ethyl glucose) was quantified as glucose and methyl-*myo*-inositol as L-quebrachitol.

4.2.1.3 Gas chromatographic-mass spectrometric (GC-MS) analysis

TMS derivatives of sugars, acids and reference compounds were analyzed with a Shimadzu QP 5000 MSD GC-MS (Kyoto, Japan) and a DB-1MS column (30 m × 0.25 mm i.d. × 0.25 μm d_f) (J & W Scientific, Agilent, Folsom, CA). A sample of 0.5 μL was injected manually into a split (1:24) injector. The flow rate of the carrier gas helium was 1.3 mL/min. The temperature of the injector and the column temperature program were the same as in the corresponding GC-FID analysis^{227,252-254}.

4.2.2 Phenolic compounds

4.2.2.1 Sample preparation

4.2.2.1.1 Anthocyanin

Berries were thawed and homogenized with a Bamix mixer (Bamix M133, Switzerland) when half-melted. About 5–7 g of slurry was weighted, extracted three times with 15 mL of MeOH/HCl (99:1), and centrifuged at 3400g for 10 min. The supernatants were combined, and the total volume was made up to 50

mL with MeOH/HCl (99:1). A portion of 1 mL of sample was taken and filtered through a syringe filter (0.45 μm) before HPLC analysis.

4.2.2.1.2 Flavonol glycosides and hydroxycinnamic acid conjugates

After being thawed and homogenized, slurry of 5 g (paper V) or 10 g (paper VI) was weighted and extracted four times with 10 mL of ethyl acetate. The four extracts were combined and evaporated. The sample was diluted in 3 mL (paper V) or 2 mL (paper VI) of MeOH and filtered (0.45 μm) before HPLC analysis.

4.2.2.2 High-performance liquid chromatographic (HPLC) analysis

4.2.2.2.1 HPLC apparatus

The analyses of black currants (paper V) were carried out with a HPLC-UV system (Shimadzu Corporation, Kyoto, Japan) and a Phenomenex Prodigy RP-18 ODS-3 column (250 \times 4.60 mm i.d., particle size 5 μm) (Torrance, CA)¹¹⁸, and those of green, red and white currants (paper VI) with an HPLC-DAD system (Shimadzu Corporation, Kyoto, Japan) and a Phenomenex Luna C18(2) 100A column (250 \times 4.60 mm i.d., particle size 5 μm) (Torrance, CA)¹¹⁹.

4.2.2.2.2 Anthocyanins

A sample of 10 μL (paper V) and 20 μL (paper VI) was injected into the HPLC-UV and HPLC-DAD system, respectively. The analysis was carried out using 5% formic acid as solvent A and acetonitrile as solvent B. For the gradient programs, please refer to the original publications^{118,119}.

Anthocyanins were detected at 520 nm. For black currants (paper V), anthocyanins were quantified using the commercial standards delphinidin-3-*O*-glucoside (Extrasynthese, Genay, France), delphinidin-3-*O*-rutinoside (Polyphenols, Sandnes, Norway), cyanidin-3-*O*-glucoside (Polyphenols, Sandnes, Norway), and cyanidin-3-*O*-rutinoside (Extrasynthese, Genay, France) as external standards for each compound. For red currants (paper VI), anthocyanins were quantified with the external standard cyanidin-3-*O*-rutinoside.

4.2.2.2.3 Flavonol glycosides and hydroxycinnamic acid conjugates

A sample of 10 μL was injected into the HPLC system. The analyses were performed by using 1% formic acid as solvent A and acetonitrile as solvent B. The eluting gradient program was: 0–20 min, 5–30% B; 20–30 min, 30–90% B;

30–35 min, 90–5% B; 35–40 min, 5% B. The flow rate of the mobile phase was 1 mL/min.

Flavonol glycosides were detected at 360 nm and hydroxycinnamic acid conjugates at 320 nm. Quantitative analysis of flavonol glycosides was carried out using available commercial standards as external standards for corresponding compounds and quercetin-3-*O*-glucoside (Extrasynthese, Genay, France) for the rest of flavonol glycosides. Ferulic, caffeic, and *p*-coumaric acids (Sigma, St. Louis, MO) were used as external standards for the quantification of hydroxycinnamic acid conjugates^{118,119}.

4.2.2.3 Liquid chromatographic-mass spectrometric (LC-MS) analysis

The extracts of anthocyanins were the same and those of flavonol glycosides and hydroxycinnamic acid conjugates were concentrated 5 times as those for HPLC analyses. The LC-MS system used was an Acquity™ Ultra Performance LC (Waters, Milford, MA) interfaced to a Waters Quattro Premier quadruple mass spectrometer. The chromatographic conditions were the same as in the quantitative analysis and the ESI-MS analyses were performed according to the method previously applied for black currant analysis in our laboratory²⁶. Data were acquired over a mass range of *m/z* 250–800 for the analysis of anthocyanins and *m/z* 100–1000 for flavonol glycosides and hydroxycinnamic acid conjugates.

4.2.3 Dry weight measurement

About 5 g of berries were accurately weighed in a watch glass in duplicate and cut with a knife. The residue on the knife was rinsed carefully into the watch glass. The berries were dried at 105°C and weighed accurately upon reaching a constant weight.

4.3 Meteorological information

The meteorological data (**Table 9**) in each growth site were collected consecutively in all the investigating years for this study. The meteorological data in Finland were provided by the Finnish Meteorological Institute (Erik Palménin aukio, FI-00560 Helsinki, Finland), and the data for Canada was provided by Environment Canada (Gatineau, Québec, Canada) and le Centre de Recherche en Horticulture, Pavillon de l'Environnement, Université Laval (Hochelaga, Québec, Canada).

Table 9 Weather variables used in the present investigations

<i>weather variables</i>
growth season period with temperature over 5 °C (day)
temperature sum over 5 °C in growth season (°C)
temperature sum over 5 °C from the start of growth season until the day of harvest (°C)
temperature sum over 5 °C in the last month before harvest (°C)
hot days (temperature > 25 °C) from the start of growth season until the day of harvest (day)
hot days (temperature > 25 °C) in the last month before harvest (day)
average temperature in the last month before harvest (°C)
average temperature in the last week before harvest (°C)
mean daily temperature difference in the last month before harvest (°C)
minimum temperature in the last month before harvest (°C)
average of daily lowest temperature in the last month before harvest (°C)
maximum temperature in the last month before harvest (°C)
average of daily highest temperature in the last month before harvest (°C)
average temperature in January, February... August, September (°C)
radiation from the start of growth season until the day of harvest (kJ/m ²)
radiation during the last month before harvest (kJ/m ²)
radiation during the last week before harvest (kJ/m ²)
radiation in January, February... August, September (kJ/m ²)
precipitation from the start of growth season until the day of harvest (mm)
precipitation in the last month before harvest (mm)
precipitation in the last week before harvest (mm)
precipitation in January, February... August, September (mm)
average humidity from the start of growth season until the day of harvest (%)
average humidity in the last month before harvest (%)
average humidity in the last week before harvest (%)
average humidity in January, February... August, September (%)
percentage of the days with relative humidity 0–10, 10–20, ... , 80–90, 90–100% from the start of growth season until the day of harvest (%)
percentage of the days with relative humidity 0–10, 10–20, ... , 80–90, 90–100% in the last month before harvest (%)

4.4 Statistical analysis

Statistical analyses were performed using SPSS 16.0.1 (SPSS Inc., Chicago, IL) and Unscrambler 9.8/10.1 (Camo Process AS, Oslo, Norway). One-way analysis of variance (ANOVA) and independent sample *t*-tests were applied to compare the currant and sea buckthorn samples of different genetic backgrounds and from different growth locations. Differences reaching a minimal confidence level of 95% were considered as being statistically significant. Principal component analysis (PCA) was applied to investigate the compositional profiles of sea buckthorn berries from different growth areas in China (paper III). Bivariate correlation analysis and partial correlation analysis were performed to study the correlation between the growth latitudes and altitudes and the composition of Chinese sea buckthorns (paper III). Partial least squares discriminant analysis (PLS-DA) was used to explain the difference between cultivars or locations according to the phenolic contents in currant berries (papers V and VI). PCA and Pearson's correlation coefficient analysis were carried out to study the effects of weather conditions on the composition of currant and sea buckthorn berries.

5 RESULTS AND DISCUSSION

5.1 Cultivar/variety/origin comparison

In order to compare the compositional differences between currant/sea buckthorn berries of different genetic backgrounds, the compositional data of the samples grown in different growth sites were combined and investigated via ANOVA and independent sample *t*-tests (Table 10–15).

5.1.1 Currants (papers I, II, V and VI)

First of all, black currant cultivars Mortti and Ola showed no differences ($p > 0.05$) in the contents of all the compounds investigated in this study (Table 10–14), suggesting similar genetic backgrounds or metabolic pathways in these two cultivars.

Citric acid (60–95% of total acids) was the most abundant acid, while fructose (39–55% of total sugars) and glucose (35–51% of total sugars) were the major sugars in all the currant cultivars investigated (Table 10). Sucrose was only found in the currant cultivars of the species *Ribes nigrum*, and absent in cultivars of the species *Ribes rubrum*. In contrast, 0.06 g kg⁻¹ of sucrose has been reported in fresh berries of red and white currants (USDA National Nutrient Database for Standard Reference (http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl)). This may have been due to the different genetic background of the berries in the two studies.

The red currant cultivar Red Dutch had the lowest value of soluble solids, and correspondingly showed the lowest amount of total sugars and total acids in the fresh berries (Table 10–11). The green currant cultivar Vertti exhibited the highest content of sugars among all the cultivars and relatively lower levels of acids. In contrast to Vertti, black currant cultivars Mortti and Ola showed the lowest sugar/acid ratio and the highest acid content.

Table 10 Comparison between currant cultivars for the contents of sugars and acids^a

Species	Variety	Fructose	Glucose	Sucrose	Total sugars (g/100 mL)	Malic acid	Citric acid	Quinic acid (mg/100 mL)	Ascorbic acid (mg/100 mL)	Total acids (g/100 mL)
<i>Ribes</i>	Mortti	3.95±0.97 bc	3.03±0.63 a	1.17±0.35 bc	8.15±1.71 b	0.19±0.06 ab	3.34±0.50 d	30±11 c	190±58 e	3.74±0.50 c
<i>Ribes</i>	Ola	3.94±0.92 bc	3.02±0.61 a	1.18±0.32 bc	8.14±1.54 b	0.20±0.04 b	3.25±0.42 d	30±8 c	179±53 e	3.65±0.41 c
<i>Ribes</i>	Melalahti	3.47±0.50 a	3.59±0.60 b	1.07±0.40 b	8.12±1.40 b	0.17±0.05 a	2.78±0.35 c	23±9 b	64±33 c	3.03±0.39 b
<i>Ribes</i>	Vertti	4.57±0.75 d	4.08±0.87 c	1.27±0.47 c	9.92±1.96 c	0.34±0.04 c	2.49±0.40 b	37±7 d	107±35 d	2.97±0.40 b
<i>Ribes</i>	Red Dutch	3.87±0.70 b	3.11±0.57 a	nd a	6.99±1.25 a	1.05±0.19 e	1.61±0.31 a	15±3 a	22±6 a	2.70±0.24 a
<i>Ribes</i>	White Dutch	4.24±0.42 c	4.38±0.50 c	nd a	8.61±0.87 b	0.48±0.10 d	2.57±0.32 b	13±8 a	39±14 b	3.11±0.38 b

^aSignificant difference ($p < 0.05$) between samples of different cultivars are marked as a–e. The sample data from southern Finland and northern Finland are combined for the cultivar comparison. Key: nd, not detected.

Table 11 Comparison between currant cultivars for the other compositional parameters^a

Species	Variety	Sugar ^o Brix	Sugar/acid	°Brix	pH	Juice yield (mL/100g)	Dry weight (%)
<i>Ribes</i>	Mortti	0.56±0.09 a	2.17±0.32 a	14.4±1.3 c	2.96±0.07 c	31.5±7.5 a	22.09±1.87 d
<i>Ribes</i>	Ola	0.55±0.08 a	2.22±0.30 a	14.9±1.3 cd	2.94±0.06 c	31.3±7.4 a	21.69±2.02 d
<i>Ribes</i>	Melalahti	0.53±0.08 a	2.69±0.37 bc	15.2±1.3 de	2.88±0.06 a	35.0±6.8 b	21.16±2.71 d
<i>Ribes</i>	Vertti	0.63±0.07 b	3.34±0.48 d	15.6±2.0 e	2.99±0.08 d	31.5±4.8 a	19.89±1.55 c
<i>Ribes</i>	Red Dutch	0.66±0.09 b	2.59±0.45 b	10.5±0.9 a	2.95±0.06 c	41.2±5.1 c	15.49±1.31 a
<i>Ribes</i>	White Dutch	0.72±0.07 c	2.80±0.34 c	12.0±0.7 b	2.91±0.07 b	39.3±6.1 c	17.20±1.17 b

^aSignificant difference ($p < 0.05$) between samples of different cultivars are marked as a–e. The sample data from southern Finland and northern Finland are combined for the cultivar comparison.

Table 12 Comparison between currant cultivars for the contents of hydroxycinnamic acid conjugates^a

Species	Variety	Caffeoyl-glucose	p-Coumaroyl-quinic acid	p-Coumaroyl-glucose	Feruloyl-glucose	Caffeic acid glucose derivative (mg/100 g fresh berry)	p-Coumaric acid glucose derivative	Ferulic acid glucose derivative	Total hydroxy-cinnamic acids
<i>Ribes nigrum</i>	Mortti	1.22±0.20 a	0.98±0.23 e	1.05±0.38 b	0.81±0.16 e	na	1.19±0.26 c	1.26±0.23 d	6.51±1.13 d
	Ola	1.24±0.22 a	1.02±0.23 e	1.06±0.33 b	0.82±0.16 e	na	1.22±0.31 c	1.21±0.23 d	6.56±1.19 d
	Melalahti	1.30±0.24 a	0.84±0.15 d	0.61±0.17 a	0.67±0.12 d	na	0.77±0.12 b	0.81±0.14 c	5.00±0.65 c
<i>Ribes rubrum</i>	Vertti	1.97±0.35 b	0.40±0.13 c	6.23±1.97 c	0.59±0.10 c	0.51±0.07 ^b	1.25±0.17 c	0.42±0.09 b	11.35±2.17 e
	Red Dutch	na	0.13±0.03 b	na	0.10±0.02 a	nd	nd a	nd a	0.23±0.04 a
	White Dutch	na	0.11±0.02 a	0.97±0.29 b	0.16±0.04 b	nd	nd a	nd a	1.25±0.34 b

^aSignificant difference ($p < 0.05$) between samples of different cultivars are marked as a–e. The sample data from southern Finland and northern Finland are combined for the cultivar comparison. ^bCaffeic acid glucose derivative coeluted with a p-coumaric acid derivative. Key: na, not analyzed; nd, not detected.

Table 13 Comparison between currant cultivars for the contents of flavonol glycosides^a

Species	Variety	Myricetin-3-O-glucoside	Quercetin-3-O-rutinoside ^b	Quercetin-3-O-glucoside	Kaempferol-3-O-rutinoside	Quercetin-3-O-(6''-malonyl)-glucoside ^c	Kaempferol-3-O-glucoside	Total flavonol glycosides
<i>Ribes nigrum</i>	Mortti	2.35±0.76 d	2.24±0.31 b	2.96±0.64 d	0.48±0.08 c	1.27±0.45 c	0.95±0.29 c	10.24±1.47 d
	Ola	2.33±0.74 d	2.17±0.39 b	2.88±0.66 d	0.50±0.11 c	1.32±0.46 c	0.94±0.28 c	10.12±1.68 d
	Melalahti	1.33±0.50 c	2.93±0.58 c	3.21±0.67 d	0.56±0.13 d	1.17±0.41 bc	1.50±0.46 d	10.70±1.59 d
<i>Ribes rubrum</i>	Vertti	0.39±0.10 b	2.76±0.69 c	2.32±0.56 c	0.76±0.19 e	1.69±0.59 d	0.94±0.18 c	8.88±1.88 c
	Red Dutch	na	0.79±0.39 a	0.56±0.31 b	nd a	1.00±0.56 b	nd a	2.35±1.23 b
	White Dutch	0.07±0.01 a	0.66±0.27 a	0.29±0.16 a	0.30±0.08 b	nd a	0.08±0.03 b	1.40±0.52 a

^aSignificant difference ($p < 0.05$) between samples of different cultivars are marked as a–e. The sample data from southern Finland and northern Finland are combined for the cultivar comparison. ^bQuercetin-3-O-rutinoside coeluted with myricetin-3-O-arabinoside, myricetin-3-O-(6''-malonyl)-glucoside and auresidin glucoside in black currants, with myricetin-3-O-(6''-malonyl)-glucoside and auresidin glucoside in Vertti, and with myricetin-3-O-(6''-malonyl)-glucoside in Red Dutch. ^cQuercetin-3-O-(6''-malonyl)-glucoside coeluted with quercetin-3-O-arabinoside in black currants. Key: na, not analyzed; nd, not detected.

Table 14 Comparison between currant cultivars for the contents of anthocyanins^a

Species	Variety	Delphinidin-3-O-glucoside		Cyanidin-3-O-glucoside		Cyanidin-3-O-sophoroside (mg/100 g fresh berry)		Cyanidin-3-O-xylosyl-rutinoside		Total Anthocyanins	Total phenolic compounds ^b
		O-rutinoside	O-rutinoside	O-glucoside	O-rutinoside	O-sophoroside	glucosyl-rutinoside	O-xylosyl-rutinoside	sambubioside		
<i>Ribes</i>	Mortti	34.10±8.79 c	159.36±48.07 c	16.49±2.89 c	124.01±25.88 d	nd	nd	nd	nd	333.96±78.91 d	350.71±78.85 e
<i>Ribes</i>	Ola	33.59±9.05 c	153.82±47.53 c	16.19±2.84 c	119.93±23.90 d	nd	nd	nd	nd	323.53±76.06 d	340.21±76.06 e
<i>Ribes</i>	Melalahti	13.22±4.87 b	95.16±25.35 b	9.38±2.44 b	107.25±21.77 c	nd	nd	nd	nd	225.01±48.49 c	240.72±48.70 d
	Vertti	nd a	nd a	nd a	nd a	nd	nd	nd	nd	nd a	20.23±3.13 b
<i>Ribes</i>	Red Dutch	nd a	nd a	nd a	2.41±1.03 b	1.94±0.42	5.28±1.42	8.04±1.94	9.38±3.16	27.06±4.63 b	29.63±5.00 c
<i>Rubrum</i>	White Dutch	nd a	nd a	nd a	nd a	nd	nd	nd	nd	nd a	2.65±0.75 a

^aSignificant difference ($p < 0.05$) between samples of different cultivars are marked as a–e. The sample data from southern Finland and northern Finland are combined for the cultivar comparison. ^bContent of total phenolic compounds was defined as the sum of anthocyanins, flavanol glycosides and hydroxycinnamic acid conjugates. Key: nd, not detected.

According to the results of the investigation on phenolic compounds in currant berries (**Table 12–14**), the currant species *R. nigrum* contained higher amounts of hydroxycinnamic acids and flavonols than the currant species *R. rubrum*. *p*-Coumaric acid derivatives and quercetin glycosides dominated and accounted for 45–87% of hydroxycinnamic acid derivatives and 63–100% of flavonol glycosides, respectively, in the six currant cultivars.

Anthocyanins only existed and dominated in black currant and red currant berries, which contributed to the higher total phenolic content in these currant cultivars in comparison to green currant and white currant berries of the same species, respectively. The lack of or suppressed expression of the genes encoding the enzymes responsible for anthocyanin biosynthesis, such as dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS)^{30,128,129}, was thereby suggested in green and white currants. However, the compositional profile of anthocyanins was significantly different between black currants and red currants, as delphinidin glycosides and cyanidin glycosides appeared in the former but only cyanidin glycosides in the latter. This indicated different metabolic pathways of these cultivars. The expression of the gene encoding flavonoid-3',5'-hydroxylase (F3',5'H) or the activity and availability of the enzyme might be suppressed in the red currant cultivar studied^{30,128,129}. Moreover, the total amount of anthocyanins in red currants was only about one tenth of that in black currants. The black currant cultivars Mortti and Ola had the highest anthocyanin levels among all the cultivars, and are proposed to be a better choice as a raw material for natural pigments or nutraceuticals.

Ascorbic acid and phenolic compounds are all characterized as health beneficial compounds with high antioxidant capacity. The black currant cultivars Mortti and Ola contained both the highest amount of ascorbic acid (**Table 10**) and the highest level of phenolic compounds (**Table 14**) and might be potential bioactive materials for food and food ingredients.

5.1.2 Sea buckthorn (papers III and IV)

Four sugar derivatives were detected in sea buckthorn berries. They were ethyl β -D-glucopyranoside (ethyl glucose) and three sugar alcohols, L-quebrachitol, methyl-*myo*-inositol, and *myo*-inositol. Sea buckthorn is a remarkably hardy bush resistant to drought and tolerant to soil salinity and cold. The appearance of sugar alcohols in sea buckthorn berries might be important for its tolerance after long adaption to extreme environmental conditions as drought and cold stress commonly occur in its habitat.

Table 15 Comparison between sea buckthorn subspecies for the contents of sugars, sugar alcohols and acids

<i>Subspecies</i>	<i>Fructose</i>	<i>Glucose</i>	<i>Ethyl glucose</i>	<i>L-Quebrachitol</i>	<i>Methyl-myo-inositol</i>	<i>Myo-inositol</i>	<i>Sucrose</i> (g/100 mL)	<i>Total sugars^a</i>	<i>Malic acid</i>	<i>Citric acid</i>	<i>Quinic acid</i>	<i>Ascorbic acid</i>	<i>Total acids</i>
<i>sinensis</i>	2.61±2.43	2.74±2.49	0.02±0.01	0.56±0.27	0.06±0.04	0.02±0.01	0.03±0.01	6.05±0.06	4.60±2.34	0.05±0.03	1.41±1.04	0.87±0.46	6.93±2.40
<i>mongolica</i>	0.95±0.71	2.52±0.72	0.09±0.09	0.19±0.08	0.02±0.01	0.02±0.01	0.02±0.03	3.80±1.15	2.22±0.91	0.03±0.01	1.51±0.33	0.10±0.06	3.87±0.86
significance	*	ns	*	*	*	*	*	*	*	*	ns	*	*
(<i>p</i> < 0.05)													

<i>Subspecies</i>	<i>Sugar/acid</i>	<i>Sugar/Brix</i>	<i>Brix</i>	<i>pH</i>	<i>Juice yield</i> (mL/100g)	<i>Berry weight</i> (g/berry)
<i>sinensis</i>	1.18±1.23	0.35±0.26	14.1±4.7	2.60±0.24	50.1±7.2	0.14±0.03
<i>mongolica</i>	1.09±0.54	0.43±0.10	8.7±0.9	2.77±0.19	55.1±5.1	0.56±0.16
significance	ns	*	*	*	*	*
(<i>p</i> < 0.05)						

^aTotal sugar content was defined as the sum of sugars, sugar derivatives, and sugar alcohols. Key: ns, no significant difference. The sample data of each subspecies from different growth sites are combined for independent sample *t*-tests.

Although the concentration of sugars and acids varied greatly among different varieties (paper IV) and different natural origins (paper III) within each sea buckthorn subspecies, statistically significant differences were detected between the two subspecies, *sinensis* and *mongolica* (**Table 15**). The subspecies *mongolica* had significantly lower sugar, sugar alcohol and organic acid levels but higher ethyl glucose level than the subspecies *sinensis*. The berry size of *H. rhamnoides* ssp. *sinensis* is remarkably smaller compared to that of *H. rhamnoides* ssp. *mongolica*. Thereby, in addition to the genetic differences in metabolic pathways, a dilution effect on the metabolites in berries of ssp. *mongolica* might contribute as another explanation for the compositional difference between these two sea buckthorn subspecies.

Within ssp. *mongolica*, the varieties Chuisakaya and Vitaminaya contained the highest amount of total sugars and the lowest amount of total acids, and had the highest value of the sugar/acid ratio ($p < 0.05$) among all the varieties studied. In contrast, the berries of the varieties Avgustinka and Botanicheskaya exhibited the lowest total sugar content and sugar/acid ratio and the highest total acid content ($p < 0.05$). With the only exception of the major sugar alcohol L-quebrachitol, all the other compounds studied in sea buckthorn berries varied considerably between different varieties of *H. rhamnoides* ssp. *mongolica*.

Within *H. rhamnoides* ssp. *sinensis*, the composition of sea buckthorn berries from different growth areas varied widely. Among the six growth areas investigated, the berries from Inner Mongolia had the highest sugar/acid ratio, as they contained the highest amounts of major sugars (fructose and glucose) and sugar alcohol (L-quebrachitol), and hence the highest total sugar content, but the lowest amount of total acids ($p < 0.05$). In contrast, samples from Sichuan showed the lowest levels of these sugars and the sugar/acid ratio ($p < 0.05$). The content of total acid in berries from Sichuan was significantly higher than in all the other samples except for the samples from Qinghai. The remarkably low sugar content (0.08 g/100 mL juice vs. 2.83–6.49 g/100 mL for fructose, 0.13 g/100 mL vs. 2.82–6.89 g/100 mL for glucose, and 0.59 g/100 mL vs. 6.41–14.53 g/100 mL for total sugars) were clear characteristics of wild Chinese sea buckthorn from the Sichuan area. The considerable differences in the composition of berries from different natural origins might be due to genetic differences between the populations and/or the adaption of plants to the local environment.

In studies on both currants and sea buckthorn, berries of different genetic backgrounds exhibited remarkable variations in their composition of sensory and nutritional components, and provided wide choices for the utilization of berry fruits as food and food materials for various purposes.

5.2 Latitudinal/altitudinal comparison

Most of the studies on the effects of agricultural practice and environmental factors on plants/fruits are conducted by changing only one variable and keeping the others constant to investigate the effects of a selected factor on the biosynthesis and content of the metabolites. However, since currants and sea buckthorn either grow wild or are commercially cultivated in open fields, the difference in environmental conditions between growth sites and between crop years is a complex combination of various factors, such as temperature, radiation, precipitation, air humidity, and soil and fertilizer composition. Thus, the interaction and co-influence of the environmental factors occurred in real life on the biosynthesis and composition of the plants and their products should be considered. This provides the grounds for this research to investigate the interactional influence of latitude/altitude and growth conditions on metabolite profiles of the berries. The comparison between samples from different growth sites is a general comparison of berry composition caused by a combination of various factors, including meteorological conditions, fertilization, and soil conditions. The location comparison based on samples collected from multiple years gives reliable trends of difference and provides guidelines for cultivation of sea buckthorn and currants in the corresponding locations studied. While the results of the study provide some basis for prediction of changing trend of berry metabolites with variation in latitude/altitude, extrapolation of the results of latitudinal/altitudinal comparison to fruits/berries and to growth locations other than the ones involved in this study should be made with great caution.

5.2.1 Latitudinal effects on currants (papers I, II, V and VI)

In the studies conducted on currants, berry samples of identical cultivars cultivated at two latitudes, i.e. southern and northern Finland, respectively, were studied in order to investigate the latitudinal effects on berry composition and quality. The altitudinal effect was ignored because of the small differences in altitudes of the two locations (less than 100 m) compared to the latitudinal difference. **Figure 4** outlines the total view of latitudinal effects on the contents of different compound groups in currant berries. Differences were observed between cultivars in relation to their compositional response to varying latitude. The contents of total flavonols, total anthocyanins, and total phenolic compounds (sum of phenolic acids, flavonols, and anthocyanins) were all higher in berries grown in the south than those grown in the north for the three black currant cultivars (**Figure 4**). In contrast, the total anthocyanin content in red currants and total phenolic compound content in green, red, and white currants were significantly higher in berries collected from northern Finland

than in those from southern Finland. In black currants, the decreases in the total anthocyanin content associated with increased latitude were mostly attributed to the considerable changes in delphinidin glycosides. The total content of delphinidin glycosides decreased significantly by 35%, 32%, and 25% in berries of the Mortti, Ola, and Melalahti cultivars, respectively, as latitude increased, while that of cyanidin glycosides decreased by only 13%, 8%, and 3%, respectively. In red currants, cyanidin glycosides existed as the only anthocyanin group and contributed to the increase in total anthocyanin content with an increase in growth latitude. In contrast to the findings in black currants, the total content of flavonols in green, red, and white currants did not vary significantly between the samples from two different latitudes. Except for the berries of the Melalahti and Red Dutch cultivars, which showed no differences ($p > 0.05$) in the total hydroxycinnamic acid content, berries grown at the higher latitude contained a higher level of hydroxycinnamic acid conjugates than those grown at a lower latitude in all the other cultivars. Concerning the total acid content, only the black currant cultivars Mortti and Ola showed significant differences between berries grown at the two latitudes, with higher values in berries grown at a lower latitude. In view of total sugar content, berries of the Vertti cultivar grown at a higher latitude exhibited higher values than those at a lower latitude, while the berries of the Ola and Red Dutch cultivars showed the opposite pattern. The other cultivars presented constant values of total sugars despite of the variation in growth latitudes.

According to the compositional differences of berries from different growth latitudes, growth site selection with the purpose of improving the sensory qualities or enriching nutrients could be applied. For example, the total acid content in the Vertti and Red Dutch cultivars did not differ between the two growth sites, but the change in total sugar content was opposite in these two cultivars as latitude increased (**Figure 4**). Therefore, in order to obtain berries with a higher amount of sugars, the green currant cultivar Vertti is recommended to be cultivated at a high latitude while the red currant cultivar Red Dutch should be grown at a low latitude. However, in order to obtain a higher phenolic content, both of the cultivars are recommended to be planted in the north rather than in the south.

In summary, in addition to its influence on the inherent composition of the berries, genetic background showed a considerable impact on the compositional response of berry fruits to different growth sites. The selection of growth location with regard to the composition and quality of berries is species/variety dependent.

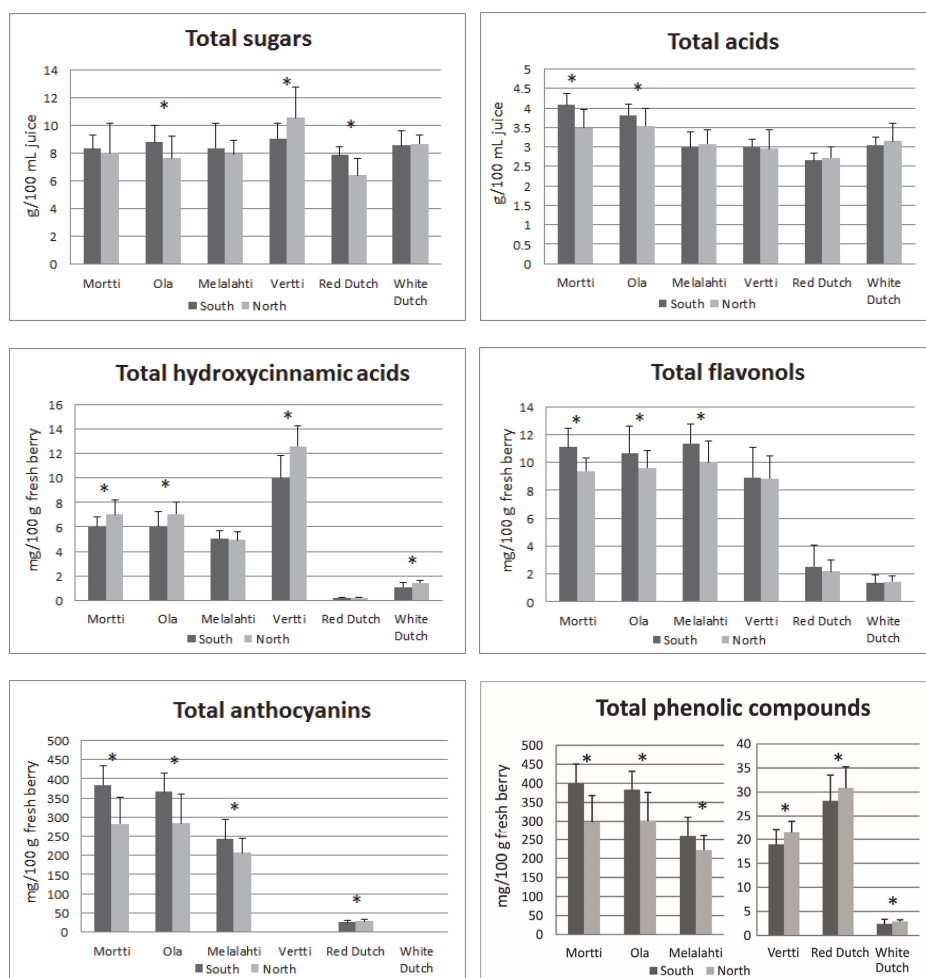


Fig. 4 Comparison of berry composition in currants collected from southern and northern Finland. * $p < 0.05$.

5.2.2 Sea buckthorn (papers III and IV)

5.2.2.1 Latitudinal \times altitudinal effect on *H. rhamnoides* ssp. *sinensis*

In the study on sea buckthorn berries of *H. rhamnoides* ssp. *sinensis* (paper III), the influence of concurrent changes in latitude and altitude on berry composition was investigated by comparing the samples from different growth locations in China. Bivariate correlation analysis showed that the contents of major sugars (fructose and glucose), the major sugar alcohol (L-quebrachitol), quinic acid (one of the major acids), and total sugars all increased as latitude increased and as altitude decreased ($p < 0.01$). In contrast, the contents of another major acid, malic acid, and total acids, as well as ascorbic acid

decreased as latitude increased and as altitude decreased ($p < 0.01$). Ethyl glucose was neither influenced by latitude nor by altitude ($p > 0.01$). It seems that latitude and altitude had opposite effects on the composition of the berries. The concurrent changes in the altitude and the latitude made it difficult to clearly distinguish the effect of altitude from that of latitude on the composition of berries. Nevertheless, partial correlation analysis indicated that latitude might have contributed more to the variation of berry composition than altitude.

This conclusion was supported by an independent investigation of the altitudinal effects on the composition of berries collected from different altitudes in Shanxi and in Sichuan with identical latitude and longitude. The results did not show clear trends in the concentration of these sensory contributing components as altitude increased or decreased. In the samples from the Sichuan area, the highest values of sugars, sugar alcohols, and acids were detected in samples grown at an altitude of 2500 m compared to those at 2000 m and 3000 m. The samples from the two altitudes in Shanxi showed no clear differences in berry composition.

5.2.2.2 Latitudinal effect on *H. rhamnoides* ssp. *mongolica*

In the study on sea buckthorn berries of *H. rhamnoides* ssp. *mongolica* (paper IV), the effects of latitude on the composition of sugars, sugar alcohols, and acids were studied by comparing the samples collected in Sammalmäki, southern Finland and in Québec, Canada, regardless of the small altitudinal difference (less than 100 m) between the two growth sites. The sample collected from Kittilä was only available in 2003 as the bushes died afterwards. Considering the stress caused by transplanting the bushes in 2003, the data for the samples from Kittilä were excluded from the analysis.

The statistical analysis showed that the content of total sugars was significantly higher while that of total acids was lower in berries grown at a lower latitude than those grown at a higher latitude. As a result, the values of the sugar/acid ratio were lower in berries collected from Finland than those from Canada. The content of ethyl glucose was higher in berries grown at a higher latitude than in those at a lower latitude, while the major sugar alcohol, L-quebrachitol, did not vary between berries collected from different locations.

The effects of the latitude and altitude of the growth location on the accumulation of compounds in plants and fruits can finally be explained by the regulation of biosynthesis and metabolism of the corresponding compounds driven by the complex effects of the growth environment, such as weather conditions, as well as the interaction between genetic and environmental factors.

5.3 Weather impact on berry composition

In order to understand the impact of environmental conditions on the chemical composition and quality of berries, currant and sea buckthorn plants of identical genetic background were naturally grown in different locations and berries were collected in several consecutive years. Weather conditions were collected systematically during the investigation period and the correlations between individual weather variables and compositional parameters were studied via correlation coefficient analysis and principal component analysis (PCA). It should be mentioned that this thesis focuses on the effects of weather conditions on the accumulation of metabolites in ripe berries. The potential influence of soil condition should not be ignored. In studies on currants (papers I, II, V and VI), the soil used for planting the bushes at the planting spots was controlled to be the same in the two growth locations (Piikkiö and Apukka) investigated. However, differences in soil condition might have contributed more to the variation in berry composition in the studies on sea buckthorns (papers III and IV) since the bushes were wild ones in all the locations.

5.3.1 Currants (papers I, II, V and VI)

Among all the currant cultivars studied, the Melalahti cultivar was the least affected by variations in weather conditions, and therefore was considered to be constant in composition and quality despite varying environmental factors and different growth locations and years. On the other hand, in addition to the similar compositional characteristics, the black currant cultivars Mortti and Ola showed a similar compositional response to varying weather conditions in terms of sugars, acids, and phenolic compounds.

Temperature had a positive impact on the contents of major sugars (fructose and glucose) and acid (citric acid) in the Mortti and Ola cultivars, but a negative impact on their contents in the White Dutch cultivar. Both temperature and radiation showed a strongly positive influence on the concentration of sugars in berries of the Red Dutch cultivar. Low relative air humidity (< 50%) seemed to induce an accumulation of major sugars and acids in Vertti, but caused a decrease in these compounds in the Mortti and Ola cultivars. In the Red Dutch and White Dutch cultivars, dry weather favored the accumulation of citric acid but suppressed the accumulation of major sugars in berries.

Although most of the literature indicated an increasing trend of ascorbic acid content in response to an increase in light intensity (**Table 6**), black currants of the Mortti, Ola, and Malalahti cultivars showed negative correlations between radiation and the content of ascorbic acid. Since the total content of vitamin C (AsA + DHA) was not investigated, it was difficult to attribute the decreasing

trends of ascorbic acid to the decreased biosynthesis of ascorbic acid or to the increased conversion of ascorbic acid to dehydroascorbic acid under increased radiation. The average temperature in March and the percentage of days with a relative humidity of 20–30% from the start of the growth season until harvest both correlated positively with the content of ascorbic acid in berries of the Melalahti, Vertti and White Dutch cultivars.

Interestingly, the studies on the effects of weather on the concentration of sugars and acids and on phenolic compounds separated the humidity conditions by different relative air humidity values. The former separated the low humidity and high humidity variables by a relative humidity of 50%, while the latter separated by 70%. This indicated a difference in the optimal conditions required for biosynthetic reactions of different metabolites in plants.

In view of the phenolic compounds, high temperature and radiation led to an increase in the contents of total anthocyanins, the major phenolic group, and total phenolic compounds in black currant berries. In contrast, the content of total anthocyanins in red currant berries decreased as temperature and radiation increased, as did the content of total phenolic compounds. Since the accumulation of anthocyanins was previously reported to be induced by both low temperature and high radiation^{139,140}, these contradictory results might indicate either a genetic difference in the biosynthetic or metabolic response to weather conditions, or a different affinity of anthocyanin biosynthetic regulation to either temperature or radiation. However, the results are more likely explained by the different responses of individual anthocyanidin groups to variations in temperature and radiation. In a study on grape berries, low light intensity and high temperatures were reported to decrease the level/proportion of trioxxygenated anthocyanins (glucosides of delphinidin, petunidin and malvidin) but increase the level/proportion of dioxygenated anthocyanins (glucosides of cyanidin and peonidin) in the skin^{37,137,169}. In accordance with these studies, delphinidin glycosides in black currants showed positive correlations, while cyanidin glycosides showed no correlation with temperature and radiation variables. Therefore, the increasing trend in total anthocyanin content in black currants with an increase in temperature and radiation was caused by an increase in the delphinidin content. The contradictory decreasing trend in total anthocyanin content in red currants was due to a decrease in the cyanidin content, the only existing anthocyanidin, in response to increased temperature and radiation. Although it is difficult to distinguish the effects of temperature from those of radiation in this study, radiation was speculated to act as the major influencing factor regarding the results in grape berries^{37,137,169}. The value of total phenolic compounds and total hydroxycinnamic acids, the major phenolic group in green and white currants, showed a negative response to temperature and radiation. Nevertheless, the total flavonol glycoside level

was less affected by weather changes in green, red, and white currants, and the black currant cultivar Melalahti, but increased as temperature increased in the black currant cultivars Mortti and Ola. In addition to temperature and radiation, weather with low relative air humidity (< 70%) had a positive impact on the total hydroxycinnamic acid content in the Mortti, Ola, Vertti, and White Dutch cultivars. However, the phenolic compounds (hydroxycinnamic acids, flavonols and anthocyanins) in the Red Dutch cultivar as well as anthocyanins in black currants were less affected by variations in relative humidity.

5.3.2 Sea buckthorn (*H. rhamnoides* ssp. *mongolica*) (paper IV)

The investigation into the effects of weather conditions on the composition of sugars and acids in sea buckthorn berries was carried out using the analytical data of samples collected from Sammalmäki, Finland and Québec, Canada. The samples collected in Kittilä, Finland were excluded from the statistical analysis because of the stress of the transplanting process of the sea buckthorn bushes in 2003 and the death of the bushes afterwards.

The contents of the major sugar (glucose) and total sugars, as well as ascorbic acid, were hardly affected by varying weather conditions in the investigation period, while all of the weather parameters investigated, including temperature, radiation, precipitation, and air humidity, showed impacts on the content of total acids in berries. Radiation and precipitation both presented a negative impact, while air humidity exhibited a positive impact on the concentrations of malic acid (the major acid) and total acids in the berries. In response to temperature variations, the malic acid content and the total acid content varied differently during different growth periods. Their contents increased as temperature increased during January–March, but decreased in the other growth periods. Differences between growth periods on the regulation of compound accumulation in terms of environmental changes have also been detected in fruits such as kiwifruit⁵⁷.

In the case of sugar derivatives, temperature showed a negative effect while relative humidity showed a positive effect on the accumulation of ethyl glucose. The content of the major sugar alcohol (L-quebrachitol) in sea buckthorn berries displayed a positive correlation with temperature, radiation, and air humidity, with the exception of radiation in September which showed a negative influence on the content of L-quebrachitol. In a previous study conducted on the wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. *sinensis*), contradictory findings regarding the effect of temperature have been reported, where the content of L-quebrachitol correlated negatively with temperature³³. This might be explained either by the difference in the regulation of metabolic pathways of the two subspecies or by the collinear

effect of radiation, precipitation, or humidity parameters and temperature parameters on the investigated compounds as a result of the open field experiments.

Overall, weather conditions had significant effects on the composition of berries. Among all the weather variables studied, temperature and radiation showed strong impacts while precipitation showed little impact on the accumulation of the studied metabolites. The findings of this research indicate that metabolic pathways and the regulation of biosynthetic pathways of metabolites vary quite a bit between berries of different species or cultivars, and may contribute to the significant differences in their response to variations in weather conditions.

6 SUMMARY AND CONCLUSION

Significant differences in the composition of berries were detected between currant cultivars and between sea buckthorn subspecies or varieties/origins. Black currant cultivars Mortti and Ola showed similar characteristics in their composition of sugars, organic acids, and phenolic compounds, which suggested a close genetic background or metabolic pathways between these two cultivars. Both cultivars contained the highest amount of ascorbic acid and phenolic compounds among all the currant cultivars investigated. Currant cultivars of the species *R. nigrum* contained an overall higher amount of hydroxycinnamic acids and flavonols than those of the species *R. rubrum*. Anthocyanins existed only in black and red currants with different compositional profiles, but was absent in green and white currants. Comparing the two sea buckthorn subspecies, *H. rhamnoides* ssp. *mongolica* had, on average, significantly lower sugars, sugar alcohols, and organic acids and higher ethyl glucose than *H. rhamnoides* ssp. *sinensis*. Nevertheless, the Chinese sea buckthorn (*H. rhamnoides* ssp. *sinensis*) collected from Sichuan Province in China was distinguished from all the other Chinese and Finnish sea buckthorn berries by an extremely low content of sugars (total sugar content 0.6 g/100 mL vs. 2.7–14.5 g/100 mL in all the other samples studied).

Latitude/altitude and weather conditions all had a significant influence on the composition of harvested berries. The response of berry composition to growth latitude and weather conditions varied considerably between cultivars or between subspecies. This indicated different metabolic pathways or different regulation of metabolite biosynthesis in plants of different genetic background, and suggested that specific consideration should be paid to different subspecies/cultivars when applying agricultural practices or selecting growth sites for commercial cultivation with a special focus on improving the sensory or health promoting attributes.

Among all the currant cultivars studied, the black currant cultivar Melalahti showed the most consistent composition at different growth sites and in different harvesting years. The black currant cultivars Mortti and Ola displayed a similar composition and response to varying weather conditions. Although temperature and radiation had a different impact on green, red and white currants regarding the accumulation of sugars and acids, they both showed a negative impact on the accumulation of major phenolic compounds (anthocyanins in red currant and hydroxycinnamic acids in green and white currants). However, in black currant berries, an increase in temperature or radiation may induce an increase in the levels of total anthocyanins and total phenolic compounds.

In the case of sea buckthorn berries, the investigation into the impact of weather on berry composition was only conducted on one subspecies of *H. rhamnoides* ssp. *mongolica*. The total sugar content was not influenced by weather conditions, while the total acid content showed a strong response to varying weather conditions. High radiation and precipitation but low air humidity may decrease the total acid content in these berries.

This study systematically investigated the berry composition of different currant cultivars and sea buckthorn subspecies/varieties and their compositional response to varying growth locations and weather conditions. The berries showed remarkable differences in genetic backgrounds regarding both the inherent composition and the compositional response to varying environmental factors. These results provide useful guidelines for berry breeding and cultivation, for growth site selection, and for commercial utilization of berries with special purposes in terms of improving the health promoting or sensory qualities. This study may also provide important information on the basic biochemical and physiological attributes of currants and sea buckthorns.

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APPENDIX: ORIGINAL PUBLICATIONS

- I. Reprinted from *Journal of Agricultural and Food Chemistry* 2009, 57, 2977–2987, with permission from American Chemical Society.
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