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Influence of Day Length and Temperature on the Content of Health-Related Compounds in Broccoli (*Brassica oleracea* L. var. *italica*)

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ABSTRACT: Vegetables grown at different latitudes are exposed to various temperatures and day lengths, which can affect the content of health- and sensory-related compounds in broccoli florets. A 2 × 2 factorial experiment was conducted under controlled growth conditions, with contrasting temperatures (15/9 and 21/15 °C) and day lengths (12 and 24 h), to investigate the effect on glucosinolates, vitamin C, flavonols, and soluble sugars. Aliphatic glucosinolates, quercetin, and kaempferol were at their highest levels at high temperatures combined with a 12 h day. Levels of total glucosinolates, D-glucose, and D-fructose were elevated by high temperatures. Conversely, the content of vitamin C was highest with a 12 h day length combined with 15/9 °C. Our results indicate that temperature and day length influence the contents of health-related compounds in broccoli florets in a complex way, suggesting no general superiority of any of the contrasting growth conditions.

KEYWORDS: *Brassica oleracea L. var. italica, broccoli, glucosinolates, flavonols, soluble sugars, vitamin C, photoperiod, temperature, day length*

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica*) is an important vegetable known for its high nutritional content. Studies have indicated that a high rate of intake of *Brassica* vegetables may reduce the risk of developing degenerative diseases such as cancer and cardiovascular diseases.[1,2] Several constituents found in this group of foods can contribute to positive health effects, including vitamins, minerals, carotenoids, glucosinolates, and phenols.[3,4] Because of the growing body of evidence of potential health benefits, consumers are interested in food that can provide bioactive compounds to prevent or delay chronic diseases and thereby improve their health.[5]

Broccoli is being grown at a wide range of latitudes, 70° N being the northernmost limit in Scandinavia. The climate conditions in the growth season at high latitudes, above the Arctic Circle, are characterized by low temperatures and light for up to 24 h. Despite the 24 h light, these areas have a distinct night period with reduced radiation and lower temperatures. In June and July (Tromsø 70° N), only approximately 10−20% of the total daily solar radiation is received from 6 p.m. until 6 a.m. and the average day and night temperatures are close to 15 and 9 °C, respectively.[6] At low latitudes (around 40° N), these vegetables are grown in the cooler seasons combined with a short day length. Previous studies have shown that certain fruits and vegetables grown at high latitudes develop different quality attributes, like taste differences for several attributes, than when grown further south.[7−10] More specifically, these studies have shown that high latitudes are associated with sweeter taste in carrots (*Daucus carota* L.),[7] a higher content of vitamin C, and a lower content of carotenoids and phenolic compounds.[8−10] Environmental factors, such as temperature and day length, influence plant growth and affect the global metabolite content and composition, including those associated with health benefits. The expression of genes regulating the biosynthesis of these metabolites is found to vary in response to different internal and external factors, including environmental factors.[11−13]

Glucosinolates make up a group of sulfur-containing secondary metabolites found in cruciferous plants that are involved in defense against herbivores and pathogens. They are synthesized from amino acids, and a number of them are important precursors of specific flavor compounds of *Brassica* species.[14] Glucosinolates have limited bioactivity, but when hydrolyzed by myrosinase, the resultant isothiocyanates, thiocyanates, and nitriles exert significant bioactivities.[15] Some of these breakdown products are proposed to protect against carcinogenesis, mutagenesis, and other forms of toxicity of electrophiles and reactive forms of oxygen.[16] Studies conducted in different seasons, years, and locations have indicated higher glucosinolate concentrations at higher temperatures.[17−20] Glucosinolates are also found to be under circadian light regulation in *Arabidopsis*.[21]

Vitamin C is an essential vitamin for humans and consists of L-ascorbic acid and the oxidized L-dehydroascorbic acid. It is a major multifunctional metabolite in plants, protecting against oxidative damage, in addition to other roles such as the regulation of photosynthetic electron transport.[22] For humans, vitamin C seems to play a dual role: a small dose is required for normal growth and development, while a dose slightly larger than the requirement appears to provide antioxidant protection against a number of chronic diseases.[23] Flavonols are a group of flavonoids that are plant secondary metabolites found in fruits and vegetables. In plants, one of their functions is to protect the

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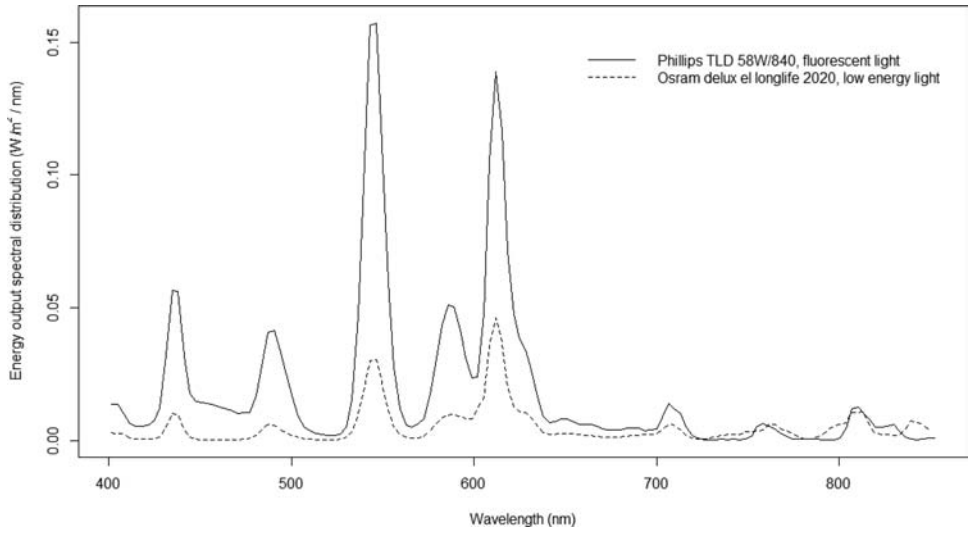

Figure 1. Wavelength range of the fluorescent light (model TLD 58W/840, Phillips), low-energy light bulbs (delux el longlife 2020, Osram, Munich, Germany) used in our experiment.

tissues from damage caused by radiation.[24] The two main flavonols found in *Brassica* vegetables are quercetin and kaempferol.[25] There is a well-documented and growing body of evidence that they may act as antioxidants or in mechanisms contributing to cardioprotective and anticarcinogenic effects in humans.[26] Both flavonols and ascorbic acid have antioxidant properties associated with protection against reactive oxygen species (ROS), levels of which can increase under stress conditions. Plants respond to higher concentrations of ROS by increasing the content of antioxidants.[27] Soluble sugars are energy-rich carbohydrates, and their content and type are key contributors to taste in vegetables, including *Brassica*.[28]

Compounds examined in this study have previously been studied in correlation with the growth season in different *Brassica* cultivars[29,30] and the influence of factors such as light and temperature.[11,31] The glucosinolate content of rapid-cycling *B. oleracea* exposed to variations in temperature, photoperiod, and light intensity showed that these conditions did have an effect, but the effect was dependent upon the part of the plant.[11] That study was conducted on roots, stems, and leaves, not florets. Schonhof et al.[31] performed an experiment on broccoli florets in a greenhouse on a range of compounds under different temperature and light intensity conditions related to climate change. They found that depending on the type of compound, an increased temperature combined with a low level of radiation in the autumn season could affect nutritional quality. However, to the best of our knowledge, there have not been any studies focusing on how the effect of contrasting temperature and day lengths interact and how this might explain the variations in content due to latitude conditions. Therefore, the main objective of this study is to determine the impact of contrasting day lengths and temperatures, and their interaction on the content of total and individual glucosinolates, the vitamin C components, flavonols, and soluble sugars in broccoli florets.

MATERIALS AND METHODS

Plant Material and Experimental Conditions. Contrasting climatic conditions were simulated in controlled climate growth chambers (Phytotron of the University of Tromsø). Diurnal temperature conditions were used to reflect different mean day and night temperatures, in combination with contrasting photoperiods found at high and low latitudes. Prior to experimental treatments, broccoli (*B. oleracea* L. var. *italica* cv. 'Lord') seeds were germinated (21 °C and light for 24 h) in light chambers, in a peat/perlite soil mixture (1:1). After 4 days, germinated seedlings were moved to 15 °C and light for 24 h. Three weeks after germinating, the plants were transferred to 12 L pots with a mixture of sand/compost and perlite (4:1 by volume). The pots were watered to 8.5 kg of total weight and fertilized with 12 g of a mineral nutrient [Yara, Fullgjødsel 11-5-18 (NPK)] in the upper 5 cm of the soil. In addition, the plants were refertilized with 3 g of calcium nitrate (Yara, Bor-Kalksalpeter, 15.4% N) every 6 week after the start of the treatment.

The treatments consisted of two levels of temperature [21/15 and 15/9 °C (day/night, respectively)] and two different day lengths (24 and 12 h), combined in a 2 × 2 factorial experimental design. There were four chambers with one treatment combination in each chamber, where the plants received only artificial light. In the short day treatment (12 h), a fluorescent light (model TLD 58W/840, Phillips, Eindhoven, The Netherlands) was used at an irradiance of 160−180 μmol m^{-2} s^{-1} (Figure 1). For long day treatments (24 h), the photoperiod was extended by an additional 12 h by using a low irradiance of 4−11 μmol m^{-2} s^{-1} (delux el longlife 2020, Osram, Munich, Germany) (Figure 1). The decrease in temperature at night occurred for 12 h and corresponded with a period of low-irradiance light. Within each treatment, 8 of 16 plants were selected for analysis. These plants had reached a standard fully developmental stage and a similar degree of maturity and uniformity between treatments. All plants within each treatment were harvested on the same day, and all plants were harvested at the same time of day (10 a.m., 2 h after light switch). Before the plants had been harvested, the plant height and head diameter were measured. The broccoli heads were cut off, including 5 cm of stem, and weighed. The florets, including 2 cm of stem, were cut off the main stem, frozen in liquid nitrogen, and coarsely ground with a mortar and pestle. Samples were vacuum-packed and stored at −80 °C. A portion of the ground plant material was freeze-dried and further ground to a very fine powder using a porcelain mortar and pestle. All compound analyses except that of vitamin C were performed on freeze-dried material.

Extraction of Glucosinolates and Flavonols. Approximately 0.200 ± 0.010 g of freeze-dried sample was weighed in a 15 mL tube, mixed with 4.50 mL of 70% (v/v) methanol (73 °C), and incubated (73 °C for 3 min). The internal standard, 0.100 mg of glucotropaeolin, was added, and the tubes were cooled to room temperature.

The tubes were centrifuged (4400 rpm) for 15 min at 20 °C. The supernatant was decanted into a new 15 mL tube; 70% methanol (3.00 mL, room temperature) was added, and the pellet was resuspended with a spatula and vortexed for approximately 15 s. The samples were

Table 1. Contents of Individual Glucosinolates in Broccoli Florets Grown at Different Temperatures and Day Lengths (Milligrams per Gram of Dry Matter ± Standard Error)[a]

temp (°C) (day/night)	day length (h)	glucoiberin	glucoraphanin	GB	4-OH-GB	4-Me-GB	Neo-GB	*n*
21/15	24	1.16 ± 0.08 b	9.8 ± 0.7 b	5.73 ± 0.28 ab	2.85 ± 0.19 b	1.89 ± 0.10 a	11.1 ± 1.1 a	7
21/15	12	1.67 ± 0.06 a	14.0 ± 0.5 a	4.94 ± 0.31 b	3.95 ± 0.12 a	1.54 ± 0.04 b	4.6 ± 0.4 b	7
15/9	24	1.03 ± 0.04 b	11.0 ± 0.5 b	6.43 ± 0.22 a	1.24 ± 0.04 c	1.03 ± 0.04 c	1.9 ± 0.2 c	8
15/9	12	0.97 ± 0.07 b	11.2 ± 0.6 b	6.59 ± 0.28 a	1.36 ± 0.06 c	1.11 ± 0.07 c	2.4 ± 1.2 bc	8

[a]Abbreviations: Neo-GB, neoglucobrassicin; GB, glucobrassicin; 4-Me-GB, 4-methoxyglucobrassicin; 4-OH-GB, 4-hydroxyglucobrassicin. Means with no common letter within columns are significantly different ($p \leq 0.05$).

Table 2. Percentage of Total Variation (Sum of Squares) Ascribed to Day Length, Temperature, and Temperature × Day Length Interaction on the Concentration of Different Glucosinolate, Ascorbic Acid, Flavonols, and Soluble Sugars in Broccoli Grown at Four Different Combinations of Temperature and Day Length

	day length (DL)		temperature (*T*)		DL × *T*		
compound[a]	% of variation	*p* value	% of variation	*p* value	% of variation	*p* value	R^2
sum GLS	0.2	(0.7)	60.1	(9.1×10^{-7})	1.4	(0.3)	0.62
aliphatic GLS	6.4	(6.7×10^{-4})	5.0	(0.1)	22.7	(1.4×10^{-3})	0.54
glucoiberin	13.9	(1.1×10^{-3})	40.6	(1.3×10^{-6})	18.5	(2.6×10^{-6})	0.73
glucoraphanin	28.1	(6.3×10^{-4})	2.9	(0.2)	20.6	(2.6×10^{-3})	0.52
indolic GLS	9.7	(3.1×10^{-3})	52.9	(4.2×10^{-8})	13.8	(6.2×10^{-4})	0.76
GB	3.1	(0.2)	36.9	(2.8×10^{-4})	6.1	(0.1)	0.46
4-OH-GB	7.6	(2.4×10^{-5})	80.5	(1.9×10^{-15})	4.5	(5.4×10^{-4})	0.93
4-Me-GB	3.8	(3.2×10^{-2})	69.4	(4.3×10^{-10})	7.2	(4.0×10^{-3})	0.81
Neo-GB	15.9	(3.5×10^{-5})	48.9	(3.2×10^{-9})	18.5	(1.2×10^{-5})	0.83
vitamin C	7.8	(1.7×10^{-2})	46.9	(1.6×10^{-6})	11.2	(6.0×10^{-3})	0.66
ascorbic acid	7.7	(1.5×10^{-2})	42.6	(2.0×10^{-6})	14.3	(2.7×10^{-3})	0.61
DHA	13.5	(0.1)	1.0	(0.6)	3.0	(0.4)	0.17
flavonols							
kaempferol	35.0	(6.5×10^{-5})	20.2	(1.3×10^{-3})	2.7	(0.2)	0.58
quercetin	29.4	(7.0×10^{-8})	53.5	(1.8×10^{-10})	2.3	(0.1)	0.85
soluble sugars	0.1	(0.7)	8.6	(0.2)	9.0	(0.1)	0.19
D-glucose	8.8	(0.1)	24.6	(7.7×10^{-3})	10.6	(0.1)	0.44
D-fructose	0.5	(0.7)	21.6	(1.9×10^{-3})	10.3	(0.1)	0.32
sucrose	0.8	(0.7)	21.2	(2.8×10^{-2})	1.4	(0.6)	0.23

[a]Abbreviations: GLS, glucosinolates; Neo-GB, neoglucobrassicin; GB, glucobrassicin; 4-Me-GB, 4-methoxyglucobrassicin; 4-OH-GB, 4-hydroxyglucobrassicin; DHA, dehydroascorbic acid.

incubated for 10 min at room temperature and centrifuged again. The combined supernatants were used for analyses of flavonols and glucosinolates. Extractions and analyses were conducted on duplicate samples.

Determination of Glucosinolates. Glucosinolates were analyzed as native substances according to the method of Tian et al.[32] as applied by Volden et al.[33]

Extraction and Determination of Vitamin C. Vitamin C, defined as L-ascorbic acid (AA) and L-dehydroascorbic acid (DHA), was analyzed according to the method of Hagen et al.[34] with some modifications. Two grams of frozen powder of plant material was accurately weighed into frozen tubes and then temperature-equilibrated at −18 °C. The frozen powder was added to a centrifuge tube containing 10 mL of ice-cold 6% MPA with 2 mM EDTA. An additional 5 mL of ice-cold 6% MPA with 2 mM EDTA was added, and the mixture was immediately stirred and homogenized (Polytron PT 1200, Kinematica AG, Lucerne, Switzerland) at 29000 rpm for 30 s. Distilled water (2.7 mL) was added, and the content was mixed. The homogenate was filtered through folded cellulose filter paper into an Erlenmeyer flask standing on ice in the dark. The extracts were weighed before being filtered through a 0.45 μm Millex-HA filter. Each extract was analyzed by high-performance liquid chromatography before and after reduction using a mobile phase with 50 mM NaH_2PO_4, 5 mM DTMA, 1.25 mM EDTA, and 2% (v/v) acetonitrile. The DHA content was calculated as the difference between the ascorbic acid content in reduced and nonreduced extracts. All samples were prepared and analyzed in duplicate.

Determination of Quercetin and Kaempferol. Contents of quercetin and kaempferol were determined as aglycons. Acid hydrolysis of flavonol glycosides in methanol extracts was performed as described by Hagen et al.[34] with minor modifications. Quantification was done using external standards.

Extraction and Determination of Soluble Sugars. Powdered freeze-dried plant material (1.00 g) was suspended in distilled water (40 mL) for 1 h at room temperature, centrifuged, and filtered. Sucrose, D-glucose, and D-fructose were quantified by enzymatic analysis using a Boehringer Mannheim test kit (R-Biopharm AG, Darmstadt, Germany).

Statistical Analysis. In all the experiments, a general linear model was used to conduct the analysis of variance (ANOVA) combined with Tukey's test for individual comparisons of treatments. ANOVA was performed using the R environment (version 2.15.0, R Development Core Team 2010, R Foundation for Statistical Computing, Vienna, Austria) for all statistical analyses.

RESULTS AND DISCUSSION

Glucosinolates. Eleven different glucosinolates were identified in the analyzed broccoli florets. They belong to three different chemical classes: six aliphatic {glucoiberin (3-methylsulfinylpropyl GLS), glucoraphanin (4-methylsulfinylbutyl GLS), gluconapoleiferin (2-hydroxypent-4-enyl GLS), glucoalyssin [(*R*)-5-methylsulfinypentyl GLS], glucoerucin (4-methylthiopropyl GLS), and epiprogoitrin [2-(*S*)-2-hydroxy-3-

Table 3. Contents of Aliphatic, Indolic, and Total Glucosinolates in Broccoli Florets Grown at Different Temperatures and Day Lengths (Milligrams per Gram of Dry Matter ± Standard Error)[a]

temp (°C) (day/night)	day length (h)	aliphatic GLS (A)	indolic GLS (I)	total GLS	A:I	*n*
21/15	24	11.6 ± 0.8 b	21.6 ± 1.5 a	33.2 ± 1.9 a	0.5 b	7
21/15	12	16.4 ± 0.5 a	15.0 ± 0.8 b	31.5 ± 0.9 a	1.1 a	7
15/9	24	12.9 ± 0.6 b	10.6 ± 0.4 c	23.5 ± 0.8 b	1.2 a	8
15/9	12	12.9 ± 0.7 b	11.5 ± 0.5 bc	24.4 ± 1.2 b	1.1 a	8

[a]Means with no common letter within columns are significantly different ($p \leq 0.05$).

butenyl GLS]}, four indolic [glucobrassicin (indol-3-ylmethyl GLS), neoglucobrassicin (1-methoxyindol-3-ylmethyl GLS), 4-hydroxyglucobrassicin (4-hydroxyindol-3-ylmethyl GLS), and 4-metoxyglucobrassicin (4-methoxyindol-3-ylmethyl GLS)], and one aromatic gluconasturtiin (2-phenylethyl GLS). The total glucosinolate content was calculated as the sum of the individual glucosinolates.

The most predominant glucosinolates were the aliphatic compound glucoraphanin and the indolic compounds neoglucobrassicin and glucobrassicin (Table 1). Gluconapoleiferin, glucoalyssin, glucoerucin, epiprogoitrin, and gluconasturtiin were detected at very low concentrations (<1.0 mg/g of dry matter), and their individual contents were not analyzed statistically. All glucosinolates, except for glucoraphanin, were significantly ($p < 0.01$) affected by temperature, and all glucosinolates except 4-methoxyglucobrassicin and glucobrassicin were significantly affected by day length (Table 2). There was a significant temperature × day length interaction for all glucosinolates except for glucobrassicin. A high temperature in combination with a 12 h day resulted in the highest content of the total glucosinolates (Table 3). This was 41% higher than the lowest content found at low temperatures combined with a 24 h day. Temperature was the main factor causing the variation in the total glucosinolate content (Table 2). Higher total glucosinolate content due to higher temperature has previously been reported by Pereira et al.[35] They found the highest total glucosinolate content at 30 and 15 °C compared to two lower temperatures (22/15 and 18/15 °C) in leaves of broccoli sprouts. The same has been reported in rapid-cycling *B. oleracea*[11] and kale (*Brassica oleracea* var. *sabellica*).[36] However, most of the variation in our study was not found in the total glucosinolate content, but within the individual glucosinolates and the grouping of aliphatic and indolic glucosinolates (Table 1).

Our study indicates that the content of aliphatic glucosinolates in broccoli florets is affected by the day length and temperature treatments and the interaction between them. The content of aliphatic glucosinolates in broccoli has previously been reported to be less affected by environmental conditions than genotypic variation compared to that of indolic glucosinolates.[37] The most abundant aliphatic glucosinolate was glucoraphanin (Table 1). Both of the aliphatic glucosinolates, glucoraphanin and glucoiberin, had the highest content at high temperatures and a 12 h day length. The variation of glucoraphanin content was mainly caused by day length and temperature × day length interaction, while the glucoiberin content was mostly affected by temperature. Charron et al.[30] reported the glucoraphanin content to be temperature insensitive in the edible part of various *Brassica* cultivars. However, they observed increasing content with increasing day length. Long day length also increased the content of aliphatic glucosinolates in stems in rapid-cycling *B. oleracea*.[11] A study by Huseby et al.[21] on *Arabidopsis* shows that glucosinolate biosynthesis can occur under diurnal regulation. The content of glucosinolates was higher during light periods. The fact that the 24 h day length in this study led to lower glucosinolate content in broccoli could be an effect of continuous light without a dark period and thus without a circadian trigger. Continuous light conditions at high latitudes in the growth season could therefore cause a decrease in glucosinolate content. This effect was not detected at low temperatures in our study, which could imply that photoperiodic monitoring is temperature-dependent. Contrasting temperature response results were reported by Schonhof et al.[31] They reported an increasing content of aliphatic glucosinolates in broccoli with a decreasing temperature (≤12 °C). However, this was found in combination with an increased level of radiation and lower temperatures. Other studies have shown higher content of aliphatic glucosinolate with increasing temperatures (from 12 to 32 °C).[11] This may indicate that high and very low temperatures can increase the content of aliphatic glucosinolates.

The content of total indolic glucosinolate, neoglucobrassicin, 4-methoxyglucobrassicin, 4-hydroxyglucobrassicin, and glucobrassicin exhibited the greatest variation between treatments (Table 1). Previous studies have also shown the strongest environmental effect within this group.[19,37] Neoglucobrassicin and 4-methoxyglucobrassicin contents were greater with a 24 h day than with a 12 h day, while the opposite was found for 4-hydroxyglucobrassicin. All of the indolic glucosinolates except for glucobrassicin showed higher levels at high temperatures than at low temperatures with a 12 h day. The variation was mostly associated with temperature, but there was also a temperature × day length effect. This significant effect of temperature on indolic glucosinolates has been previously reported.[19,30,31] Charron et al.[30] found that indolic glucosinolates in different *Brassica* cultivars increased because of the increased temperature. A similar temperature effect was reported for 4-hydroxyglucobrassicin in broccoli sprouts.[35] Neoglucobrassicin was the glucosinolate most affected by day length and temperature treatments in our study. In the 24 h day treatments, the florets had a 6-fold higher content at high temperatures than at low temperatures. The photoperiodic response was detected only at the high temperatures. An increase due to temperature conditions has previously been reported in Chinese cabbage (*Brassica rapa* L. var. *pekinensis*).[19] Furthermore, Schonhof et al.[31] in a study of temperature and irradiance effects in broccoli reported that the highest content of indolic glucosinolates occurred at high temperatures combined with low levels of radiation. These results correspond to our results on indolic glucosinolates.

A number of studies have reported differences in glucosinolate content between seasons and years.[17,18,30] In these studies, it is more difficult to distinguish what is causing the effect. However, as in our study, a higher content of glucosinolates has generally been found in seasons and years

Table 4. Contents of Ascorbic Acid, Dehydroascorbic Acid, and Vitamin C in Broccoli Florets Grown at Different Day Lengths and Temperatures (Milligrams per 100 g of Fresh Weight ± Standard Error)[a]

temp (°C) (day/night)	day length (h)	AA	DHA	vitamin C (AA+DHA)	n
21/15	24	78.9 ± 2.5 b	6.1 ± 0.6 a	85.0 ± 2.3 b	8
21/15	12	78.2 ± 2.2 b	5.8 ± 1.0 a	84.0 ± 2.6 b	8
15/9	24	85.7 ± 2.8 b	7.2 ± 0.9 a	92.8 ± 3.2 b	7
15/9	12	99.1 ± 2.3 a	8.6 ± 1.2 a	107.7 ± 2.5 a	8

[a]Abbreviations: AA, ascorbic acid; DHA, dehydroascorbic acid. Means with no common letter within columns are significantly different ($p \leq 0.05$).

Table 5. Contents of Kaempferol and Quercetin in Broccoli Florets Grown at Different Day Lengths and Temperatures (Milligrams per 100 g of Dry Matter ± Standard Error)[a]

temp (°C) (day/night)	day length (h)	kaempferol	quercetin	sum	K:Q ratio	n
21/15	24	13.1 ± 1.6 b	10.6 ± 0.9 b	23.7 ± 2.3 b	1.2 b	8
21/15	12	21.8 ± 2.3 a	16.7 ± 0.8 a	38.5 ± 2.9 a	1.3 b	7
15/9	24	9.8 ± 0.5 b	5.5 ± 0.3 c	15.3 ± 0.7 c	1.8 a	8
15/9	12	14.7 ± 0.6 b	8.8 ± 0.4 b	23.5 ± 0.9 b	1.7 a	8

[a]Means with no common letter within columns are significantly different ($p \leq 0.05$).

with higher temperatures. Our study suggests that this may be caused by a combination of varying temperature and day length conditions. The content of glucosinolates has previously been found to vary between species and ecotypes, between and within individual plants, and depend upon developmental stage and plant organs.[14] The possible mechanisms behind this have been linked to plant growth and development and to the fact that there are numerous stages in the biosynthesis that could be sensitive to external growth conditions. Knowing that the aliphatic glucosinolates are synthesized from chain-elongated forms of amino acids in contrast to indolic and aromatic glucosinolates, Sønderby et al.[38] concluded that differences in response would be related to the properties and regulation of the various enzymes in biosynthesis. The biosynthetic pathway from tryptophan to indolic glucosinolates is less complex than that from methionine to aliphatic glucosinolates and is also found to be temperature-dependent.[39] In particular, the modifications by hydroxylation or methylation could be sensitive according to our results. This could explain the great differences between treatments for the content of neoglucobrassicin in our study but also the difference in the content of 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin. Our results in combination with previous findings indicate that a higher level of glucosinolates could be expected at low latitudes in the summer season associated with high temperatures and short day lengths. Continuous light, found at high latitudes, could cause a decrease in the level of the aliphatic glucosinolates. However, the effect of the environmental conditions such as temperature and day length variations could be expected to differ between the individual glucosinolates.

Vitamin C. The highest content of ascorbic acid and vitamin C was found in the treatment with low temperatures and 12 h days (Table 4). This level was ∼25% higher than the lowest content at high temperatures. At high temperatures, we found no difference between 24 and 12 h days. At low temperatures, the level for a 24 h day was significantly lower than that for a 12 h day. Most of the variation between treatments was explained by the temperature effect, although both day length and temperature × day length interaction had an effect (Table 2). The level of dehydroascorbic acid was also higher at low temperatures.

Previous studies have also found that the ascorbic acid content is higher at lower temperatures in broccoli,[31] in pak choi (*Brassica rapa* L. ssp. *chinensis*),[40] and in cauliflower (*Brassica oleracea* L. var. *botrytis*).[41] The increases in ascorbic acid content at lower temperatures have been linked to stress and the antioxidant ability of vitamin C. Stress can cause a higher level of production of ROS. Under normal growth conditions, the pool of ascorbate is low compared to that under stress conditions.[42] Light is not found to be essential for the biosynthesis of ascorbic acid, but the light intensity during growth has been reported to affect the amount of ascorbic acid formed.[12,43,44] Studies of *Ribes* sp. grown at different latitudes have shown both an insignificant content[10] and a significantly lower content at low latitudes[9] of ascorbic acid. The decrease in vitamin C content at 24 h days in our study could be due to a possible photoperiodic regulation of biosynthesis. The fact that this is not found at high temperatures could suggest that this regulation is temperature-dependent. Also, sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) had a lower concentration of ascorbic acid when it was grown at lower latitudes.[45] Our results support previous temperature findings on vitamin C content that the levels are expected to be highest at lower growth temperatures; however, 12 h days may also increase the content in combination with low temperatures. This could imply that the low latitude in the late autumn season could be favorable for high vitamin C content in broccoli florets.

Flavonols. The content of quercetin and kaempferol was higher at high temperatures and 12 h day lengths than with other treatments (Table 5). At 21/15 °C, the content of kaempferol was 66% higher at short compared to long day lengths, and correspondingly 58% higher for quercetin. At 15/9 °C, the content of quercetin was 62% higher at 12 h than at 24 h day lengths. For kaempferol, the day length did not have an effect at low temperatures. However, the sum of the two flavonols at 12 h day lengths was 54% (15/9 °C) and 62% higher (21/15 °C) compared to that at 24 h day lengths. Kaempferol was most affected by day length, while quercetin was most affected by temperature (Table 2).

Our results indicate that noncontinuous day length and high temperatures would be most favorable for high levels of quercetin and kaempferol in broccoli. A low temperature was found both to increase (<4.5 °C)[46] and to have no effect (9.6–0.3 °C)[47] on the content of total flavonols in kale, whereas a

Table 6. Contents of Soluble Sugars in Broccoli Florets Grown at Different Day Lengths and Temperatures (Grams per 100 g of Dry Matter ± Standard Error)[a]

temp (°C) (day/night)	day length (h)	D-glucose	D-fructose	sucrose	total sugars
21/15	24	11.07 ± 0.59 a	8.65 ± 0.55 a	2.03 ± 0.41 a	21.73 ± 0.98 a
21/15	12	9.22 ± 0.20 b	7.12 ± 0.27 b	3.15 ± 0.46 a	19.55 ± 0.53 a
15/9	24	9.67 ± 0.22 ab	8.17 ± 0.19 ab	2.43 ± 0.25 a	20.23 ± 0.63 a
15/9	12	9.28 ± 0.36 b	7.88 ± 0.34 ab	3.10 ± 0.35 a	20.28 ± 0.85 a

[a]Means with no common letter within columns are significantly different ($p \leq 0.05$); $n = 5$.

decease was detected from 30 to 10 °C in apples (*Malus domestica* Borkh.).[48] An increased flavonol concentration due to low temperatures in winter wheat (*Triticum aestivum* L.) has been connected to an increased level of ROS, as a cold acclimation mechanism.[49] The same was found in lettuce (*Lactuca sativa*) when it was exposed to environmental stresses like low and high temperatures and a high light intensity.[50] In the study presented here, the temperatures were not as low as in the studies by Schmidt et al.[47] and Neugart et al.[46] Reports have suggested that the levels may be regulated by the circadian clock,[51] and as for glucosinolates, the lower content at 24 h could be due to the lack of circadian rhythm. The content of flavonols in berries grown at low latitudes where the day length is shorter and the temperatures are higher was higher than that in berries at high latitudes.[52] These results suggest that flavonol content is species-dependent and could be expected to increase at extreme low and high temperatures. Likewise, a 24 h light period seems to reduce the content. However, at nonextreme treatments as in our study, higher temperatures would favor the highest content. Our results indicate that low latitudes in the spring and summer season could be favorable for high contents of flavonols compared to the low temperature and 24 h light as found at high latitudes.

Soluble Sugars. The total content of soluble sugars in the florets of fresh broccoli was not affected by the treatments. D-Glucose was the most abundant sugar (47−51% of total sugar, depending on treatment) followed by D-fructose and sucrose (Table 6). Temperature was the factor explaining most of the variation (Table 2). Levels of D-glucose and D-fructose were significantly ($p < 0.01$) higher at long than at 12 h day lengths at 21/15 °C but not at 15/9 °C. Sucrose content was unchanged. Our results for soluble sugars coincide with findings about berries grown at different latitudes.[9,10] Zheng et al.[10] found a higher content of sugars in currant cultivars grown in southern Finland compared to the northern regions. The same was found in sea buckthorn.[45] Rosa et al.[28] found higher levels of sugar in three varieties of *B. oleracea* grown in spring and summer season than in those grown in the summer and winter season. It could therefore be that higher temperatures within the range of normal growth temperatures could give higher sugar content and possibly influence the taste. However, this effect is not expected to be strong, so that the variation in soluble sugar content between high and low latitudes is not expected to be severe.

Hence, our study emphasizes the importance of evaluating temperature and day length simultaneously to reveal their complex influence on health-related compounds in broccoli and possibly also in other vegetables. Our data suggest that contrasting temperature and day length regimes found at high and low latitudes could be expected to cause variation in the content of health-related compounds in broccoli florets.

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ABBREVIATIONS USED

4-Me-GB, 4-methoxyglucobrassicin; 4-OH-GB, 4-hydroxyglucobrassicin; AA, L-ascorbic acid; DHA, L-dehydroascorbic acid; DTMA, *n*-dodecyltrimethylammonium chloride; EDTA, Na_2H_2-ethylenediaminetetraacetic acid; GB, glucobrassicin; GLS, glucosinolate; MPA, metaphosphoric acid; Neo-GB, neoglucobrassicin; ROS, reactive oxygen species

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