UiT
THE ARCTIC
UNIVERSITY
OF NORWAY

Faculty of Biosciences, Fisheries and Economics

Effects of latitudinal climate conditions on quality attributes *Brassica oleracea*

—

Anne Linn Hykkerud Steindal

A dissertation for the degree of Philosophiae Doctor – November 2013

Abstract

Brassica is a genus of plants that includes vegetables that are widely used for human consumption and contain high levels of health-promoting compounds. These vegetables are grown in large parts of the world, and the environmental conditions where they are cultivated can affect several quality attributes. The research presented in this thesis examined how variation in growth conditions with latitudinal location can influence both the content of specific health-promoting compounds and sensory attributes. Two cultivars of the species *Brassica oleracea*, broccoli and kale, were studied under controlled and semi-field conditions. Interest was focused on the variation in day lengths and temperatures associated with different latitudinal conditions, and also light quality and cold acclimation in relation to latitudinal changes. In broccoli, it was found that levels of glucosinolates, vitamin C, and flavonols were sensitive to both temperature and day length. Furthermore, the content of aliphatic glucosinolates and flavonols was highest at high temperatures (21/15 °C) and short days (12 h), although in some experiments the levels of aliphatic glucosinolates were highest at lower growth temperatures. Far-red light seemed to reduce the content of glucosinolates and flavonols. For vitamin C, the assessments showed that levels were insensitive to the field conditions and higher at low temperatures. A number of differences in sensory attributes were also observed. For example, several of the sensory attributes did not vary between study locations in Norway (Tromsø and Grimstad) but did differ from those noted in the southernmost summer location (Berlin). The content of glucosinolates in kale was affected less by pre-acclimation growth conditions and more extensively by cold acclimation temperatures. Thus the results provided by the work underlying this thesis indicate that the quality of *Brassica* vegetables is affected by the latitudinal location of cultivation, and this finding can help optimize quality of the plant and increase the awareness of differences related to this aspect.

Acknowledgements

This thesis is based on studies which have been carried out at Norwegian Institute for Agriculture and Environmental Research (Bioforsk) and University of Tromsø in the period 2009-2013. Thanks to the Norwegian council of research for the founding. I would like to thank the people who have been involved in this project for their help en support. I especially would like to thank:

My supervisors Laura, Jørgen and Tor for their support and guidance.

Gunnar, Sidsel, Mona, Ane and Anna and all the other people at the lab on Nofima-mat for help en guidance with the chemical analysis, and Leidulf at the University of Tromsø for helpful guidance at the phytotron and for taking well care of my plants.

All my present and former colleagues at Bioforsk are thanked for creating a friendly, inspiring environment. I would like to give a special thank Sigridur for mental support and the technicians for taking care of my plants.

I thank friends and family for their support. Especially my husband for being encouraging and helpful in this process and taking good care of our kinds in times of traveling, and my three kinds Ingeborg Sofie, Anna Ovidia and Hilmar, for being so good and patient, this work is dedicated to them.

Tromsø, November 2013

Anne Linn Hykkerud Steindal

Contents

Abstract **I**

Acknowledgements **III**

List of papers **VII**

1 Introduction **1**

1.1 Background . . . 1
1.2 *Brassica oleracea* . . . 3
1.2.1 Broccoli . . . 3
1.2.2 Kale . . . 4
1.3 Environmental effects . . . 4
1.3.1 Temperature . . . 5
1.3.2 Light . . . 6
1.4 Health- and taste-related compounds . . . 9
1.4.1 Glucosinolates . . . 9
1.4.2 Vitamin C . . . 13
1.4.3 Phenolic compounds . . . 15
1.4.4 Fatty acids . . . 17
1.4.5 Soluble sugars . . . 18
1.5 Sensory attributes . . . 19
1.5.1 Sensory descriptive analysis . . . 19
1.6 Objectives . . . 20

2 Materials and methods **21**

2.1 Methodological approaches . . . 21
2.2 Analysis . . . 22
2.2.1 Chemical analysis . . . 22

2.2.2 Sensory profiling 23
2.3 Experimental set-up 23
2.3.1 Paper I 23
2.3.2 Paper II 24
2.3.3 Paper III 24
2.3.4 Paper IV 24
2.3.5 Paper V 25

3 Results and discussion **27**
3.1 Health- and taste-related compounds 29
3.1.1 Glucosinolates 29
3.1.2 Ascorbic acid 33
3.1.3 Flavonols 34
3.1.4 Fatty acid 36
3.1.5 Soluble sugars 37
3.2 Sensory attributes 38
3.3 Main conclusions and future prospects 39

List of papers

This thesis is based on the following papers:

I **A. L. H. Steindal**, J. Mølmann, G. B. Bengtsson and T. J. Johansen: "Influence of day length and temperature on the content of health-related compounds in broccoli (*Brassica oleracea* L. var. *italica*)", *J. Agric. Food Chem.*, 2013, **61**, 10779-10786

II **A. L. H. Steindal**, T. J. Johansen, G. B. Bengtsson, S. F. Hagen and J. Mølmann: "Effects of supplement LED light qualities and temperature on health-related compounds in broccoli florets". Manuscript.

III J. Mølmann, **A. L. H. Steindal**, R. Seljåsen, G. Bengtsson, J. Skaret, P. Lea, and T. J. Johansen: "Effects of day length and temperature on sensory and nutritional quality of broccoli florets". Manuscript.

IV R. Seljåsen, **A. L. H. Steindal**, J. B. Mølmann, G. B. Bengtsson, M. Schreiner, P. Velasco, P. Lea, J. Skaret, S. F. Hagen, M. E. Cartea and T. J. Johansen : "Sensory quality and health-related compounds of broccoli (*Brassica* sp.) grown at different latitudes with standardized soil and fertilization conditions". Manuscript in preparation

V **A. L. H. Steindal**, R. Rødven, E. Hansen and J. Mølmann: "Effects of photoperiod, temperature and cold acclimation period on the content of glucosinolates, sugars, and fatty acids in curly kale". Manuscript.

Chapter 1

Introduction

1.1 Background

In recent years, consumers have become increasingly interested in the quality and health benefits of foods. Considering vegetables in particular, sensory attributes generally have the greatest impact on consumer preferences (Gunden and Thomas, 2012). Nutritional value and the nature of health-promoting constituents are also important in this context (Nayga et al., 1999; Pollard et al., 2002), and in Norway there is a national goal to increase the consumption of vegetables. Therefore, enhancing the sensory and health-related quality of vegetables might attract attention of consumers and offer competitive advantage to growers.

Vegetables of the genus *Brassica* are cultivated and consumed in large quantities worldwide. The species *Brassica oleracea* belongs to the family Brassicaceae and includes many well-known vegetables, such as cabbage, broccoli, cauliflower, kale, and Brussels sprouts. Epidemiological studies have recognized a positive correlation between the intake of fruits and vegetables and prevention of diseases like atherosclerosis, cancer, diabetes, and arthritis (Kaur and Kapoor, 2001; Fisher and Hollenberg, 2005; Crozier et al., 2009). *Brassica* vegetables in particular are known to have positive health effects (Herr and Büchler, 2010), and increased consumption of these plants has been recommended as a measure to prevent human cancer (National Research Council, 1982).

The positive effect on health have been attributed to bioactive compounds, such as vitamins, glucosinolates, carotenoids and flavonoids several of which are found at high levels in *Brassica* (Jahangir et al., 2009). Research has shown that the content of many of the bioactive compounds is influenced by environmental growth conditions like light and temperature (Engelen-Eigles et al., 2006; Charron and Sams, 2004; Schonhof et al., 2007), and those conditions vary at different latitudes. During the summer season, high latitude locations have lower growth temperatures, longer day length and disparate composition of solar radiation compared to locations at lower latitudes. At lower latitudes (40°N), *Brassica* vegetables are often cultivated in the cooler seasons with short day length and lower temperatures (Kałużewicz et al., 2010), and previous field studies have suggested that quality varies in vegetables and berries grown at different latitudes (Hårdh et al., 1977; Zheng et al., 2009a, 2012a).

Evaluation of the effects of climatic conditions on vegetable quality requires accurate chemical and sensory analyses, as well as both field and controlled studies. Field studies can be conducted to document the impact of environmental conditions, although it is difficult to identify the factors that actually give rise to the variations observed in such studies. The natural conditions can also fluctuate considerably between years. On the other hand, use of controlled experimental conditions can make it possible to test different growth factors separately, but in such an approach it is difficult to exactly mimic various environmental conditions. Accordingly, a combination of field and controlled experiments is ideal to obtain comprehensive information concerning the effects of environmental factors on specific aspects related to the quality of plants.

1.2 *Brassica oleracea*

Plants of the species *Brassica oleracea* grow wild in the Mediterranean region, Western Europe, and temperate areas of Asia. This species was first brought to the eastern part of the Mediterranean region between the first and second millennium BC, and during that period it became fully domesticated and underwent an extensive diversification that gave rise to a range of cultivated varieties (Rangavajhyala et al., 1998). *Brassica* vegetables have a characteristic taste that is associated with the sulfur containing compounds called glucosinolates (Fenwick et al., 1983). A moderate temperature of around 20 °C is optimum for the growth of most *Brassica oleracea* vegetables. However, these plants can also tolerate cooler temperatures, and thus, many of them can be grown up to a latitude of 70°N in Norway.

1.2.1 Broccoli

Broccoli is the *Brassica* vegetable that is most widely consumed in the world, and hence it is of substantial commercial importance. The word broccoli originates from the Latin “brachium”, which means an arm or branch. The head of the broccoli plant consists of a single large terminal inflorescence, and the plant is harvested when the flowering head is immature and still compact (Rangavajhyala et al., 1998). Broccoli is to some extent sensitive to temperature, and the optimal range for vegetative growth is not the same as for inflorescence development. Warm weather (>25 °C) can result in abnormal inflorescences and loose bud clusters (Bjorkman and Pearson, 1998; Grevsen, 1998). By comparison, frost damage can reduce the yield and quality of broccoli, although some cultivars withstand short periods of frost depending on the developmental stage (Tan et al., 1999; Kałużewicz et al., 2010).

1.2.2 Kale

Kale is a non-heading *Brassica oleracea* with an erect stem that bears large leaves, and it is a close relative to wild cabbage. Kale is a biannual crop that is important in traditional farming systems in Southern Europe (Cartea et al., 2002) and is also becoming increasingly popular in Northern Europe and North America. Regarding nutritional value, kale has a prominent rank among vegetables because of its high content of vitamins and minerals. Kale is cultivated year-round in temperate areas, but it does not grow well in warm weather (Vaughan and Geissler, 1997) and is therefore seldom grown as a summer crop at low latitudes.

1.3 Environmental effects

In food plants environmental growth factors can have an impact not only on yield, but also on quality-related features such as the content of health-promoting compounds and various sensory attributes (Tan et al., 2000; Francisco et al., 2011). All of these quality characteristics are genetically controlled, but they vary widely between and even within species and varieties (Vallejo et al., 2003a; Farnham et al., 2004). There are many environmental factors that can affect the regulation of the biosynthetic pathways involved in production of bioactive compounds (Gliszczyńska-Świglo et al., 2007; Gu et al., 2012). Abiotic environmental growth conditions vary in relation to differences in several factors such as location (e.g, latitudes, altitude and inland/coastal distance).

Compared to low latitudes regions, areas at high latitudes generally have lower temperatures and a shorter growing season. During the summer months in high-latitude areas (June and July), the midnight sun provides a 24 h photoperiod, and the solar angle also affects the wavelength distribution (Kaurin et al., 1985). A larger part of the land located north of the Arctic Circle (66°N) is not suited for crop production due to low temperatures. However, the northern part of Scandinavia has a milder climate because of the effect of the Gulf Stream, which makes it the northernmost area in the world (latitudes up to 70°N) where vegetables can be grown.

It has been stated that vegetables grown at high and low latitudes differ with regard to taste and other quality attributes. Therefore, interest in gaining scientific evidence on this matter has increased, and a few studies on this topic have been conducted. In Finland, a large investigation of several different vegetables in the 1970s showed that those grown at high latitudes had higher levels of sugar and vitamin C but lower amounts of carotene content compared to those grown at low latitudes (Hårdh and Hårdh, 1977). In carrots (*Daucus carota* L.), low growth temperatures have been found to result in sweeter taste and high temperatures have led to more bitter taste (Rosenfeld et al., 1997). It has also been reported that currant berries (*Ribes* sp.) have a higher content of phenolic compounds when grown at lower latitudes (Zheng et al., 2012a) and higher level of vitamin C at high latitudes (Zheng et al., 2009a).

1.3.1 Temperature

Temperature affects chemical reactions and physical properties in plants at both the cellular and the whole-organism level, and temperature requirements have been determined for various plant species (Berry and Björkman, 1980; Luo, 2011). Plant growth can be limited by low and high temperatures, and the range in between represents the optimum temperatures for maximum yields. Plants have adapted to potential changes in temperature and can adjust to conditions slightly below and above the optimum range by inducing numerous genetically regulated mechanisms (Berry and Björkman, 1980; Taiz and Zeiger, 2002).

Solar energy is distributed over a smaller surface area in equatorial regions, and thus the average temperature is higher in adjacent areas than at the high latitudes. The average temperatures during the growing season at high latitudes are characterized with temperatures between 9-14 °C whereas areas at low latitudes closer to the equator are too warm for production of many *Brassica* species. Several reports have described the effects of temperature on the production and accumulation of secondary metabolites and primary compounds in *Brassica* plants (Rosa and Rodrigues, 1998; Lefsrud et al., 2005; Pék et al., 2012).

Low temperature is considered to be one of the environmental conditions that limit the global distribution of plants, and it is responsible for significant reductions in the yields and quality of important food crops. When cold-tolerant plants are exposed to low temperatures, they can induce cold acclimation programs that lead to enhanced cold tolerance (Levitt, 1980; Alberdi and Corcuera, 1991). Exposure to low temperatures and frost can damage and reduce the yields of broccoli, whereas such temperatures can increase the tenderness and flavor of the leaves of kale plants. Those effects on kale may be related to the conversion of polysaccharides to monosaccharides, which is a mechanism that improves the tolerance to frost (Hagen et al., 2009).

1.3.2 Light

Plants depend on sunlight as their source of energy, and they can sense the light environments they grow in and detect almost all aspects of light, such as the photoperiod and specific wavelengths. In plants, light is one of the most important variables affecting production of health-promoting compounds, as well as other important functions (Kopsell and Kopsell, 2008; Pérez-Balibrea et al., 2008). Light conditions that have an impact on plants include light intensity, photoperiod and wavelength distribution (Kaurin et al., 1985).

Photoperiod

A photoperiod is defined as the extent of light and darkness in a 24 h cycle. The variation in the photoperiod at the equator is essentially zero, with 12 h light and 12 h darkness all year long. Due to the difference in the angle of the earth's axis in relation to the sun at increasing distance from the equator towards either of the earth's poles, the length of the light and dark periods vary and becomes unequal divisions of the 24 h cycle. The light period becomes longer in summer and shorter in winter, and the summer solstice (around 20/21 June for the Northern hemisphere) is the point at which the length of the day is at the annual maximum for a particular latitude; conversely, the winter solstice (21/22 December) is the time with the shortest day length (Jackson, 2009). The difference between the lengths of light and dark period increases with increasing latitude. North of the Arctic Circle (66 °N), the sun is always above the horizon during the summer months, thus representing a 24 h photoperiod (Kaurin et al., 1985; Jackson, 2009). The length of the photoperiod can influence a number of physiological processes in plants, such as biomass production, flowering and variation in secondary metabolites (Riihimäki and Savolainen, 2004; Velez-Ramirez et al., 2011).

The changes in the photoperiod affect the circadian clock, which is a mechanism that allows an organism to coordinate biological processes with specific times of the day or night. This mechanism consists of interlocked transcriptional feedback loops that control downstream targets, lead clock input signals, and interact with other signaling pathways (Pruneda-Paz and Kay, 2010). The circadian clock is based on oscillators that generate behavior which is affected by environmental input, and gene expression is then based on behavior of the oscillators (Hotta et al., 2007).

Light quality

Light quality is described in terms of wavelengths, and the visible light spectrum includes wavelengths of about 400-750 nm. In photosynthesis, plants mainly use light in the blue and red wavelengths, and reflect green wavelengths (Taiz and Zeiger, 2002). The amount and quality of solar radiation vary with increasing latitude in the Northern and Southern hemispheres, and this is due to difference in the curvature of the earth and the fact that the radiations from the sun has to pass further through more atmosphere to reach the surface of the earth. One of the changes caused by decreasing solar elevation in the higher latitudes is a decrease in the red:far-red ratio (Kaurin et al., 1985).

Plant pigments can sense specific wavelengths of visible light. These pigments are sensory photoreceptors called phytochromes, cryptochromes, and phototropins, and they allow plants to react and alter their growth in response to the light environment. Phytochromes are the photoreceptors that have been studied most extensively, and they detect light in the red (660 nm) and far red (730 nm) region (Quail, 2002). Phytochromes are involved in regulation of several cellular responses in plants (Mathews, 2006). By switching between the red-absorbing (P_r) and the far-red-absorbing (P_{fr}) form, phytochromes regulate gene expression in plants. P_r is the biologically inactive form that is quickly converted to P_{fr} upon stimulation, and P_{fr} is the biologically active form that regulates levels of transcription of various genes (Batschauer, 1999). The other two types of light-sensing pigments, cryptochromes and phototropins, detect wavelengths in the blue part of the spectrum (Chen et al., 2004). Cryptochromes play an important role during de-etiolation and flowering in the circadian system.

Specific wavelengths have complex effects on the biosynthesis of specific compounds in plants, and attempts to study these aspects have often provided mixed results (Bartoli et al., 2009; Li and Kubota, 2009; Lin et al., 2013). UV-radiation is another important factor that can have an impact on plants and their content of secondary metabolites. The amount of UV-radiation is influenced by season and latitude, with highest levels at the summer solstice and higher levels at low latitudes.

Light intensity

Light intensity is crucial for plant growth and production of primary and secondary metabolites. Even though there is 24 h of daylight at high latitudes in summer, the total irradiance in such regions is lower. On a sunny day above the Arctic Circle, only about 20% of the total daily solar radiation occurs from 6 p.m. to 6 a.m. (Kaurin et al., 1985).

1.4 Health- and taste-related compounds

Vegetables and fruits are known to contain several types of health-promoting compounds, among them are essential minerals, vitamins, and other antioxidants. Many of the mechanisms by which these substances contribute to positive health effects (e.g., antioxidant properties) have been studied comprehensively. There are also other bioactive compounds that protect against cardiovascular diseases or exhibit anticarcinogenic activity, but their mechanisms of action are less understood. Glucosinolates and some phenolic compounds are examples of such constituents that are attracting increasing attention (Brandt et al., 2004). Bioactivity is defined as a beneficial or adverse effect of a compound on a living organism or tissue.

1.4.1 Glucosinolates

Glucosinolates are a large group sulfur-containing glycosides, that are derived from amino acids and appear in all varieties of *Brassica* vegetables. More than 120 glucosinolates have been described, approximately 15 of which are found in species of the genus *Brassica* (Halkier and Gershenzon, 2006). Thirteen different glucosinolates were detected in the present studies (Table 1.1). Of the various cabbage varieties grown for human consumption, kale has the highest levels of glucosinolates and broccoli the second highest. The primary role of glucosinolates in plants is not well known, although it has been shown that the hydrolysis products are involved in the interactions between the plants and insects, herbivores, and pathogens (Fahey et al., 2001; Redovnikovic et al., 2008).

Table 1.1: Glucosinolates found in *Brassica oleracea* vegetables in studies included this thesis

Chemical structure of the side chain	Trivial name	Glucosinolate side chain
Aliphatic		
	Glucoiberin	3-methylsulhinylpropyl
	Glucoraphanin	4-methylsulphinylbutyl
	Glucoallysin	(R)-5-methylsulphinypentyl
	Progoitrin	2-hydroxy-3-butenyl
	Epiprogoitrin	2-(S)-2-hydroxy-3-butenyl
	Gluconapoleiferin	2-hydroxypent-4-enyl
	Glucoerucin	4-methylthiopropyl
	Sinigrin	2-propenyl
Aromatic		
	Gluconasturtiin	2-phenylethyl
Indolyl		
	Neoglucobrassicin	1-methoxyindol-3-ylmetyl
	4-methoxyglucobrassicin	4-methoxyindol-3-ylmethyl
	4-hydroxyglucobrassicin	4-hydroxyindol-3-ylmethyl
	Glucobrassicin	3-indolmetyl

The generalized structure of glucosinolates is shown in Figure 1.1. Glucosinolates are characterized by a core structure consisting of a β-D-thioglucose group, a sulfonated oxime moiety, and a variable side chain derived from amino acids (Mithen et al., 2000).

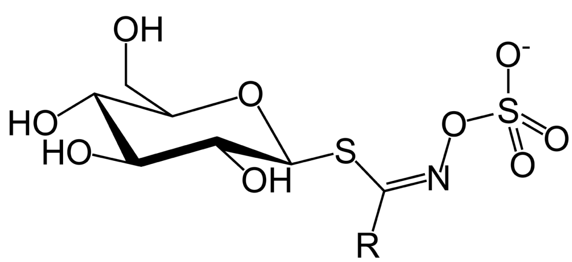

Figure 1.1: Generalized structure of glucosinolates

The biosynthesis of glucosinolates is controlled by genes that (i) regulate elongation of side chain amino acids (alanine, leucine, isoleucine, valine, phenylalanine, tyrosine and tryptophan), (ii) convert amino acids to the core structure of glucosinolates, and (iii) modify the secondary side-chain (Halkier and Gershenzon, 2006). When plant tissue is damaged by herbivores, the glucosinolates are hydrolyzed by an endogenous thioglucosidase called myrosinase, which releases a range of degradation products. Depending on conditions, such as the protein present and the pH, the aglycone can undergo intramolecular rearrangement and/or fragmentation to give rise to products such as thiocyanates, isothiocyanates, nitriles, cyanides, and ozazlidine-2-thiones (Bones and Rossiter, 1996; Halkier and Gershenzon, 2006).

Epidemiological studies have shown that the anticarcinogenic activity in *Brassica* vegetables can be ascribed to glucosinolates (Higdon et al., 2007; Kristal and Lampe, 2009), and thus producing vegetables with a high content of these compounds might provide substantial health benefits. The glucosinolate breakdown products from glucoraphanin, glucoiberin, glucobrassicin and gluconasturtiin in particular have been linked to the anticarcinogenic activity. These compounds strengthen the cellular defense against carcinogens by up-regulating enzymes involved with protective mechanisms (Talalay et al., 1995; Mithen et al., 2000).

The characteristic flavor and astringency of *Brassica* vegetables has been related to the glucosinolate breakdown products (Fenwick et al., 1983), although a sensory study of broccoli found no significant link between taste and amounts of these compounds (Baik et al., 2003). The food industry has previously tried to lower the content of glucosinolate through breeding in order to reduce the bitter taste (Drewnowski and Gomez-Carneros, 2000). However, the opposite has now become the goal due to the recent findings regarding the health-promoting effects of glucosinolates. Varieties with especially high levels of the glucosinolates that are known to have the greatest impact on human health are appearing on the market (e.g. Beneforté 'super broccoli').

Several reports have shown that the content and the composition of glucosinolates are affected by both the genotype of the plant and environmental factors (Farnham et al., 2004; Charron et al., 2005; Kang et al., 2006). The latter include climatic conditions, such as temperature, light, and rainfall (Fenwick et al., 1983; Rosa et al., 1996; Rosa and Rodrigues, 1998; Farnham et al., 2004; Velasco et al., 2007). Agronomic factors, like soil type and mineral nutrient availability, are also known to affect the glucosinolate content (Vallejo et al., 2003a). Biosynthesis of glucosinolates is a complex process that comprises several steps during which the metabolism can be affected by environmental conditions. Studies have demonstrated that the content of glucosinolates is influenced by seasons and years (Rosa et al., 1996; Ciska et al., 2000; Velasco et al., 2007), and the highest levels have been observed in seasons with higher average temperature and more hours of sunlight (Rosa et al., 1997; Vallejo et al., 2003a). Also, a study concerning the impact of temperature showed that both high (32 °C) and low (12 °C) temperatures increased the glucosinolate content (Charron and Sams, 2004). On the other hand, Schonhof et al. (2007) found that decreasing growth temperature (>12 °C) led to augmented accumulation of glucoraphanin in broccoli, and also observed larger amounts of indolic glucosinolate at higher temperatures. Accumulation of myrosinases is favored by high temperatures (Yen and Wei, 1993), although this does not seem to interfere with the increase in glucosinolate content.

Biosynthesis of glucosinolates has been found to be controlled by light (Pérez-Balibrea et al., 2008; Huseby et al., 2013). In *Brassica oleracea* plants, Charron et al. (2005) showed that the concentrations of total glucosinolates, indole glucosinolates, and glucoraphanin differed greatly during the growing season as a result of light conditions, and the observed variation was noted to be associated with photosynthetic photon flux, day length, and temperature. There have also been reports of increased levels of gluconasturtiin caused by longer photoperiods of up to 18 h (Engelen-Eigles et al., 2006). In addition, a few studies have assessed the effect of different light qualities on levels of glucosinolates, which showed that blue light increased the content of glucosinolates in sprouting broccoli (Kopsell and Sams, 2013), and red light led to a higher content of the glucosinolate gluconasturtiin compared to far-red light in watercress (*Nasturtium officinale*) (Engelen-Eigles et al., 2006).

1.4.2 Vitamin C

Vitamin C is a common term for ascorbic acid (Figure 1.2) and its oxidized form dehydroascorbic acid (DHA). Vitamin C is a water-soluble antioxidant that has chain-breaking properties and can donate electrons to superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl and peroxyl radicals, and singlet oxygen (Hancock and Viola, 2005).

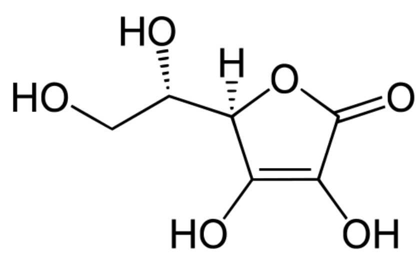

Figure 1.2: Molecular structure of L-ascorbic acid

Plants have several pathways for biosynthesis of ascorbic acid, and there is strong evidence supporting the existence of the L-galactose pathway. The enzyme L-galactose dehydrogenase catalyzes the oxidation of L-galactose to L-galactono-1,4-lactone by opening the lactone ring and forming a new bond between the carbonyl and the hydroxyl group. Thereafter a mitochondrial enzyme reacts with L-galactono-1,4-lactone and resulting in ascorbic acid (Smirnoff and Wheeler, 2000; Hancock and Viola, 2005).

Reactive oxygen species (ROS) are byproducts of normal cellular metabolism, but concentrations of ROS are elevated during stress conditions. ROS can initiate a cascade of reactions that lead to production of the hydroxyl radical and other destructive species that can damage cells. Therefore, aerobic organisms have evolved numerous mechanisms to diminish the toxic effects of ROS, and vitamin C is one of these components in plants (Davey et al., 2000). The pool of ascorbate is low under normal growth conditions and increases under stress (Smirnoff and Wheeler, 2000; Urzica et al., 2012).

Besides functioning as an antioxidant, vitamin C serves as an enzyme co-factor in plants that is an electron donor or acceptor either at the plasma membrane or in chloroplasts (Smirnoff and Wheeler, 2000). Humans cannot synthesize vitamin C and hence depend on dietary sources to cover requirements. In the human body, this vitamin is involved in numerous biological activities (Davey et al., 2000): it can act as an enzyme co-factor, as a scavenger of radicals, and as a mediator in the electron transport across the plasma membrane, and it can also regenerate α-tocopherol. Vitamin C is essential for prevention of scurvy, and it plays a critical role in immune responses and protection against cancer, coronary heart diseases, and cataract (Lee and Kader, 2000).

The content of ascorbic acid in plants has been found to be affected by both light and temperature conditions. High latitudes have been associated with elevated levels of ascorbic acid in berries and various vegetables (Hårdh and Hårdh, 1977; Zheng et al., 2009b, 2012b), and plants grown at low temperatures have been observed to accumulate larger amounts of ascorbic acid (Mahmud et al., 1999; Lo Scalzo et al., 2007; Schonhof et al., 2007). This effect of low temperature on ascorbic acid levels has been related to stress and the antioxidant ability of this compound.

Light is not essential for the biosynthesis of ascorbic acid, but it has been reported that light intensity during growth affects the amount of ascorbic acid formed (Davey et al., 2000; Weerakkody, 2003; Pérez-Balibrea et al., 2008). One investigation showed that broccoli sprouts grown in light had 88% higher vitamin C content compared to those grown in darkness (Pérez-Balibrea et al., 2008). It has also been suggested that the level of ascorbic acid is regulated by phytochromes (Biringer and Schopfer, 1970; Mastropasqua et al., 2012). However, studies examining the effect of different light qualities on the content of ascorbic acid in various plant species have provided contradictory results. In short, the content of vitamin C content was found to be unaffected (Li and Kubota, 2009) or higher (Bartoli et al., 2009) at an increasing red:far-red ratio, and higher under blue and red/blue light than under red and white light (Ohashi-Kaneko et al., 2007).

1.4.3 Phenolic compounds

Phenolics are secondary metabolites characterized by a carbon structure with one or more phenyl groups (Figure 1.3). There are several classes of these compounds, including phenolic acids, flavonoids, stilbenes, and lignans (Crozier et al., 2009).

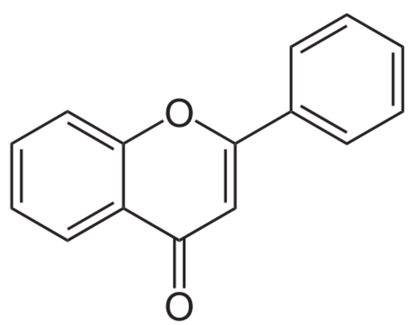

Figure 1.3: Molecular structure of the flavone backbone (2-phenyl-1,4-benzopyrone)

Flavonoids

The flavonoids are phenolic compounds derived from 2-phenyl-chromen-4-one, and they represent the most widespread and diverse class of phenolics and are found in almost all plant parts. Flavonols are one of the six main sub-groups of flavonoids. (Bohm, 1998). The biosynthetic pathways of phenolic compounds include complex series of biochemical reactions. Most phenolics are synthesized via the phenylpropanoid pathway, which initially involves conversion of phenylpropanoid to cinnamic acid by phenylalanine-ammonia lyase. The cinnamic acid is subsequently converted to coumaric acid and further to 4-coumaroyl-CoA, and the latter is then condensed with three molecules of malonyl-CoA by the action of chalcone synthase at the beginning of the general flavonoid pathway (Parr and Bolwell, 2000). After synthesis, the flavonoids are transported to vacuoles or cell walls. Flavonols are yellow pigments that have the 3-hydroxyflavone backbone, and qurecetin and kaempferol glycosides are the most common flavonols in *Brassica* vegetables.

The main functions of flavonoids in plants are as pigments to attract pollinators, as defense against pathogens, and as protection from stress caused by light or other factors. More specifically, levels of flavonoids in plants have been related to stress factors such as UV-B radiation, high intensity light, low temperature, drought, and attack by pathogens (Dixon and Paiva, 1995).

Flavonoids have long been recognized for their antioxidant properties. Some epidemiological studies have provided evidence that high dietary intake of flavonoids can prevent cancer and cardiovascular and neurodegenerative diseases (Adebamowo et al., 2005; Kroon and Williamson, 2005). Thus the flavonoids are highly interesting as compounds in the human diet.

The accumulation of flavonoids in plants can be influenced by climate conditions, such as temperature and radiation. Low temperatures have been found both to increase (<4.5 °C) (Neugart et al., 2012) and to have no effect (0.3-9.6 °C) (Schmidt et al., 2010) on the content of total flavonols in kale, and other studies have shown that the content increases with rising temperature (Bakhshi and Arakawa, 2006; Harbaum-Piayda et al., 2010). By comparison, Harbaum-Piayda et al. (2010) noted that the concentration of kaempferol in pak choi (*Brassica rapa* var. *chinensis*) was higher at 9 °C than at 22 °C. Elevated levels of flavonols caused by low temperature have been correlated with a cold acclimation mechanism involving increased levels of ROS (Klimov et al., 2008).

Currant berries growing at lower latitudes have been observed to accumulate larger amounts of flavonols compared to those grown at higher latitudes (Zheng et al., 2012a). In addition to the temperature effect, it has been reported that the biosynthesis of flavonols is light dependent (Harborne, 1967; Gliszczyńska-Świglo et al., 2007). Flavonols are known to be involved in protection against damaging radiation, and a pronounced increase in these compounds have been documented in plants exposed to UV radiation (Winkel-Shirley, 2002).

1.4.4 Fatty acids

Most of the lipids in plants are located in the chloroplasts, and palmitic (C16:0), linoleic (C18:2 n-6), and α-linolenic (C18:3 n-3) acid constitute approximately 95% of those compounds (Hawke, 1973). The biosynthesis of fatty acids in plants can be described as a disconnected system in which separate genes produce different proteins that each catalyzes a single step in the pathway. The different biosynthetic pathways provide fatty acids of different chain lengths as well as unsaturated fatty acids, iso- and ante-isobranched-chain fatty acids, and hydroxyl fatty acids (White et al., 2005).

The plant cell membrane represents a major site of freeze-induced injury, because the fluidity of this organelle is directly affected by changes in temperature. During the cold acclimation process, the plant stabilizes its cell membranes to prevent damage that can lead to cell death, which can occur in non-acclimated tissue. This acclimation process enables cold-tolerant plants to alter other processes that affect the composition of lipids and fatty acids in the cell membrane (Bradshaw and Hardwick, 1989). This capacity of plants to withstand low temperature stress increases markedly upon continuous exposure to cold temperatures (Dominguez et al., 2010). The increasing content of unsaturated fatty acids compared to saturated fatty acids, which is induced by the activity of desaturases, plays a critical role during cold stress, because it generates ion permeability, appropriate fluidity, and stability in cell membranes.

Linoleic (C18:2 n-6) and α-linolenic (C18:3 n-3) acid are essential fatty acids that cannot be synthesized by mammals and thus must be supplemented in the diets of humans. There is evidence that consumption of polyunsaturated fatty acids can decrease the level of low-density lipoprotein cholesterol in blood and thereby reduce the risk of death from cardiac arrest (Bommareddy et al., 2006; Mozaffarian et al., 2008).

Besides conditions that induce cold acclimation, there are other environmental growth factors that can affect the content and composition of fatty acids in plants. Lo Scalzo et al. (2007) found a higher ratio of unsaturated to saturated fatty acids in cauliflower late compared to early in the growing season. It has also been reported that moderate growth temperatures are optimal for a high content of fatty acids in plants, and that levels of these compounds decrease with increasing temperature (Werteker et al., 2010; Mao et al., 2012).

1.4.5 Soluble sugars

Soluble sugars are commonly defined as mono- and disaccharides, and they are produced by photosynthesis and play an essential role in all living cells. Soluble sugars are also key factors in low-temperature acclimation in cold-tolerant plants (Levitt, 1980). To avoid loss of water, the cells in these plants can accumulate low-molecular-weight osmolytes, including soluble sugars m(Sasaki et al., 1996; Savitch et al., 2000).

In addition to their role in cold acclimation, soluble sugars are important taste and flavor components in *Brassica* vegetables. The primary function of these sugars in humans is to serve as a source of energy, although they also affect satiety, blood glucose, insulin, protein glycosylation, lipids, bile acids, large bowel function, and colonocyte and hepatic metabolism (Cummings et al., 1997).

Considering the strong link between soluble sugars and photosynthetic activity, it is likely that the content of these compounds varies with the environmental growth conditions. Levels of glucose, fructose, and sucrose in vegetables are most likely influenced by factors such as light intensity, temperature, and water stress (Rosa et al., 2001).

1.5 Sensory attributes

Evaluation of the sensory attributes of food products requires more than chemical analysis of compounds that are expected to affect those characteristics. Although chemical assessments do provide interesting information, they cannot describe the sensory attributes of food as they are perceived by humans.

1.5.1 Sensory descriptive analysis

A discipline called sensory descriptive analysis exploits the human senses to classify sensory attributes (Varela and Ares, 2012). Such evaluation entails a strict experimental design and is performed by a panel of human assessors that are screened and trained to recognize and rank the attributes. Members of the panel use a defined sensory language, and the trials are repeated in order to achieve a full quantitative description. There are several different approaches, for example, the following: classification based on differences and similarities, verbal description, and ranking using a set of pre-selected attributes or a set of attributes based on intensity. Before conducting a test, the assessors are exposed to a range of products in the evaluated category to create a common reference point. Statistical methods are subsequently used to distinguish differences. Depending of the number of attributes that are to be evaluated, these types of analysis can provide a nearly complete description of the sensory characteristics of the evaluated product. Indeed, sensory descriptive analysis has long been used by the food industry, but has only more recently been applied in academic research to gain a better understanding of the mechanisms involved in changes in texture, flavor, aroma, and/or structural and microstructural features (Moussaoui and Varela, 2010).

1.6 Objectives

The project upon which this thesis is based aims to document quality characteristics between *Brassica* vegetables grown at variable environmental conditions associated with high and low latitudes. More specifically the main aim covered in Paper I-IV was to determine how the effect of latitudinal conditions can affect the content of specific health-promoting compounds and sensory attributes in broccoli under both controlled and semi-field conditions. In addition, growth temperature and photoperiod, and cold acclimation effect on the content of health-promoting compounds and compounds associated with cold acclimation was investigated in kale (Paper V).

Chapter 2

Materials and methods

2.1 Methodological approaches

In the work underlying this thesis, two main experimental approaches were used to document differences in quality between *Brassica* vegetables grown at high and low latitudes: controlled conditions (natural and artificial light) and field conditions (localities from 70°N to 40°N). Furthermore, two key quality characteristics were investigated: content of health-promoting compounds and sensory attributes.

All plants were harvested at the growth stage considered to be optimum according to commercial standards. Plant material for chemical analysis was frozen in liquid nitrogen immediately after collection and stored at -80 °C, and was later freeze-dried before determination all compounds except vitamin C. The sensory analysis was conducted on blanched broccoli florets that had been stored at -20 °C and re-heated before the evaluation.

2.2 Analysis

2.2.1 Chemical analysis

Glucosinolates

Two different methods were used to determine the content of glucosinolates (Papers I-V), both methods were built upon a methanol extraction. In Papers I and III glucosinolates were quantified and separated using an internal standard and LC-MS/MS[1] set up at Nofima Mat, Ås, Norway. A similar LC-MS/MS-method was used in Paper V, set up in Marbio, Tromsø, Norway. In Papers II and IV the extracted glucosinolates were desulfated before being quantified and separated using an internal standard and HPLC[2] at Nofima Mat lab, Ås, Norway.

Vitamin C

The content of vitamin C was investigated in Papers I-IV. Reduced and oxidized ascorbic acid (ascorbic acid and dehydroascorbic acid) were quantified and determined using a method based upon external standard and HPLC detection. All papers used the same method to quantify and separate ascorbic acid and dehydroascorbic acid, with only slight modifications to optimize the method over time, set up at Nofima Mat lab, Ås, Norway.

Flavonols

The content of the two main flavonols found in *Brassica* vegetables, quercetin and kaempferol, were determined in Papers I-IV. Samples were extracted with methanol and then hydrolyzed and later quantified using external standards at HPLC in Nofima Mat lab, Ås, Norway. The same method was used in Paper I-IV.

[1] Liquid chromatography-tandem mass spectrometry
[2] High-performance liquid chromatography

Fatty acids

Fatty acid content was only determined in Paper V. The fatty acids were extracted with methanolic HCl, quantified and separated by an internal standard and GC-FID[3].

Soluble sugars

In Papers I, II and V soluble sugars were extracted by using an enzymatic kit (Boehringer Mannheim test kit, R-Biopharm AG, Darmstadt, Germany) and quantified and detected by using a spectrophotometer.

2.2.2 Sensory profiling

Sensory profiling in Paper III and IV was performed by a trained, professional sensory panel at Nofima Mat, Ås, Norway, using sensory descriptive analysis according to ISO 6564:1985. The attributes were ranked on a set of pre-selected attributes.

2.3 Experimental set-up

2.3.1 Paper I

The experiment described in Paper I was conducted in temperature- and humidity-controlled darkrooms with broccoli plants under fluorescent light. A 2 × 2 factorial design was used, combined with a high and a low temperature (21/15 and 15/9 °C), and a long and a short day (24 and 12 h). The contents of the health- and sensory-related compounds glucosinolates, vitamin C, flavonols, and soluble sugars were investigated as quality attributes.

[3]Gas chromatography-Flame ionization detector

2.3.2 Paper II

The research reported in Paper II was conducted on broccoli plants grown in temperature- and humidity-controlled darkrooms with supplemental light from light emitting diodes (LED) lamps (red, far-red, red+far-red, and blue light) and to fluorescent lamps. In addition, two controlled treatments with no supplemental LED lighting were performed using 12 and 24 h day length. The experiment was run with relatively high irradiation to ascertain whether any of the light-sensing pigments are involved in the regulation of glucosinolates, vitamin C, flavonols, and soluble sugars.

2.3.3 Paper III

In the work presented in Paper III, broccoli plants were grown under both semi-field and controlled conditions. In the semi-field experiments broccoli plants were grown in 12 L pots placed under insect nets in two locations in Norway (Tromsø and Grimstad). In the latter case, the experiment was performed in phytotron chambers under controlled temperatures and natural light conditions (Tromsø, 70°N, June-August). Both the sensory quality and chemical content of glucosinolates, vitamin C and flavonols, were determined.

2.3.4 Paper IV

In the research described in Paper IV, the broccoli plants were grown outdoors under different latitudes in a semi-field experiment. This was done in specially designed pots, each fitted with a lid and covered by insect netting to avoid variation in external factors other than light and temperature conditions. The experiment was carried out over three years at four locations: Tromsø and Grimstad, Norway; Berlin, Germany; Pontevedra, Spain. Sensory profiling and chemical composition of glucosinolates, vitamin C, and flavonols were determined. The content of health-promoting compounds was analyzed only in one-year samples.

2.3.5 Paper V

In the study reported in Paper V, kale plants were grown under pre-acclimation growth treatments followed by a cold acclimation period. A 2 × 2 × 2 factorial design was used in which all plants received either higher or lower temperature (21/15 and 15/9 °C) combined with a long and a short day (24 and 12 h). Half of the plants were harvested and analyzed after this growth period, and the other half were exposed to a cold acclimation period under non-photosynthetic active radiation light (12 h) and decreasing temperatures down to 3 °C. Both before and after the cold acclimation period, the content of glucosinolates, fatty acids, and soluble sugars were measured and a freeze test was conducted.

Chapter 3

Results and discussion

The work reported in this thesis shows that the quality of *Brassica* vegetables can be affected by latitude at which the plants are grown. The biosynthetic pathways in plants are regulated by genetic factors, but they are also influenced by environmental and physiological conditions. In the current experiments (Papers I-V), variation in natural and simulated latitudinal conditions led to differences in the content of health-promoting compounds and sensory quality in broccoli and kale. In general, temperature was the main factor responsible for these effects, although both day length (Paper I) and specific wavelengths of the light (Paper II) also increased or diminished the variation. When investigating the climatic effect of different latitude conditions there are several factors to consider, such as temperature, day length, and light quality. Experiments were performed under controlled conditions to explore in detail how conditions found at different latitudes can affect the quality of broccoli and kale (Papers I-III and V). In two investigations (Papers III and IV) an experiment was set up under semi-field conditions including control of non-climatic factors such as soil, watering, precipitation, fertilization, and pests. The results obtained under the field and controlled conditions were not always consistent, which demonstrates the importance of conducting both types of studies.

The range in levels of health-promoting compounds in broccoli varied between the studies included in the thesis (Papers I-IV, Table 3.1). Considering glucosinolates, the content of indolic glucosinolates differed distinctly between the studies in which native glucosinolates were detected (Papers I and III) and the investigations in which glucosinolates were desulfated prior to detection (Papers II and IV). Earlier studies have also found large variation in the content of glucosinolates detected in broccoli, and Table 3.1 outlines the results regarding glucosinolates obtained in the present compared to previous studies. Previous studies presented in Table 3.1 were all analyzed by using enzymatic desulfation. By comparison, the content of ascorbic acid was within the same range in our experiments and previous results from broccoli (Table 3.1). Furthermore, the content of flavonols in broccoli was much higher when grown outdoors (Papers III and IV) than those grown under controlled conditions (Papers I and II) without addition of UV light and with lower radiation.

Table 3.1: Range in levels of mean values of health-promoting compound in broccoli

Study:	Aliphatic GLS (μmol/g dw)	Indolic GLS (μmol/g dw)	Ascorbic acid (mg/100 g fw)	Flavonols (mg/100 g dw)
Paper I	22.3-32.2	21.3-46.1	78.2-99.1	15.3-38.5
Paper II	3.2-14.1	4.7-7.3	75.6-92.2	11.6-21.1
Paper III	33.4-44.4	13.7-21.9	119-196	18.9-78.5
Paper IV	12.3-16.7	3.4-6.4	74.2-101.0	68.1-123.5
Hansen et al. (1997)	21.4-50.1	15.8-47.3	-	-
Kushad et al. (1999)	3.0-31.4	0.4-6.2	-	-
Brown et al. (2002)	1.3-26.3	0.7-5.9	-	-
Vallejo et al. (2002)	1.3-8.3	1.7-20.0	-	-
Vallejo et al. (2003a)	1.4-8.3	17.2-76.7	-	-
Borowski et al. (2008)	18.2-28.8	12.2-17.1	-	-
Aires et al. (2011)	10.8-21.5	7.8-26.6	-	-
Kurilich et al. (1999)	-	-	54.0-119.8	-
Vallejo et al. (2003b)	-	-	34.3-91.9	-
Schonhof et al. (2007)	-	-	80.9-119.9	-
Kaur et al. (2007)	-	-	74.2-112.4	-
Koh et al. (2009)	-	-	13.0-110.3	-
Alarcón-Flores et al. (2014)	-	-	-	34.5-65

GLS=glucosinolates.

3.1 Health- and taste-related compounds

3.1.1 Glucosinolates

In the study conducted at different latitudes (Paper IV), total glucosinolate content was highest at lower latitude in the summer season (Berlin), and this was due to variation in a few of the individual glucosinolates, neoglucobrassicin and 4-methoxyglucobrassicin. Levels of the indolic glucosinolates increased with decreasing latitude, and the content of neoglucobrassicin was highest at the low-latitude/summer location (Berlin). The aliphatic glucosinolates, on the other hand, were not affected by the growth location.

The total glucosinolate content in broccoli florets was also higher in plants grown at higher temperatures (18 and 21/15 °C; Papers I and III). An increase in the total glucosinolate content at higher growth temperatures has previously been found in leaves of broccoli sprouts by Pereira et al. (2002), with the highest level detected at 30/15 °C compared to lower temperatures (22/15 and 18/15 °C). The same trend has been observed in a rapid-cycling population of *Brassica oleracea* (Charron and Sams, 2004) and in kale (Velasco et al., 2007).

The current studies of broccoli under controlled conditions (Papers I-III) showed conflicting effects of temperature on aliphatic glucosinolates. In one of the investigations (Paper I), the content of aliphatic glucosinolates was higher at high temperature regime (21/15 °C-12 h day length) than at the lower temperature regime (15/9 °C-12 h day length). The two other studies showed contrasting results, 18 °C/24 h resulted in the lowest content of glucoraphanin in Paper III, and in Paper II the content of glucoraphanin was lowest at 15 °C compared to 12 °C. There were two main differences between the investigation described in Paper I and the other two studies (Papers II and III) that might explain the contradictory results: a diurnal temperature regime was used in Paper I and natural light conditions rather than fluorescent light was used in Paper III. Other studies have also found conflicting effect of temperature on the content of aliphatic glucosinolates, particularly glucoraphanin. Charron et al. (2005) observed that the glucoraphanin content in the edible part of various *Brassica* cultivars including broccoli, was insensitive to temperature. Schonhof et al. (2007) noted an increase in the content of aliphatic glucosinolates in broccoli with decreasing temperature ($\leq$ 12 °C) in combination with increased radiation. Other studies have detected the highest content of aliphatic glucosinolates at a higher temperature (32 °C compared to 12 °C) (Charron and Sams, 2004). Our results and the findings of some other studies of *Brassica* vegetables indicate that temperatures below and above the optimum growth temperature can increase the content of aliphatic glucosinolates.

All of the present studies under controlled conditions (Papers I-III and V) showed that exposure to 24 h of light decreases the content of the aliphatic glucosinolate, glucoraphanin. Although the day length effect described in Papers II and V was not significant, there was a tendency toward lower glucoraphanin content in plants grown under 24 h of light. These results are in contrast to the investigations by Charron and Sams (2004) and Charron et al. (2005) conducted to examine the impact of different day lengths on the content of aliphatic glucosinolates in different *Brassica* crops. However, Charron et al. (2005) did not use continuous light and Charron and Sams (2004) found the day length effect in stems but not the roots or leaves of rapid-cycling *Brassica oleracea*. This may explain the discrepancy between our findings and those reported by Charron and co-workers. Also of interest, a study of *Arabidopsis* showed that the biosynthesis of glucosinolates was under diurnal regulation and that the content of these compounds was highest during light periods (Huseby et al., 2013). The lower level of glucosinolates found in broccoli under continuous light might be explained by the lack of circadian trigger in these plants, corresponding to the uninterrupted light at high latitudes in the summer season. In addition, it seemed that the decreased aliphatic glucosinolate content upon exposure to 24 h of light was temperature-dependent, because it was detected only at higher (Paper I) or lower (Paper III) temperatures.

The content of indolic glucosinolates was highest in broccoli florets grown at higher temperatures (Papers I and III). Of all the glucosinolates that were analyzed, the largest variation was noted for neoglucobrassicin, while glucobrassicin was the least influenced by the different treatments. Notwithstanding, as indicated in Paper II, no difference was found between the two temperatures 12 and 15 °C, possibly because of the relatively small difference in temperature compared with what was used in the other studies in our project. Other studies have also found the highest levels of the indolic glucosinolates at higher temperatures (Charron and Sams, 2004; Charron et al., 2005; Schonhof et al., 2007). At high temperatures neoglucbrassicin and 4-methoxyglucobrassicin were highest at 24 h day length, which is in contrast to the levels of aliphatic glucosinolates (Paper I). When comparing aliphatic and indolic glucosinolates under both controlled (Paper I) and semi-field (Paper IV) conditions, the aliphatic glucosinolates showed less variation between treatments. The content of aliphatic glucosinolates (as compared to indolic glucosinolates) in broccoli has been reported to be less affected by environmental conditions than by genotypic variations (Brown et al., 2002; Farnham et al., 2004). The aliphatic glucosinolates are synthesized from chain elongated forms of amino acids, whereas the biosynthesis of indolic and aromatic glucosinolates is less complex (Sønderby et al., 2010). Accordingly, differences in response between the respective groups of glucosinolates can be related to the properties and regulation of the various enzymes in the biosynthesis pathways. Large variation was found for the indolic glucosinolate, neoglucobrassicin, in three of the present studies (Papers I, III and IV).

In addition to the observed temperature and day length effect, the content of glucosinolates was influenced by variation in light quality. The level of glucoraphanin in broccoli florets was reduced by supplemental far-red light

(Paper II), whereas the amount of glucoraphanin was not affected by exposure to red light as compared to no supplemental LED light. There was also evident that some of the indolic glucosinolates were influenced interactions between temperature and light quality treatments. Far-red light has previously been reported to lower the level of glucosinolates, as illustrated by data obtained by Engelen-Eigles et al. (2006) showing that a reduction in the aromatic gluconasturtiin was induced by supplemental far-red light, but not by red light. The response to far-red light indicates that phytochrome mediated signaling is involved in the regulation of glucosinolates, which might result in lower content of glucosinolates at high latitudes. However, it seems that the effect depends on the plant part and the species in question, thus suggesting that the metabolic pathways or modes of regulation of metabolite biosynthesis vary in different species.

In the frost tolerant kale plants (Paper V), the levels of all glucosinolates, except sinigrin, decreased after a cold acclimation period, and also under specific pre-acclimation conditions. It is possible that the reduction seen after the acclimation period could due to degradation, although our results indicate that it is unlikely that such acclimation would cause pronounced changes in the glucosinolate content.

The mean levels of aliphatic and indolic glucosinolates found in broccoli florets varies markedly between previously published studies (Table 3.1), and the discrepancies might be explained by differences in the analytical methods used. Moreover, the results of the studies listed in Table 3.1 emphasize that the glucosinolate content varies considerably among different cultivars (Vallejo et al., 2002, 2003a), both between individual florets (Borowski et al., 2008) and between seasons (Aires et al., 2011). This variation makes it difficult to distinguish the specific effect that growth location might have in relation to the health-related compounds. Nevertheless, our results indicate that there is a potential to increase the levels of health-promoting glucosinolates by optimizing the growth conditions.

3.1.2 Ascorbic acid

In the semi-field study of broccoli (Paper IV), the latitudinal location did not affect the content of ascorbic acid/vitamin C. On the other hand, the experiments focused on broccoli under controlled conditions (Papers I and II), showed that the ascorbic acid content tended to be highest at the low temperatures. In agreement with results from Papers I and II, previous studies generally demonstrate higher levels of ascorbic acid/vitamin C in various *Brassica* vegetables grown under conditions characterized by lower temperatures (Mahmud et al., 1999; Lo Scalzo et al., 2007; Schonhof et al., 2007). Despite those findings, near frost temperatures have been reported to reduce the content of ascorbic acid by 50% in kale (Hagen et al., 2009). The increase in ascorbic acid contents at low temperatures has been associated with the antioxidant capacity of ascorbic acid exhibited in response to the higher production of ROS under conditions of environmental stress (Smirnoff and Pallanca, 1996).

As described in Paper I, a day length effect on the level of ascorbic acid was observed in broccoli florets, noted as a lower content at 24 h than at 12 h light. Previous studies, however, have shown greater amounts of ascorbic acid with increasing duration of the photoperiod and with increasing light intensity (Mozafar, 1994; Dumas et al., 2003). As for glucosinolates, this disparity might be related to a lack of circadian rhythm. It seemed that the day length effect was temperature-dependent, because it was detected only at low temperatures. The mentioned results suggest that low-latitude conditions during winter seasons could be favorable for high content of vitamin C, although this was not observed in the semi-field study (Paper IV).

The content of vitamin C in broccoli was not affected by light quality in the present study (Paper II), whereas other studies have contradictory results. In komatsuna (*Brassica rapa* var. *perviridis*), it was found that the level of ascorbic acid was higher under blue and red/blue light than under red and white light (Ohashi-Kaneko et al., 2007). In lettuce (*Phaseolus vulgaris)*, Li and Kubota (2009) noted that the content of vitamin C was unaffected by different light quality treatments, and Bartoli et al. (2009) showed that the levels of vitamin C increased with an increasing ratio of red to far-red. In oat leaves (*Avena sativa* L.), it has also been suggested that there is a phytochrome effect, because the content of ascorbic acid was observed to rise upon exposure to both blue and red light (Mastropasqua et al., 2012). These findings indicate that even though the broccoli florets in our study were not affected by light spectral composition, it is possible that phytochrome involvement in the regulation is species-dependent.

Other studies have shown the same range of ascorbic acid levels in broccoli as observed in our studies (Table 3.1). In our project, the highest level of ascorbic acid per fresh weight was found in the broccoli under treatment conditions entailing temperatures down to 6 °C at the end of the growing season (Paper III), but these plants also had the highest percentage dry weight. Furthermore, the controlled experiment seemed to suggest that the content of ascorbic acid could be increased under low temperature conditions (Paper I).

3.1.3 Flavonols

The content of flavonols was examined in broccoli florets at various latitudes and detected highest levels in plants grown in Tromsø and Berlin (Paper IV). Considering other plant species, Zheng et al. (2012a) observed a higher content of flavonols in currant berries (*Ribes* spp.) grown at lower latitudes. Lätti et al. (2010) on the other hand, observed a trend towards increasing amount of flavonols in bog bilberries, with the highest levels in the berries collected in high latitudes (66°03' to 68°34' N) compared to southern populations (60°23' to 61°53' N). The latter study was conducted on wild plant material grown in their original growth location.

Under controlled conditions in the present studies (Papers I, II and III), elevated temperature generally led to the highest content of the flavonols kaempferol and quercetin in broccoli. Levels of those two compounds were affected by both temperature and day length, but not by interactions between those two factors (Paper I). Also, day length had a greater impact on kaempferol than on quercetin. Our results indicate that growth conditions associated with 12 h light and high temperatures, characteristics of low latitudes in the summer season, would primarily favor high levels of quercetin and kaempferol. Low temperature has been found both to increase (<4.5 °C; Neugart et al. (2012)) and to have no effect (from 9.6 down to 0.3 °C; Schmidt et al. (2010)) on the content of total flavonols in kale. An increase in flavonol concentrations, induced by low temperature, has been related to the antioxidant properties of these compounds and to elevated levels of ROS (Klimov et al., 2008; Oh et al., 2009). In the studies of broccoli (Paper I-IV), the temperature was not as low as in the studies of kale performed by Schmidt et al. (2010) and Neugart et al. (2012), of which the results suggest that exposure to stress temperatures causes up-regulation of the biosynthesis of flavonols. However, higher temperatures within the range of normal growth conditions would favor a higher content of flavonols.

In addition to the temperature effect, a day length effect was detected, and the level of flavonols was highest when there was a break in the photoperiodic regime. Studies have demonstrated that the biosynthesis of flavonols is light sensitive, and this also applies to broccoli (Koes et al., 1994; Gliszczyńska-Świglo et al., 2007). The content of both of the flavonols analyzed (quercetin and kaempferol) was lower at a 24 h day length (Paper I), and it has been reported that the levels of flavonoids may be regulated by the circadian clock (Thain et al., 2002). Regardless of the temperature treatments, flavonols level was highest without continuous light (Paper I).

In the present study it was also found that exposure to additional red LED light elevated the content of both quercetin and kaempferol in broccoli (Paper II). Similarly, Li and Kubota (2009) noted that supplemental red light resulted in the highest content of phenolic compounds in baby leaf lettuce (*Lactuca sativa* L.). These findings may suggest that phytochrome-mediated regulation is involved in the biosynthesis of flavonols. One of the main functions of flavonols is to provide UV protection, and hence concentrations of these compounds rise in plants exposed to increased UV radiation (Treutter, 2005; Koyama et al., 2012). Together, the mentioned observations indicate that the levels of flavonols also in broccoli are influenced by different environmental growth conditions. However, this interaction seems to be complex and require further investigation.

3.1.4 Fatty acid

In the present project, the content of fatty acids was investigated only in kale (Paper V). Since this vegetable can tolerate temperatures below freezing it was of interest to evaluate the effects of cold acclimation temperatures on the levels and composition of fatty acids. The results revealed a decrease in the total fatty acid content during cold acclimation, which was may be due to degradation of chloroplasts. The saturated fatty acid 22:0 was the only fatty acid detected that actually increased after cold acclimation, which caused the ratio of unsaturated to saturated fatty acids to be unaffected by the acclimation. However, when excluding 22:0 and performing the assessment only on unsaturated fatty acids in relation to 18:0, there was a clear shift in the fatty acid composition from before to after cold acclimation, and the decrease in the content of the unsaturated fatty acids, dominated by 18:3, was less compared to 18:0. The exact function of 22:0 in plants is not known, and this fatty acid is not a common component in cell membranes (Millar et al., 2000), thus it is possible that the composition of fatty acids is associated with increased frost tolerance in kale. However, the degradation process can reduce the general content of membrane fatty acids (Paper V).

Pre-acclimation day length and temperature also caused some significant differences in fatty levels and composition, although no general trend was found. There have been reports of such effects, indicating higher contents of fatty acids at medium growth temperatures (Mao et al., 2012) and decreasing concentrations of fatty acids with increasing temperature (Werteker et al., 2010). In cauliflower, the ratio of unsaturated to saturated fatty acids has been found to be higher when grown late compared to early in the growing season (Lo Scalzo et al., 2007). The findings presented in Paper V suggest that the content of fatty acids can be affected by preharvest temperatures and photoperiod. However, such effects are probably not substantial, and long photoperiods with a short dark period might compensate for the lower temperature.

3.1.5 Soluble sugars

The amounts and types of soluble sugars present in *Brassica* vegetables can influence the flavor and also contribute to increased frost tolerance. All our investigations of the levels of soluble sugar (Paper I, II and V) indicated the following ranking D-glucose>D-frucrose>sucrose, which agrees with other studies of kale and broccoli (Ayaz et al., 2006; Rosa et al., 2001). The content of soluble sugars is directly associated with photosynthesis, for which the optimum temperature differs between species and in relation to growth and environmental conditions. In addition, increased levels of soluble sugars are linked to enhanced cold tolerance. In one of our studies (Paper I) the content of D-glucose in broccoli was highest at 21/15 °C combined with 24 h of light. However, the total content of soluble sugars did not differ with varying growth temperature and day lengths. In another experiment on broccoli (Paper II) the low temperature treatments (12 °C) led to the highest sucrose content, which implies that either there was no marked variation in photosynthesis or a cold acclimation was induced for the low temperature conditions. Regarding kale, plants subjected to cold acclimation temperatures had an increased content of all soluble sugars compared to the pre-acclimation levels, and the largest increase was noted for sucrose (Paper V). Such rise in sucrose is often detected in the autumn season, and this plays a special role in the development of frost tolerance (Sasaki et al., 1996). Our results indicate that an increase in the content of soluble sugars is a part of the cold acclimation program in kale, and greater tenderness and sweetness of the leaves is an effect of this mechanism.

3.2 Sensory attributes

The influence of contrasting temperature and day length conditions was investigated in relation to sensory attributes of broccoli grown under controlled temperature conditions compared to semi-field conditions (Paper III). The results showed significant effects on several sensory attributes, including bud size, color, and sweetness, and the highest scores were noted at 24 h of light combined with a low temperature of 12 °C.

In the study performed at different latitudes, growth location affected 23 out of 30 attributes (Paper IV). Appearance was clearly different between the locations, with a high-to-low latitude gradient for all attributes except the uniformity of bud size, which was similar at all sites. Sour odor and green odor differed, with higher intensities in plants from the two locations in Norway than in those grown in Berlin. The differences in taste and flavor attributes were insignificant between the two Norwegian locations, but many of these attributes differed from those observed in Berlin. Broccoli from the northern locations tasted more fresh, had a more evident green flavor, was less bitter, and had less of a cabbage, stale and watery flavor. Furthermore, broccoli grown in the two Norwegian locations was more firm, crunchier, crispier and juicier and had higher values for toughness and fibrousness compared to that grown in Berlin.

To our knowledge no earlier studies in the literature have used sensory descriptive analysis of *Brassica* vegetables to describe changes in sensory attributes caused by growth conditions. Nevertheless, one investigation did show that flavor compounds in cabbage cultivars differ as a results of cultivation conditions (MacLeod and Nussbaum, 1977), and some studies have examined the sensory difference between varieties (Francisco et al., 2009) and chemical compounds known to influence taste (Schonhof et al., 2004), as well as a combinations of these aspects (Padilla et al., 2007). The use of instrumental analyses to identify factors that impact flavor is a valuable approach, but further information can be gained by performing sensory descriptive analysis to evaluate a range of sensory attributes of vegetables. Based on the results of such assessment, latitudinal growth conditions can be expected to change the sensory quality of broccoli, and it will be up to consumers to decide which attributes are preferable.

3.3 Main conclusions and future prospects

In conclusion, the present controlled experiments revealed that day length, temperature, and specific light qualities, associated with different latitudinal conditions influence the content of several health-promoting compounds in broccoli. The highest levels of most glucosinolates and flavonols were found in plants grown at higher temperatures and at short day length. The results suggest that far-red light can reduce the content of glucosinolates in broccoli, and that the content of these compounds is lower in regions and seasons associated with such growth conditions. Regarding vitamin C, the highest level was detected at a lower growth temperature and short day length. Hence, the present findings emphasize the importance of evaluating temperature and light simultaneously in order to reveal the complex influence of these factors on health-promoting compounds in broccoli, and possibly other vegetables as well. Our semi-field study of broccoli conducted at different latitudes detected differences in the contents of glucosinolates and flavonols. The glucosinolate content was highest in plants grown in Berlin in summer, whereas no difference in levels of vitamin C was found between the locations. These results also demonstrate the importance of conducting both controlled and field experiments. It would be interesting to further investigate the regulation of these health-promoting compounds throughout the day to gain more knowledge about the effects of circadian rhythm. However, if the goal is to improve the content of health-promoting compounds, it is also necessary to consider growth site and season.

The highest levels of the soluble sugars D-fructose and D-glucose were found in broccoli grown at higher temperatures, and to be part of the cold acclimation process in kale. Composition of fatty acids was also observed to be a part of the acclimation process in kale. Sensory differences were also identified between broccoli grown under high- and low- latitude conditions, and it was found that plants from two locations in Norway had several positive tastes and texture attributes, such as being less bitter, less watery in flavor, crunchier, crispier and juicier. For further investigations consumer tests can be performed to identify preferences for certain attributes. Today, there is only minimal production of vegetables in the Arctic latitudes of Scandinavia, but the results from the present studies indicate that high- latitude conditions are well suited for growing *Brassica* vegetables. Our findings can promote awareness of importance of growth site in relation to the quality of vegetables.

Bibliography

Adebamowo, C. A., Cho, E., Sampson, L., Katan, M. B., Spiegelman, D., Willett, W. C., and Holmes, M. D. (2005). Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *Int. J. Cancer*, 114:693–633.

Aires, A., Fernandes, C., Carvalho, R., Bennett, R. N., Saavedra, M. J., and Rosa, E. A. (2011). Seasona leffects on bioactive compounds and antioxidant capacity of six economically important *Brassica* vegetables. *Molecules*, 16:6816–6832.

Alarcón-Flores, M. I., Roberto Romero-Gonález, J. Luis Martínez Vidal, F. J. E. G., and Frenich, A. G. (2014). Monitoring of phytochemicals in fresh and fresh-cut vegetables: A comparison. *Food Chem.*, 142:392–399.

Alberdi, M. and Corcuera, L. J. (1991). Cold acclimation in plants. *Phytochemistry*, 30:3177–3184.

Ayaz, F. A., Glew, R. H., Millson, M., Huang, H. S., Chuang, L. T., and Hayırlıoglu-Ayaz, C. S. S. (2006). Nutrient contents of kale (*Brassica oleraceae* L. var. *acephala* DC.). *Food Chem.*, 96:572–579.

Baik, H. Y., Juvik, J. A., Jeffery, E. H., Wallig, M., Kushad, M., and Klein, B. (2003). Relating glucosinolate content and flavor of broccoli cultivars. *J. Food Sci.*, 68:1043–1050.

Bakhshi, D. and Arakawa, O. (2006). Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples. *J. Appl. Hortic.*, 8:101–104.

Bartoli, C. G., Tambussi, E. A., Diego, F., and Foyer, C. H. (2009). Control of ascorbic acid synthesis and accumulation and glutathione by the incident light red/far red ratio in *Phaseolus vulgaris* leaves. *FEBS lett.*, 583:118–122.

Batschauer, A. (1999). Light perception in higher plants. *Cell. Mol. Life Sci.*, 55:153–165.

Berry, J. and Björkman, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.*, 31:491–543.

Biringer, I. and Schopfer, P. (1970). Photomodulation by phytochrome of the rate of accumulation of ascorbic acid in mustard seedlings (*Sinapsis alba* L.). *Planta*, 93:152–159.

Bjorkman, T. and Pearson, K. (1998). High temperature arrest of inflorescence development in broccoli (*Brassica oleracea* var.*italica* L.). *J. Exp. Bot.*, 49:101–106.

Bohm, B. A. (1998). *Introduction to Flavonoids*. Harwood Academic Publishers, Amsterdam, The Netherland.

Bommareddy, A., Arasada, B. L., Mathees, D. P., and Dwivedi, C. (2006). Chemopreventive effects of dietary flaxseed on colon tumour development. *Nutr. Cancer*, 54:216–222.

Bones, A. M. and Rossiter, J. T. (1996). The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiol. Plantarum*, 97:194–208.

Borowski, J., Szajdek, A., Borowska, E. J., Ciska, E., and Zieliński, H. (2008). Content of selected bioactive components and antioxidant properties of broccoli (*Brassica oleracea* L.). *Eur. Food. Res. Technol.*, 226:459–465.

Bradshaw, A. D. and Hardwick, K. (1989). Evolution and stress-genotypic and phenotypic components. *Biol. J. Linn. Soc.*, 37:137–155.

Brandt, K., Christensen, L. P., Hansen-Møller, J., Hansen, S. L., Haraldsdottir, J., Jespersen, L., Purup, S., Kharazmi, A., Barkholt, V., Frøkiær, H., and Kobæk-Larsen, M. (2004). Health promoting compounds in vegetables and fruits: A systematic approach for identifying plant components with impact on human health. *Trends Food Sci. Tech.*, 15:384–393.

Brown, A. F., Yousef, G. G., Jeffery, E. H., Klein, B. P., Wallig, M. A., Kushad, M. M., and Juvik, J. A. (2002). Glucosinolate profiles in broccoli: Variation in levels and implications in breeding for cancer chemoprotection. *J. Am. Soc. Hortic. Sci.*, 127:807–813.

Cartea, M. E., Picoaga, A., Soengas, P., and Ordas, A. (2002). Morphological characterization of kale populations from northwestern Spain. *Euphytica*, 129:25–32.

Charron, C. S. and Sams, C. E. (2004). Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *J. Am. Soc. Hortic. Sci.*, 129:321–330.

Charron, C. S., Saxton, A. M., and Sams, C. E. (2005). Relationship of climate and genotype to seasonal variation in the glucosinolate-myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J. Sci. Food Agric.*, 85:671–681.

Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher plants. *Annu. Rev. Genet.*, 38:87–117.

Ciska, E., Martyniak-Przybyszewska, B., and Kozlowska, H. (2000). Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *J. Agric. Food Chem.*, 48:2862–2867.

Crozier, A., Jaganath, I. B., and Clifford, M. N. (2009). Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.*, 26:1001–1043.

Cummings, J. H., Roberfroid, M. B., Andersson, H., Barth, C., Ferro-Luzzi, A., Ghoos, Y., Gibney, M., Hermonsen, K., James, W. P. T., Korver, O., Lairon, D., Pascal, G., and Voragen, A. G. S. (1997). A new look at dietary carbohydrate: Chemistry, physiology and health. *Eur. J. Clin. Nutr.*, 51:417–423.

Davey, M. W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J. J., Strain, J. J., Favell, D., and Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.*, 80:825–860.

Dixon, R. A. and Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7:1085–1097.

Dominguez, J., Aira, M., and Gómez-Brandón, M. (2010). Increasing omega-3 desaturase expression in tomato results in altered aroma profile and enhanced resistance to cold stress. *Plant Physiol.*, 153:655–665.

Drewnowski, A. and Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.*, 72:1424–1435.

Dumas, Y., Dadomo, Di Lucca, G., and Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric.*, 83:369–382.

Engelen-Eigles, G., Holden, G., Cohen, J. D., and Gardner, G. (2006). The effect of temperature, photoperiod, and light quality on gluconasturtiin concentration in watercress (*Nasturtium officinale* R. Br.). *J. Agric. Food Chem.*, 54:328–334.

Fahey, J. W., Zalcmann, A. T., and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, 56:5–51.

Farnham, M. W., Wilson, P. E., Stephenson, K. K., and Fahey, J. W. (2004). Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. *Plant Breeding*, 123:60–65.

Fenwick, G. R., Heaney, R. K., and Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Cr. Rev. Food Sci.*, 18:123–201.

Fisher, N. D. L. and Hollenberg, N. K. (2005). Flavanols for cardiovascular health: the science behind the sweetness. *J. Hypertens.*, 23:1453–1459.

Francisco, M., Cartea, M. E., Soengas, P., and Velasco, P. (2011). Effect of genotype and environmental conditions on health-promoting compounds in *Brassica rapa*. *J. Agric. Food Chem.*, 59:2421–2431.

Francisco, M., Velasco, P., Romero, A., Vázquez, L., and Cartea, M. E. (2009). Sensory quality of turnip greens and turnip tops grown in northwestern Spain. *Eur. Food. Res. Technol.*, 230:281–290.

Gliszczyńska-Świglo, A., Kalużewicz, A., Lemańska, K., Knaflewski, M., and Tyrakowska, B. (2007). The effect of solar radiation on the flavonol content in broccoli inflorescence. *Food Chem.*, 100:241–245.

Grevsen, K. (1998). Effects of temperature on head growth of broccoli (*Brassica oleracea* L. var. *italica*): Parameter estimates for a predictive model. *J. Hortic. Sci. Biotech.*, 73:235–244.

Gu, Z., Guo, Q., and Gu, Y. (2012). Factors influencing glucoraphanin and sulforaphane formation in brassica plants: a review. *J. Integra. Agric.*, 11:1804–1816.

Gunden, C. and Thomas, T. (2012). Assessing consumer attitudes towards fresh fruit and vegetable attributes. *J. Food Agric. Environ.*, 10:85–88.

Hagen, S. F., Borge, G. I. A., Solhaug, K. A., and Bengtsson, G. B. (2009). Effect of cold storage and harvest date on bioactive compounds in curly kale (*Brassica oleracea* L. var. *acephala*). *Postharvest Biol. Tec.*, 51:36–42.

Halkier, B. A. and Gershenzon, J. (2006). Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.*, 57:303–333.

Hancock, R. D. and Viola, R. (2005). Biosynthesis and catabolism of L-ascorbic acid in plants. *Crit. Rev. Plant Sci.*, 24:167–188.

Hansen, M., Laustsen, A. M., Olsen, C. E., Poll, L., and Sørensen, H. (1997). Chemical and sensory quality of broccoli (*Brassica oleracea* L. var *italica*). *J. Food Quality*, 20:441–459.

Harbaum-Piayda, B., Walter, B., Bengtsson, G. B., Hubbermanna, E. M., , Bilger, W., and Schwarz, K. (2010). Influence of pre-harvest UV-B irradiation and normal or controlled atmosphere storage on flavonoid and hydroxycinnamic acid contents of pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*). *Postharvest Biol. Tec.*, 56:202–208.

Harborne, J. (1967). *Comparative Biochemistry of the Flavonoids*. Academic Press, London.

Hårdh, J. E., Persson, A. R., and Ottosson, L. (1977). Quality of vegetables cultivated at different latitudes in Scandinavia. *Acta Agr. Scand.*, 27:81–86.

Hårdh, K. and Hårdh, J. E. (1977). Studies on quality of vegetables and strawberries at different latitudes in Finland. *Ann. Agr. Fenn.*, 16:19–26.

Hawke, J. C. (1973). Lipids. In Butler, G. W. and Bailey, R. W., editors, *Chemistry and biochemistry of herbage Volume 1*, pages 213–263. London Academic Press, London.

Herr, I. and Büchler, M. W. (2010). Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer. *Cancer Treat. Rev.*, 36:377–383.

Higdon, J. V., Delage, B., Williams, D. E., and Dashwood, R. H. (2007). Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol. Res.*, 55:224–236.

Hotta, C. T., Gardner, M. J., Hubbard, K. E., Baek, S. J., Dalchau, N., Suhita, D., Dodd, A. N., and Webb, A. A. R. (2007). Modulation of environmental responses of plants by circadian clocks. *Plant Cell*, 30:333–349.

Huseby, S., Koprivova, A., Lee, B.-R., Saha, S., Mithen, R., Wold, A.-B., Bengtsson, G. B., and Kopriva, S. (2013). Diurnal and light regulation of sulphur assimilation and glucosinolate biosynthesis in *Arabidopsis*. *J. Exp. Bot.*, 64:1039–1048.

Jackson, S. D. (2009). Plant responses to photoperiod. *New Phytol.*, 181:517–531.

Jahangir, M., Kim, H. K., Choi, Y. H., and Verpoorte, R. (2009). Health-affecting compounds in *Brassicaceae*. *CRC Cr. Rev. Food Sci.*, 8:31–43.

Kałużewicz, A., W. Krzesiński, M. K., Lisiecka, J., Spiżewski, T., and Frąszczak, B. (2010). The effect of temperature on the broccoli yield and length of the period from the head initiation to harvest. *Acta Sci. Pol. Hortorum Cultus*, 9:167–174.

Kang, J. Y., Ibrahim, K. E., Juvik, J. A., Kim, D. H., and Kang, W. J. (2006). Genetic and environmental variation of glucosinolate content in chinese cabbage. *HortScience*, 41:13–82–1385.

Kaur, C. and Kapoor, H. C. (2001). Antioxidants in fruits and vegetables - The millennium's health. *Int. J. Food. Sci. Tech.*, 36:703–725.

Kaur, C., Kumar, K., Anil, D., and Kapoor, H. (2007). Variations in antioxydant activity in broccoli (*Brassica oleracea* L.) cultivars. *J. Food Biochem.*, 31:621–638.

Kaurin, Å., Junttila, O., and Nilsen, J. (1985). *Plant production in the north.* Universitetsforlaget.

Klimov, S. V., Burakhanova, E. A., Dubinina, I. M., Alieva, G. P., Salnikova, E. B., Olenichenko, N. A., Zagoskina, N. V., and Trunova, T. I. (2008). Suppression of the source activity affects carbon distribution and frost hardiness of vegetating winter wheat plants. *Russ. J. Plant Physl.+*, 55:308–314.

Koes, R. E., Quattrocchio, F., and Mol, J. N. M. (1994). The flavonoid biosynthetic pathway in plants: Function and evolution. *BioEssays*, 16:123–132.

Koh, E., Wimalasiri, K. M. S., Chassy, A. W., and Mitchell, A. E. (2009). Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli. *J. Food Compos. Anal.*, 22:637–643.

Kopsell, D. A. and Kopsell, D. E. (2008). Genetic and environmental factors affecting plant lutein / zeaxanthin. *Agro Food Ind. Hi-Tech*, 19:44–46.

Kopsell, D. A. and Sams, C. E. (2013). Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *J. Am. Soc. Hortic. Sci.*, 138:31–37.

Koyama, K., Ikeda, H., Poudel, P. R., and Goto-Yamamoto, N. (2012). Light quality affects flavonoid biosynthesis in young berries of cabernet sauvignon grape. *Phytochemistry*, 78:54–64.

Kristal, A. R. and Lampe, J. W. (2009). *Brassica* vegetables and prostate cancer risk: A review of the epidemiological evidence. *Nutr. Cancer*, 42:1–9.

Kroon, P. and Williamson, G. (2005). Polyphenols: dietary components with established benefits to health? *J. Sci. Food Agric.*, 85:1239–1240.

Kurilich, A. C., Tsau, G. J., Brown, A., Howard, L., Klein, B. P., Jeffery, E. H., Kushad, M., Wallig, M. A., and Juvik, J. A. (1999). Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *J. Agric. Food Chem.*, 47:1576–1581.

Kushad, M. M., Brown, A. F., Kurilich, A. C., Juvik, J. A., Klein, B. P., Wallig, M. A., and Jeffery, E. H. (1999). Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *J. Agric. Food Chem.*, 47:1541–1548.

Lätti, A. K., Jaakola, L., Riihinen, K. R., and Kainulainen, P. S. (2010). Anthocyanin and flavonol variation in bog bilberries (*Vaccinium uliginosum* L.) in Finland. *J. Agric. Food Chem.*, 58:427–433.

Lee, S. K. and Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Tec.*, 20:207–220.

Lefsrud, M. G., Kopsell, D. A., Kopsell, D. E., and Curran-Celentano, J. (2005). Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. *HortScience*, 40:2026–2030.

Levitt, J. (1980). *Responses of plants to environmental stresses, Vol. 1.* New York: Academic press.

Li, Q. and Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.*, 67:59–64.

Lin, K.-H., Huang, M.-Y., nd Ming-Huang Hsu, W.-D. H., Yang, Z.-W., and Yang, C.-M. (2013). The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*). *Sci. Hortic.*, 150:86–91.

Lo Scalzo, R., Bianchi, G., Genna, A., and Summa, C. (2007). Antioxidant properties and lipidic profile as quality indexes of cauliflower (*Brassica oleracea* L. var. *botrytis*) in relation to harvest time. *Food Chem.*, 100:1019–1025.

Luo, Q. (2011). Temperature thresholds and crop production: a review. *Climate Change*, 109:538–598.

MacLeod, A. J. and Nussbaum, M. L. (1977). The effects of different horticultural practices on the chemical flavour composition of some cabbage cultivars. *Phytochemistry*, 16:861–865.

Mahmud, T. M. M., Atherton, J. G., Wright, C. J., Ramlan, M. F., and Ahmad, S. H. (1999). Pak Choi (*Brassica rapa* ssp Chinensis L) quality response to pre-harvest salinity and temperature. *J. Sci. Food Agric.*, 79:1698–1702.

Mao, Z., Fu, H., and Wan, C. (2012). Effect of temperature on fatty acid content in *Vicia sativa*. *J. Verbr. Lebensm.*, 7:133–135.

Mastropasqua, L., Borraccino, G., Bianco, L., and Paciolla, C. (2012). Light qualities and dose influence ascorbate pool size in detached oat leaves. *Phytochemistry*, 183:57–64.

Mathews, S. (2006). Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. *Mol. Ecol.*, 15:3483–3503.

Millar, A. A., Smith, M. A., and Kunst, L. (2000). All fatty acids are not equal: discrimination in plant membrane lipids. *Trends Plant. Sci.*, 5:95–101.

Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S., and Johnson, I. T. (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *J. Sci. Food Agric.*, 80:967–984.

Moussaoui, K. A. and Varela, P. (2010). Exploring consumer product profiling techniques and their linkage to a quantitative descriptive analysis. *Food Qual. Prefer.*, 21:1088–1099.

Mozafar, A. (1994). *Plant vitamins: Agronomical, physiological and nutritional aspects.* CRC Press.

Mozaffarian, D., Stein, P. K., Prineas, R. J., and Siscovick, D. S. (2008). Dietary fish and omega-3 fatty acid consumption and heart rate variability in us adults. *Circulation*, 117:1130–1137.

National Research Council (1982). Inhibitors of carcinogenesis. In *Diet, Nutrition, and Cancer*, page 358. National Academy Press.

Nayga, R. M., Tepper, B. J., and Rosenzweig, L. (1999). Assessing the importance of health and nutrition related factors on food demand: a variable preference investigation. *Appl. Econ.*, 31:1541–1549.

Neugart, S., Kläring, H.-P., Zietz, M., Schreiner, M., Rohn, S., Kroh, L. W., and Krumbein, A. (2012). The effect of temperature and radiation on flavonol aglycones and flavonol glycosides of kale (*Brassica oleracea* var. *sabellica*). *Food Chem.*, 133:1456–1465.

Oh, M.-M., Trick, H., and Rajashekar, C. B. (2009). Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. *J. Plant Physiol.*, 166:108–191.

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., and Kurata, K. (2007). Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environ. Control Biol.*, 45:189–198.

Padilla, G., Cartea, M. E., Velasco, P., de Haro, A., and Ordás, A. (2007). Variation of glucosinolates in vegetable crops of *Brassica rapa*. *Phytochemistry*, 68:536–545.

Parr, A. J. and Bolwell, G. P. (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.*, 80:985–1012.

Pék, Z., Daood, H., Nagyné, M. G., Berki, M., Tóthné, M. M., Neményi, A., and Helyes, L. (2012). Yield and phytochemical compounds of broccoli as affected by temperature, irrigation, and foliar sulfur supplementation. *HortScience*, 47:1646–1652.

Pereira, F. M. V., Rosa, E., Fahey, J. W., Stephenson, K., Carvalho, R., and Aires, A. (2002). Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* var. *italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. *J. Agric. Food Chem.*, 50:6239–6244.

Pérez-Balibrea, S., Moreno, D. A., and García-Viguera, C. (2008). Influence of light on health-promoting phytochemicals of broccoli sprouts. *J. Sci. Food Agric.*, 88:904–910.

Pollard, J., Kirk, S. F. L., and Cade, J. E. (2002). Factors affecting food choice in relation to fruit and vegetable intake: a review. *Nutr. Res. Rev.*, 15:373–387.

Pruneda-Paz, J. L. and Kay, S. A. (2010). An expanding universe of circadian networks in higher plants. *Trends Plant. Sci.*, 15:259–265.

Quail, P. H. (2002). Photosensory perception and signalling in plant cells: new paradigms? *Nat. Rev. Mol. Cell Biol.*, 3:85–93.

Rangavajhyala, N., Ghorpade, V. M., and Kadam, S. S. (1998). Broccoli. In Salunkhe, D. K. and Kadam, S. S., editors, *Handbook of vegetable science and* *technology: Production, composition, storage and processing*, pages 337–358. New York : Marcel Dekker.

Redovnikovic, I. R., Glivetic, T., Delonga, K., and Vorkapic-Furac, J. (2008). Glucosinolates and their potential role in plant. *Period. Biol.*, 110:297–309.

Riihimäki, M. and Savolainen, O. (2004). Environmental and genetic effects on flowering differences between northern and southern populations of *Arabidopsis lyrata* (*Brassicaceae*). *Am. J. Bot.*, 91:1036–45.

Rosa, E., David, M., and Gomes, M. H. (2001). Glucose, fructose and sucrose content in broccoli, white cabbage and Portuguese cabbage grown in early and late seasons. *J. Sci. Food Agric.*, 81:1145–1149.

Rosa, E. A. S., Heaney, G. K., Fenwick, G. R., and Portas, C. (1997). Glucosinolates in crop plants. *Hortic. Rev.*, 19:99–215.

Rosa, E. A. S., Heaney, R. K., Portas, C. A. M., and Fenwick, G. R. (1996). Changes in glucosinolate concentrations in brassica crops (*B. oleracea* and *B. napus*) throughout growing seasons. *J. Sci. Food Agric.*, 71:237–244.

Rosa, E. A. S. and Rodrigues, P. M. F. (1998). The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. *J. Sci. Food Agric.*, 78:208–212.

Rosenfeld, H. J., Risvik, E., Samuelsen, R. T., and Rødbotten, M. (1997). Sensory profiling of carrots from northern latitudes. *Food Res. Int.*, 30:593–601.

Sasaki, H., Ichimura, K., and Oda, M. (1996). Changes in sugar content during cold acclimation and deacclimation of cabbage seedlings. *Ann. Bot.-London*, 78:365–369.

Savitch, L. V., Harney, T., and Huner, N. P. A. (2000). Sucrose metabolism in spring and winter wheat in response to high irradiance, cold stress and cold acclimation. *Physiol. Plantarum*, 108:270–278.

Schmidt, S., Zietz, M., Schreiner, M., Rohn, S., Kroh, L. W., and Krumbein, A. (2010). Genotypic and climatic influences on the concentration and composition of flavonoids in kale (*Brassica oleracea* var. *sabellica*). *Food Chem.*, 119:1293–1299.

Schonhof, I., Kläring, H.-P., Krumbein, A., Claußen, W., and Schreiner, M. (2007). Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. *Agr. Ecosyst. Environ.*, 119:103–111.

Schonhof, I., Krumbein, A., and Bruckner, B. (2004). Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. *Nahrung.*, 48:25–33.

Smirnoff, N. and Pallanca, J. E. (1996). Ascorbate metabolism in relation to oxidative stress. *Biochem. Soc. T.*, 24:472–478.

Smirnoff, N. and Wheeler, G. L. (2000). Ascorbic acid in plants: Biosynthesis and function. *Crit. Rev. Plant Sci.*, 19:267–290.

Sønderby, I. E., Geu-Flores, F., and Halkier, B. A. (2010). Biosynthesis of glucosinolates - gene discovery and beyond. *Trends Plant. Sci.*, 15:283–290.

Taiz, L. and Zeiger, E. (2002). *Plant physiology.* Sinauer Associates Inc. Publishers.

Talalay, P., Fahey, J. W., Holtzclaw, W. D., Prestera, T., and Zhang, Y. (1995). Chemoprotection against cancer by phase 2 enzyme induction. *Toxicol. Lett.*, 82-83:173–179.

Tan, D. K. Y., Birch, C., Wearing, A. H., and Rickert, K. G. (2000). Predicting broccoli development I. development is predominantly determined by temperature rather than photoperiod. *Sci. Hortic.*, 84:227–243.

Tan, D. K. Y., Wearing, A. H., Rickert, K. G., Birch, C. J., and Joyce, D. C. (1999). Freeze-induced reduction of broccoli yield and quality. *Aust. J. Exp. Agri.*, 39:771–780.

Thain, S. C., Murtas, G., Lynn, J. R., McGrath, R. B., and Millar, A. J. (2002). The circadian clock that controls gene expression in Arabidopsis is tissue specific. *Plant Physiol.*, 130:102–110.

Treutter, D. (2005). Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Breeding*, 7:581–591.

Urzica, E. I., Adler, L. N., Page, M. D., Linster, C. L., Arbing, M. A., Casero, D., Pellegrini, M., Merchant, S. S., and Clarke, S. G. (2012). Impact of oxidative stress on ascorbate biosynthesis in chlamydomonas via regulation of the VTC2 gene encoding a GDP-L-galactose phosphorylase. *J. Biol. Chem.*, 287:14234–14245.

Vallejo, F., s Barbeá n, F. A. T., and a Viguera, C. G. (2002). Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. *J. Sci. Food Agric.*, 82:1293–1297.

Vallejo, F., Tomás-Barberán, F. A., Benavente-García, A. G., and García-Viguera, C. (2003a). Total and individual glucosinolate contents in inflorescences of eight broccoli cultivars grown under various climatic and fertilisation conditions. *J. Sci. Food Agric.*, 83:307–313.

Vallejo, F., Tomas-Barberan, F. A., and Garcia-Viguera, C. (2003b). Effect of climatic and sulphur fertilisation conditions,on phenolic compounds and vitamin C, in the inflorescences of eight broccoli cultivars. *Eur. Food. Res. Technol.*, 216:395–241.

Varela, P. and Ares, G. (2012). Sensory profiling, the blurred line between sensory and consumer science. a review of novel methods for product characterization. *Food Res. Int.*, 48:893–908.

Vaughan, J. G. and Geissler, C. A. (1997). *The new Oxford book of food plants.* Oxford University press.

Velasco, P., Cartea, M. E., González, C., Vilar, M., and Ordás, A. (2007). Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem.*, 55:955–962.

Velez-Ramirez, A. I., van Ieperen, W., Vreugdenhi, D., and Millenaar, F. F. (2011). Plants under continuous light. *Trends Plant. Sci.*, 16:311–318.

Weerakkody, W. A. P. (2003). Nutritional value of fresh leafy vegetables as affected by pre-harvest factors. *Acta Hortic.*, 604:511–515.

Werteker, M., Lorenz, A., Johannes, H., Berghofer, E., and Findlay, C. S. (2010). Environmental and varietal influences on the fatty acid composition of rapeseed, soybeans and sunflowers. *J. Agron. Crop Sci.*, 196:20–27.

White, S. W., Zheng, J., Zhang, Y.-M., and Rock, C. O. (2005). The structural-biology of type ii fatty acid biosynthesis. *Annu. Rev. Biochem.*, 74:791–831.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Curr. Opin. Plant. Biol.*, 5:218–223.

Yen, G.-C. and Wei, Q.-K. (1993). Myrosinase activity and total glucosinolate content of cruciferous vegetables, and some properties of cabbage myrosinase in Taiwan. *J. Sci. Food Agric.*, 61:471–475.

Zheng, J., Kallio, H., and Yang, B. (2009a). Effects of latitude and weather conditions on sugars, fruit acids and ascorbic acid in currant (*Ribes* sp.) cultivars. *J. Sci. Food Agric.*, 89:2011–2023.

Zheng, J., Yang, B., Ruusunen, V., Laaksonen, O., Tahvonen, R., Hellsten, J., and Kallio, H. (2012a). Compositional differences of phenolic compounds between black currant (*Ribes nigrum* L.) cultivars and their response to latitude and weather conditions. *J. Agric. Food Chem.*, 60:6581–6593.

Zheng, J., Yang, B., Toumasjukka, S., Ou, S., and Kallio, H. (2009b). 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.*, 57:2977–2987.

Zheng, J., Yang, B., Trépanier, M., and Kallio, H. (2012b). 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.*, 60:3190–3189.

Paper 1

Paper 2

Paper 3

Paper 4

Paper 5