Food Chemistry 119 (2010) 1293–1299

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Genotypic and climatic influences on the concentration and composition of flavonoids in kale (*Brassica oleracea* var. *sabellica*)

Susanne Schmidt [a], Michaela Zietz [b], Monika Schreiner [a], Sascha Rohn [b], Lothar W. Kroh [b], Angelika Krumbein [a,*]

[a] Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V., Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany
[b] Institute of Food Technology and Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, 13355 Berlin, Germany

ARTICLE INFO

Article history:
Received 8 April 2009
Received in revised form 13 July 2009
Accepted 1 September 2009

Keywords:
Curly kale
Quercetin
Kaempferol
Isorhamnetin
Cultivar
Radiation
Temperature

ABSTRACT

The aim of this study was to determine the composition and concentration of flavonoid aglycones in kale, the dependence on genotype and their interaction with decreasing temperature and global radiation. Eight kale cultivars, comprising hybrid and traditional, old cultivars, were grown in a field experiment and harvested four times at 4-week intervals. The traditional, old cultivars in particular contained high concentrations of flavonoids. In all of the investigated cultivars, kaempferol was the main flavonoid aglycone, followed by quercetin and isorhamnetin, which was quantified in six of the eight cultivars. Furthermore, in six of the eight cultivars, the total concentration of flavonoids remained unchanged with decreasing temperature and global radiation. The quercetin concentration increased in five of these six cultivars, whereas the kaempferol concentration decreased. Interestingly, the quercetin-to-kaempferol ratio increased in all of the investigated cultivars, despite the fact that the radiation level decreased, suggesting that the impact of the decline in temperature could be responsible for this effect.

1. Introduction

Kale (*Brassica oleracea* var. *sabellica*), which is commonly cultivated in Central and Northern Europe and North America, belongs to the Brassicaceae family. As a leafy vegetable, kale has higher concentrations of flavonoids than other vegetables, such as onion, green bean, broccoli or leek (Hertog, Hollman, & Katan, 1992).

Due to their molecular structure, flavonoids have a health-promoting effect on humans. Flavonoids possess a variety of biological activities, which may contribute to protecting humans against chronic diseases (Gerhäuser, 2001; Knekt et al., 2002). Quercetin, a main aglycone in human nutrition, is a potent free radical scavenger due to the di-hydroxylated B-ring, unsaturation at the C-ring and a 4-oxo function at the C-ring (Williams, Spencer, & Rice-Evans, 2004), and is thus considered to protect humans against several types of cancer (Knekt et al., 2002) and cardiovascular diseases (Chu, Chang, & Hsu, 2000). In addition, kaempferol also revealed a strong antioxidant potential (Kim, Liu, Guo, & Meydani, 2006), and higher intakes resulted in a lower risk of coronary heart disease (Lin et al., 2007). It has been demonstrated recently that quercetin and kaempferol synergistically suppress cell proliferation in human gut cancer lines (Ackland, Van de Waarsenburg, & Jones, 2005). Furthermore, isorhamnetin revealed distinct vasodilator effects in animal models, suggesting vascular protective effects in human cardiovascular diseases (Ibarra et al., 2003). Quercetin, kaempferol and isorhamnetin were shown to have an anti-inflammatory effect on activated macrophages (Hämäläinen, Nieminen, Vuorela, Heinonen, & Moilanen, 2007).

Flavonoids consist of two aromatic rings and one heterocyclic ring containing an oxygen atom (Fig. 1). In plants, flavonoids are commonly present as glycosides, usually conjugated with glucose. The flavavone naringenin, which is the precursor of various flavonoid groups, e.g., flavonols, was formed by several enzymes in the flavonoid biosynthesis pathway including phenylalanine ammonium lyase, cinnamate-4-hydroxylase, chalcone synthase, and chalcone isomerase. Flavonols, e.g., monohydroxylated kaempferol, are synthesised by the introduction of a hydroxyl group and a double bond in the heterocyclic ring catalysed by flavanone 3-β-hydroxylase and flavonol synthase, respectively. The flavonol 3′-hydroxylase introduces a hydroxyl group at the 3′ position of the B-ring, resulting in the formation of di-hydroxylated quercetin. Isorhamnetin, the methylised form of quercetin, is formed by *O*-methyltransferase implementing a methyl group at the 3′ position (Edreva, 2005; Kim, Kim, Kim, Lee, & Ahn, 2008; Yonekura-Sakakibara et al., 2008).

Huang, Wang, Eaves, Shikany, and Pace (2007) found kaempferol to be the main flavonoid aglycone in curly kale (*B. oleracea* var.

* Corresponding author. Fax: +49 (0)33701 55391.
E-mail address: Krumbein@igzev.de (A. Krumbein).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.09.004

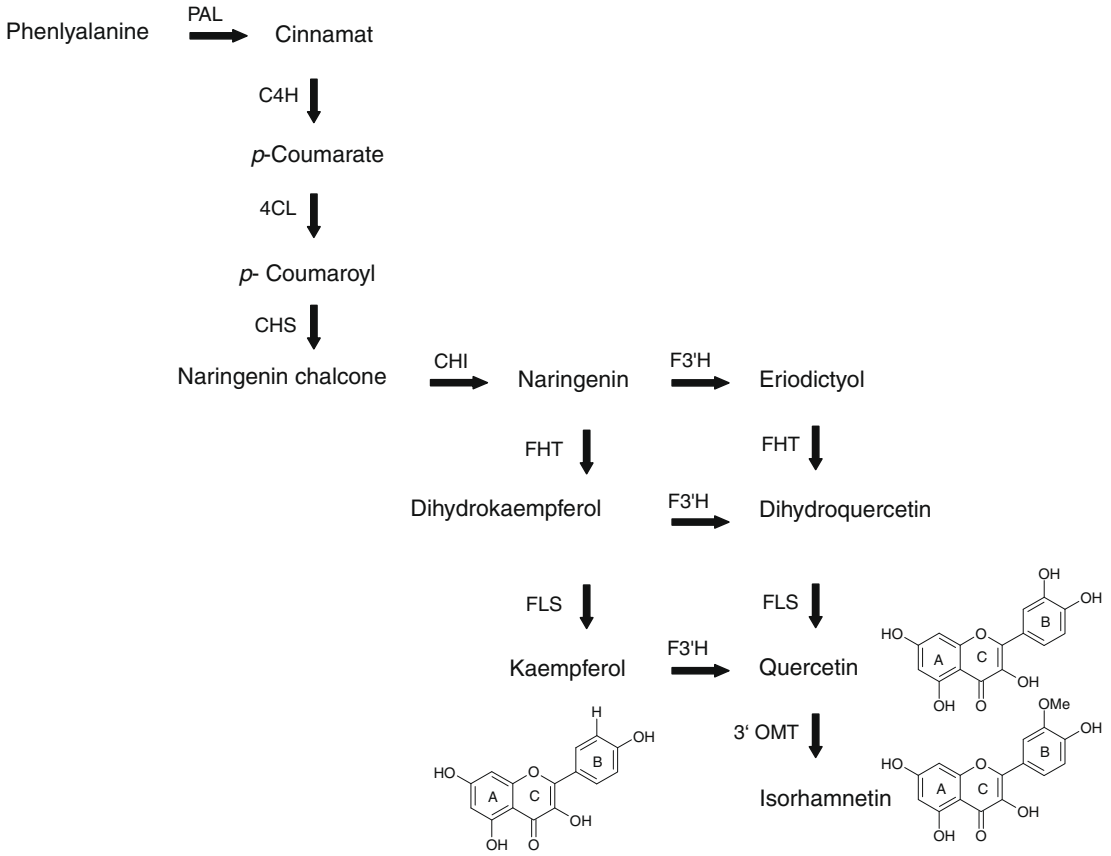

Fig. 1. Biosynthetic pathway and structure of kaempferol, quercetin and isorhamnetin (PAL, phenylalanine ammonia lyase; C4H, cinnamate4-hydroxylase; 4CL, 4-coumaroyl:CoA-ligase; CHS, chalcone synthase; CHI, chalcone isomerase; FHT, flavanone 3β-hydroxylase; F3′H, flavonoid 3′-hydoxylase; FLS, flavonol synthase; 3′OMT, 3′-*O*-methyltransferase).

acephala), followed by quercetin and isorhamnetin. Heimler, Vignolini, Dini, Vincieri, and Romani (2006) described kaempferol-3-[2-sinapoylglucopiranosyl (1,2) glucopiranoside]-7-[glucopiranosyl (1,4) glucopiranoside] and kaempferol-3-[2-feruloylglucopiranosyl (1,2) glucopiranoside]-7-[glucopiranosyl (1,4) glucopiranoside] and other kaempferol glycosides, as well as one quercetin glycoside, to be the main flavonoid glycosides.

Flavonoids can be influenced by the genetic potential. Different *Brassica* species and cultivars (cvs) usually show a similar flavonoid composition but quantitative differences in their flavonoid concentrations (Krumbein, Saeger-Fink, & Schonhof, 2007; Rochefort, Imsic, Jones, Trenerry, & Thomkins, 2006). Information about flavonoid concentrations in various kale cultivars (*B. oleracea* var. *sabellica*) is still rather lacking. Zhang et al. (2003), and more recently Murtaza et al. (2005), studied the genotype-induced effect in various kale cultivars. However, these results are only provide limited information since cultivar (cv) details were missing and local kale cultivars were used, respectively.

Furthermore, phenolic substances, such as flavonoids, can be influenced by temperature and radiation. Low temperatures are associated with higher concentrations of flavonoids due to the higher quantities of reactive oxygen species (ROS) (Klimov et al., 2008). At lower temperatures, the mRNA of phenylalanine ammonia lyase and chalcone synthase are enhanced or accumulated in maize seedlings and *Arabidopsis thaliana* (Christie, Alfenito, & Walbot, 1994; Leyva, Jarillo, Salinas, & Martinezzapater, 1995). Phenolic substances are responsible for protecting the plants from damage caused by radiation, in particular UV-B, which is part of global radiation (Edreva, 2005). Flavonoids act as shielding components, as they are strong scavengers of ROS (Edreva, 2005). Increasing global radiation induces the synthesis and accumulation of shielding components, such as flavonoids and hydroxycinnamic acids, especially in the epidermis of leaves (Edreva, 2005). With regard to radiation and polyphenols, many investigations have been carried out under enhanced UV-B radiation levels. Otherwise, climate simulations for Central and Northern Europe indicate in various scenario runs that, as a result of the greenhouse gases in the atmosphere, warming in these regions will be most noticeable in late autumn and winter, whereas radiation is still being degraded in this season (Raisanen et al., 2004). To our knowledge none of the literature deals with the climatic conditions of low temperature combined with low radiation for vegetables cultivated in Central Europe in the winter season.

To obtain a more detailed knowledge about flavonoid concentration and composition in kale, we investigated a highly various genotype pool including both hybrid cultivars and traditional, old cultivars. The aim of this study, therefore, was to determine the quality and quantity of flavonoid aglycones in kale, dependent on genotype to characterise the flavonoid profile and their interaction with the climatic factors temperature and global radiation to develop strategies for different cultivation conditions of kale cultivars with a high anti-oxidative potential dependent on climate changes.

2. Materials and methods

2.1. Plant material and experimental design

To analyse the influence of genotype, eight kale cultivars (F1 hybrids: 'Winterbor' (by Bruno Nebelung, Norken, Germany),

'Redbor' (by Chrestensen, Erfurt, Germany), 'Winnetou' (by Bruno Nebelung, Norken, Germany) and 'Arsis' (by Gärtner Pötschke, Kaarst, Germany); traditional, old cultivars: 'Altmärker Braunkohl' (with red and green coloured leaves) (by Dulcamara-Samen Carmzow-Wallmow, Germany), 'Halbhoher grüner Krauser' (by Chrestensen, Erfurt, Germany), and 'Lerchenzunge' (by Gärtner Pötschke, Kaarst, Germany) and the cultivar Frostara (by Bruno Nebelung, Norken, Germany)) were set in a randomised block design with three replicates on the experimental fields at the Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren and Erfurt e.V. (Grossbeeren, Germany). The sowing date for all of the kale plants in 2007 was 18 June; plants in the four-leaf stadium were transplanted into the field on 17 July. The spacing between plants was 0.4 × 0.5 m. Six fully developed plants of each cultivar were harvested four times per replication at 4-week intervals (23–25 October, 19–21 November, 17–19 December and 15–17 January). The daily mean temperature and daily mean global radiation were calculated from measurements taken every 2 and 5 min, respectively, throughout the 4-week prior to each harvest (October: 9.7 °C, 713 μmol/m^2 s; November 6.0 °C; 285 μmol/m^2 s; December: 3.8 °C, 179 μmol/m^2 s; January: 0.3 °C, 172 μmol/m^2 s).

2.2. Sample preparation

A mixture of leaves (without the middle rib) from six plants per replication was frozen (−40 °C), lyophilised and ground. Afterwards, samples were stored at room temperature until analysis. For analysis, 0.5 g of the lyophilised kale sample was hydrolysed with 50% aqueous methanol and 1.6 M HCl (40 ml of 62.5% aqueous methanol and 10 ml of 8 M HCl) in duplicate. After refluxing at 90 °C for 2 h, the extract was cooled down to room temperature, adjusted to 100 ml with 50% aqueous methanol and sonicated for 5 min. The extract was then filtered through a 0.45 μm PTFE filter for HPLC analysis (Krumbein et al., 2007).

2.3. HPLC-DAD-ESI-MS^2

Flavonoids were determined as aglycones after acid hydrolysis, using a modified HPLC-DAD-ESI-MS^2 method described by Krumbein et al. (2007). A HPLC series 1100 from Agilent (Waldbronn, Germany), consisting of a degaser, binary pump, autosampler, thermostat and a photodiode array detector was used to determine the composition and concentration of the flavonoid aglycones. The extracts were separated on a Prodigy (ODS 3, 150 × 3.0 mm, 5 μm, 100 Å) column (Phenomenex, Aschaffenburg, Germany) with a security guard C18 (ODS 3, 4 × 3.0 mm, 5 μm, 100 Å) at a temperature of 25 °C using a water/acetonitrile gradient. Solvent A consisted of 99.5% water and 0.5% acetic acid, whereas solvent B contained 100% acetonitrile. The following gradient was used: 30–35% B (5 min), 35–39% B (12 min), 39–90% B (5 min), 90% B isocratic (5 min), 90–30% B (5 min), and 30% B isocratic (5 min). The flow was performed using 0.3 ml/min, and the measured detector wavelength was 370 nm. The standards dihydroquercetin, kaempferol and isorhamnetin (Carl Roth GmbH, Karlsruhe, Germany) were used to obtain an external calibration curve in the range of 0.1–10 mg/100 ml. The total concentration of flavonoids was calculated as the sum of the concentration of the individual flavonoid aglycones quercetin, kaempferol and isorhamnetin.

Quercetin, kaempferol and isorhamnetin were identified as deprotonated molecular ions and characteristic mass fragment ions by HPLC-DAD-ESI-MS^2, using an Agilent series 1100 MSD (ion trap) with ESI as an ion source in negative ionisation mode. Nitrogen was used as the dry gas (12 l/min, 350 °C) and nebuliser gas (40 psi). Helium was used as the collision gas in the ion trap. The mass optimisation was performed for quercetin $[M-H]^-$ m/z 301.

2.4. Statistical analysis

For the analysis of variance (ANOVA), Tukey's Honest Significant Difference (HSD) test was used to calculate significant differences. All tests were performed at a significance level of $p \leqslant 0.05$. Calculations were performed using Statistica (version 6.1, Statsoft Inc., Tulsa, Okla).

3. Results and discussion

3.1. Influence of genotype on flavonoid concentration and composition

Eight kale cultivars harvested in October were investigated to determine the genotypic influence on flavonoid concentration and composition. The three flavonoid aglycones quercetin, kaempferol and isorhamnetin, which belong to the group of flavonols, were determined after acid hydrolysis. The flavonoid aglycones were identified by their deprotonated molecular ions m/z 301 for quercetin, m/z 285 for kaempferol and m/z 315 for isorhamnetin. The characteristic mass fragment ions in MS^2 were m/z 151 and 179 for quercetin, m/z 151 for kaempferol and m/z 300 for isorhamnetin. The sum of these flavonoid aglycones ranged between 6.0 and 14.8 mg/g dry matter (dm), which is related to 97.4–298.5 mg/100 g fresh matter (fm)) (Fig. 2). This genotypic variation reveals that traditional, old cvs Altmärker Braunkohl, Halbhoher grüner Krauser and Lerchenzunge are characterised by relatively high flavonoid concentrations, while lower flavonoid concentrations were found in the hybrids 'Arsis' and 'Winterbor', as well as in the cv Frostara. The main flavonoid in all tested kale cultivars in October was kaempferol, which varied from 3.8 to 9.1 mg/g dm (61.7–187.5 mg/100 g fm) (Fig. 3), followed by quercetin with concentrations from 0.9 to 4.0 mg/g dm (15.6–82.2 mg/100 g fm). Isorhamnetin varied from 0.6 to 3.0 mg/g dm (9.8–60.0 mg/100 g fm), but was not quantitatively detected in 'Winterbor' and 'Frostara'.

Murtaza et al. (2005) found total phenolic concentrations between 145 and 286 mg/100 g fm in local Indian kale cultivars, corresponding to the total flavonoid concentrations determined in our tested kale cultivars. Comparable concentrations to our results were also determined by Huang et al. (2007) in curly kale (*B. oleracea* var. *acephala*), with 90.5, 31.8 and 23.6 mg/100 g fm for kaempferol, quercetin and isorhamnetin, respectively. Furthermore, similar quercetin concentrations (7.7–24.4 mg/100 g fm) were detected in curly kale, but the kaempferol concentrations were much lower (21–47 mg/100 g fm) compared to our investigated cultivars, whereas isorhamnetin was not detected in these kale varieties (Hertog et al., 1992; Justesen, Knuthsen, & Leth, 1998; Zhang et al., 2003). Our investigations showed that isorhamnetin occurred in both traditional, old cultivars and hybrid cultivars, even if they were found in quantitatively non-detectable traces in 'Winterbor' and 'Frostara'. Furthermore, the flavonoid aglycones kaempferol, quercetin and isorhamnetin were assessed in all cultivars (Fig. 3), but the composition varied as Krumbein et al. (2007) and Rochefort et al. (2006) described in previous investigations for the *Brassica* species broccoli and pak choi, respectively.

3.2. Influence of the climatic factors temperature and global radiation on flavonoid concentration and composition

During the harvest period from October 2007 to January 2008, daily mean temperatures and daily mean global radiations decreased from 9.7 to 0.3 °C and from 713 to 172 μmol/m^2 s, respectively. In six cultivars ('Winterbor', 'Redbor', 'Frostara', 'Altmärker Braunkohl', 'Halbhoher grüner Krauser', and 'Lerchenzunge'), the

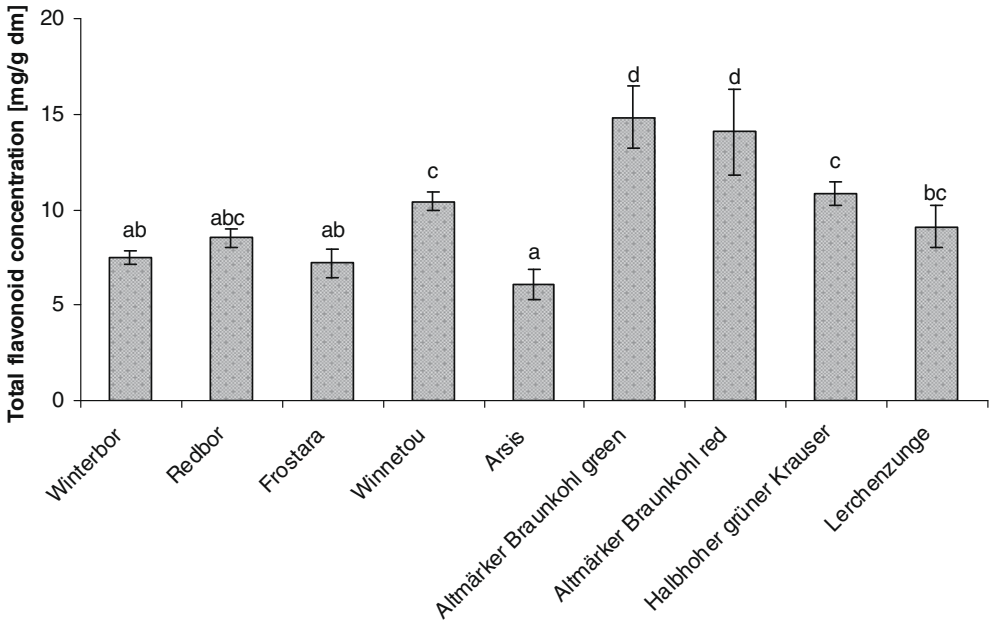

Fig. 2. Total flavonoid concentration in various genotypes of kale. Different letters indicate significant differences between cultivars (* green and red describes the leaf colour). Different letters indicate significant differences between cultivars for each flavonoid ($p \leqslant 0.05$ by Tukey's HSD test). Each value represents the mean of three replicates.

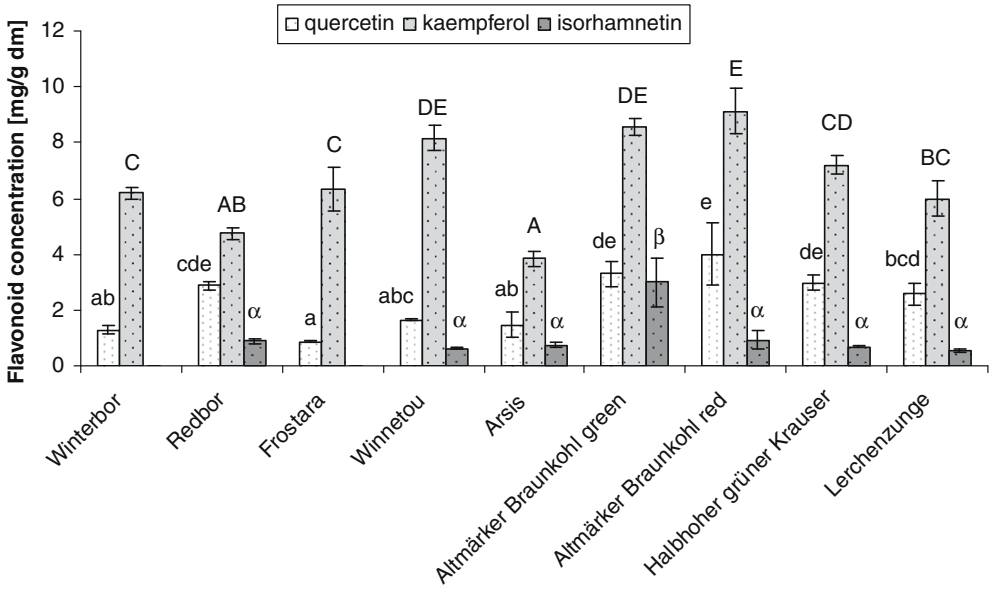

Fig. 3. Concentrations of the flavonoid aglycones quercetin, kaempferol and isorhamnetin in various genotypes of kale (* green and red describes the leaf colour). Different letters indicate significant differences between cultivars for each flavonoid ($p \leqslant 0.05$ by Tukey's HSD test). Each value represents the mean of three replicates.

total flavonoid concentration was unaffected by the different harvest times, and hence by varying climatic conditions, whereas 'Arsis' showed increasing flavonoid concentrations with decreasing temperature and radiation intensity (Table 1). In five of these cultivars ('Winterbor', 'Redbor', 'Altmärker Braunkohl' with green leaves, 'Halbhoher grüner Krauser', and 'Lerchenzunge'), quercetin was increased and kaempferol decreased, while temperature and global radiation declined. Furthermore, the isorhamnetin concentration increased in four of these five cultivars ('Winterbor', 'Redbor', 'Halbhoher grüner Krauser', and 'Lerchenzunge'). This effect is exemplarily shown for 'Winterbor' (Fig. 4). The kaempferol concentration did not change, although the quercetin concentration increased within a temperature range of between 9.7 and 3.8 °C combined with radiation intensities from 713 to 179 μmol/m^2 s (October–December). In contrast, the kaempferol concentration declined while the quercetin concentration remained constant and the isorhamnetin concentration increased in kale plants cultivated down to 0.3 °C and 172 μmol/m^2 s (January).

Due to an enhanced ROS level at lower temperatures during cultivation, an enhancement of the total flavonoids in winter wheat was determined (Klimov et al., 2008), as we found for quercetin in kale. The increase of epidermal located phenolic compounds, such as flavonoids, could also be due to a cold-induced biosynthesis based on the induction of phenylalanine ammonium lyase and chalcone synthase (Christie et al., 1994; Leyva et al., 1995). Enhanced quercetin concentrations also occurred in cold stressed

Table 1
Total flavonoid concentration (mg/g dm) in various kale genotypes dependent on climate conditions at different harvest times.

Cultivar	October 2007 9.7 °C 713 μmol/m² s	November 2007 6.0 °C 285 μmol/m² s	December 2007 3.8 °C 179 μmol/m² s	January 2008 0.3 °C 172 μmol/m² s
Winterbor	7.49 ± 0.37a	8.26 ± 0.29a	8.07 ± 0.79a	8.13 ± 0.51a
Redbor	8.51 ± 0.46a	7.39 ± 1.16a	8.89 ± 0.68a	8.02 ± 0.53a
Frostara	7.18 ± 0.79a	7.26 ± 1.08a	7.80 ± 0.84a	7.49 ± 0.56a
Winnetou	10.43 ± 0.52b	8.66 ± 0.99a	10.43 ± 0.45b	10.21 ± 0.64ab
Arsis	6.05 ± 0.80ab	5.87 ± 0.49a	7.23 ± 0.73b	8.26 ± 0.53c
Altmärker Braunkohl (green leaves)	14.84 ± 1.61a	14.47 ± 3.30a	18.53 ± 4.32a	16.83 ± 2.92a
Altmärker Braunkohl (red leaves)	14.06 ± 2.24a	13.11 ± 2.67a	15.82 ± 0.96a	14.46 ± 1.32a
Halbhoher grüner Krauser	10.86 ± 0.63a	9.47 ± 0.90a	10.90 ± 1.68a	9.92 ± 1.05a
Lerchenzunge	9.11 ± 1.07a	9.31 ± 1.23a	9.33 ± 1.37a	10.09 ± 0.27a

Different letters indicate significant differences between harvest times for each cultivar ($p \leqslant 0.05$ by Tukey's HSD test). Each value represents the mean of three replicates.

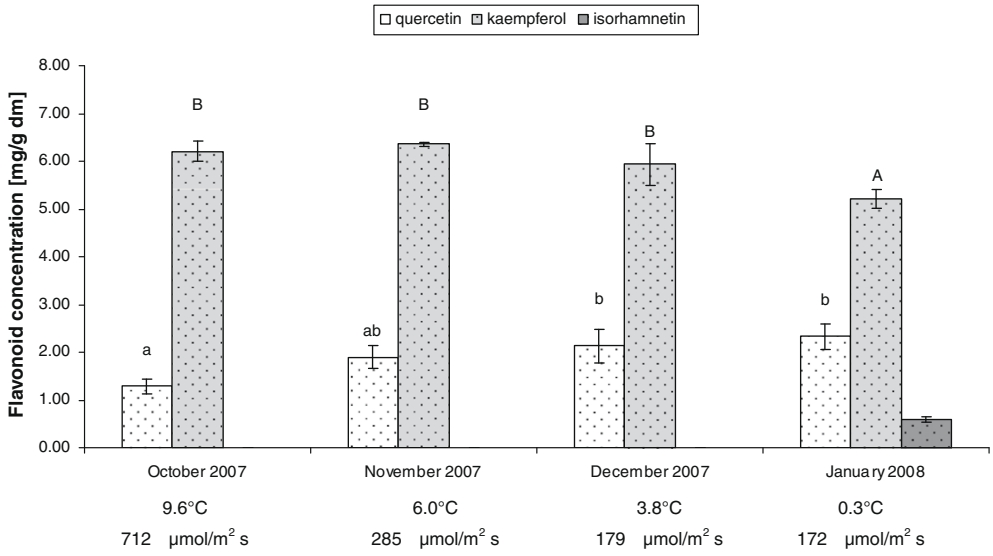

Fig. 4. Concentrations of the flavonoid aglycones quercetin, kaempferol and isorhamnetin in the kale cv Winterbor, dependent on climate conditions at different harvest times. Different letters indicate significant differences between harvest times for each flavonoid aglycone ($p \leqslant 0.05$ by Tukey's HSD test). Each value represents the mean of three replicates.

buckwheat plants (Suzuki, Honda, & Mukasa, 2005). These results indicate that quercetin, as a dihydroxyflavonoid, is a stronger antioxidant, after a reversible redox transition to a quinone, in contrast to kaempferol (Edreva, 2005). In contrast, 'Granny Smith' apples showed a lower quercetin concentration grown at 4 °C compared to those grown at 20 °C (Reay, 1999). Otherwise, kaempferol decreased in our results while temperatures decreased, but to our knowledge there is no literature that explains the effect of low temperatures on kaempferol concentration.

Exposure to global radiation enhanced the synthesis and accumulation of shielding compounds, such as flavonoids, in the adaxial epidermis of fully developed leaves (Edreva, 2005). Tsormpatsidis et al. (2008) demonstrated that Lollo Rosso lettuce 'Revolution' had lower concentrations of total flavonoids in June compared to July, dependent on the higher radiation levels in July. Accordingly, in our experiment we expected lower concentrations of flavonoids in low light for January (172 μmol/m² s) than in October (713 μmol/m² s), which was only found for kaempferol but not for quercetin or isorhamnetin, which increased. Furthermore, the total concentration of flavonoids in the investigated kale cultivars remained unchanged while global radiation decreased. In white mustard (*Sinapis alba*) Reifenrath and Müller (2007) assessed a sevenfold increase in quercetin and a doubling of kaempferol under global radiation compared to the exclusion of UV radiation under greenhouse conditions. In leaves of bilberry exposed to sunlight, the expression of genes encoding phenylalanine ammonium lyase, chalcone synthase and flavanone 3-β-hydroxylase was enhanced causing flavonol (quercetin and kaempferol) concentrations to increase (Jaakola, Maatta-Riihinen, Kärenlampi, & Hohtola, 2004). In contrast, in our experiment decreasing global radiation, and hence also decreasing UV radiation, resulted in higher quercetin and isorhamnetin concentrations. The kaempferol concentration fell, as expected. In leaves of the perennial, woody species *Ligustrum vulgare* and *Phillyrea latifolia*, the phenylalanine ammonium lyase activity was distinctly higher at 100% sunlight exposure, compared to 12% sunlight exposure, indirectly causing an increased concentration of *ortho*-di-hydroxylated B-ring flavonoid glycosides, such as quercetin-3-*O*-rutinoside and luteolin-7-*O*-glucosid after the application of full sunlight; in contrast, the concentration of apigenin-7-*O*-glucoside, an *ortho*-mono-hydroxylated flavonone glycoside comparable to kaempferol, showed a decreasing concentration at 100% sunlight (Tattini et al., 2005), suggesting that quercetin and kaempferol potentially differ in their response to radiation at low radiation, as in our experiment. However, in our experiment decreased global radiation did not result in a decreased quercetin concentration but in a decreased kaempferol concentration. In soybean, however, global radiation enhanced the concentrations of all of the assessed quercetin glycosides compare radiation to excluding UV radiation, whereas the kaempferol-based glycosides did not change, and only one of four isorhamnetin

Table 2
Quercetin-to-kaempferol ratio in various kale genotypes, dependent on climate conditions at different harvest times.

Cultivar	October 2007 9.7 °C 713 μmol/m^2 s	November 2007 6.0 °C 285 μmol/m^2 s	December 2007 3.8 °C 179 μmol/m^2 s	January 2008 0.3 °C 172 μmol/m^2 s
Winterbor	0.21	0.30	0.36	0.45
Redbor	0.61	0.68	1.02	1.19
Frostara	0.14	0.16	0.17	0.22
Winnetou	0.20	0.23	0.38	0.43
Arsis	0.38	0.47	0.64	0.81
Altmärker Braunkohl (green leaves)	0.39	0.65	0.94	1.37
Altmärker Braunkohl (red leaves)	0.44	0.53	0.52	0.62
Halbhoher grüner Krauser	0.41	0.56	0.82	0.81
Lerchenzunge	0.43	0.61	0.92	1.13

Each value represents the mean of three replicates.

glycosides increased under the global radiation level (Winter & Rostas, 2008). These results indicate that quercetin was more sensitive to radiation than kaempferol. In our experiments, isorhamnetin showed a corresponding response to the radiation level as quercetin. Interestingly, in our experiment the quercetin-to-kaempferol ratio in all of the investigated cultivars increased as quercetin increased and kaempferol decreased, with a prolonging cultivation duration corresponding to the reduction of the radiation and temperature levels (Table 2). Accompanied with the lowest temperature and global radiation levels (0.3 °C and 172 μmol/m^2 s in January) in the cvs. Redbor, Altmärker Braunkohl with green coloured leaves and Lerchenzunge, quercetin became the predominant flavonoid aglycone, as indicated by a ratio value $\geqslant 1$; for 'Redbor' this was already determined at 3.8 °C and 179 μmol/m^2 s (December). In *Petunia* wild-type and transgenic-lines, an approximately 80% increase in the quercetin-to-kaempferol ratio between plants grown without UV-B and plants with UV-B 25% above the ambient global radiation level was determined due to an increasing expression of the gene-encoding flavonol 3′-hydroxylase (Ryan et al., 1998), which promoted the formation of quercetin. Similar results have been demonstrated by Zhang et al. (2003), where an enhanced quercetin-to-kaempferol ratio was detected in the kale cv Vates with supplemented UV-B (25% above maximum average global radiation). Flavonoids act as shielding components against UV-B because they are strong scavengers of ROS (Edreva, 2005). Ryan et al. (1998) and Zhang et al. (2003) used supplemented UV-B to enhance the quercetin-to-kaempferol ratio, which contrasted with our investigated decreasing low radiation levels, suggesting that the decreasing temperature in our experiment could have led to the enhancement of the quercetin-to-kaempferol ratio, due to higher ROS, which is associated with lower temperatures (Klimov et al., 2008). Additionally, with ongoing plant development an increase in ROS is accompanied by physiological aging (Vogt & Gulz, 1994). In our experiment, the number of newly developed leaves between October and January was low: the mean maximum number of leaves (including all cultivars) increased from 39, 41, 44 and 44 in 18-, 22-, 26- and 30-week-old plants, respectively. However, the existing leaves were physiological aging. A higher number of hydroxyl groups, as in the trihydroxylated myricetin aglycone, compared with the di-hydroxylated quercetin is associated with higher antioxidant activity (Vogt & Gulz, 1994). According to our results, a shift from kaempferol (mono-hydroxylated) to quercetin (di-hydroxylated) in the investigated kale plants could possibly provide a better protection against ROS caused in particular by lower temperatures in addition to the physiological aging of the plants. The decreasing radiation only seemed to have a weak effect on the flavonoid biosynthesis, if at all.

Our results revealed that the total flavonoid concentration did not differ, although the composition of the three flavonoid aglycones kaempferol, quercetin and isorhamnetin changed, dependent on varying climatic conditions, in particular low temperature levels. In general, at a low radiation level the quercetin-to-kaempferol level increased in all of the investigated cultivars, suggesting that the enhanced ROS, resulting only from a lower temperature (Klimov et al., 2008), could be responsible for this effect. It is aimed to carry out further research under well-defined conditions in climate chambers to determine the individual influence of temperature and radiation. Another aim is to enhance the flavonoids in kale and to determine the parameters by means of producing a functional food.

Acknowledgements

We sincerely thank Elke Büsch and Ursula Zentner for conducting the growth experiments and technical assistance. This study was funded by the DFG (projects: KR-2066/3-1 and KR-1452/12-1).

References

Ackland, M. L., Van de Waarsenburg, S., & Jones, R. (2005). Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. *In Vivo, 19*, 69–76.

Christie, P. J., Alfenito, M. R., & Walbot, V. (1994). Impact of low-temperature stress on general phenylpropaoid and anthocyanin pathways – Enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. *Planta, 194*, 541–549.

Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

Edreva, A. (2005). The importance of non-photosynthetic pigments and cinnamic acid derivatives in the photoprotection. *Agriculture Ecosystems and Environment, 106*, 135–146.

Gerhäuser, C. (2001). Flavonoids and other dietary plant constituents. What is of practical relevance? Should we modify our eating habits? *Aktuelle Ernährungsmedizin, 26*, 137–143.

Hämäläinen, M. R., Nieminen, R., Vuorela, P., Heinonen, M., & Moilanen, E. (2007). Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappa B activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappa B activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators of Inflammation*. doi:10.1155/2007/45673.

Heimler, D., Vignolini, P., Dini, M. G., Vincieri, F. F., & Romani, A. (2006). Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. *Food Chemistry, 99*, 464–469.

Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry, 40*, 2379–2383.

Huang, Z. L., Wang, B. W., Eaves, D. H., Shikany, J. M., & Pace, R. D. (2007). Phenolic compound profile of selected vegetables frequently consumed by African Americans in the southeast United States. *Food Chemistry, 103*, 1395–1402.

Ibarra, M., Moreno, L., Vera, R., Cogolludo, A., Duarte, J., Tamargo, J., et al. (2003). Effects of the flavonoid quercetin and its methylated metabolite isorhamnetin in isolated arteries from spontaneously hypertensive rats. *Planta Medica, 69*, 995–1000.

Jaakola, L., Maatta-Riihinen, K., Kärenlampi, S., & Hohtola, A. (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta, 218*, 721–728.

Justesen, U., Knuthsen, P., & Leth, T. (1998). Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass-spectrometric detection. *Journal of Chromatography A, 799*, 101–110.

Kim, J. D., Liu, L. P., Guo, W. M., & Meydani, M. (2006). Chemical structure of flavonols in relation to modulation and angiogenesis and immune-endothelial cell adhesion. *Journal of Nutritional Biochemistry, 17*, 165–176.

Kim, B. G., Kim, J. H., Kim, J., Lee, C., & Ahn, J. H. (2008). Accumulation of flavonols in response to ultraviolet-B irradiation in soybean is related to induction of flavanone 3-beta-hydroxylase and flavonol synthase. *Molecules and Cells, 25*, 247–252.

Klimov, S. V., Burakhanova, E. A., Dubinina, I. M., Alieva, G. P., Saĺnikova, E. B., Olenichenko, N. A., et al. (2008). Suppression of the source activity affects carbon distribution and frost hardiness of vegetating winter wheat plants. *Russian Journal of Plant Physiology, 55*, 308–314.

Knekt, A., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliovaara, M., Reunanen, A., et al. (2002). Flavonoid intake and risk of chronic diseases. *American Journal of Clinical Nutrition, 76*, 560–568.

Krumbein, A., Saeger-Fink, H., & Schonhof, I. (2007). Changes in quercetin and kaempferol concentrations during broccoli head ontogeny in three brokkoli cultivars. *Journal of Applied Botany and Food Quality, 81*, 136–139.

Leyva, A., Jarillo, J. A., Salinas, J., & Martinezzapater, J. M. (1995). Low-temperature induces the accumulation of phenylalanine ammonia-lyase and chalcone synthase messenger-RNAs of *Arabidopsis thaliana* in a light-dependent manner. *Plant Physiology, 108*, 39–46.

Lin, J., Rexrode, K. M., Hu, F., Albert, C. M., Chae, C. U., Rimm, E. B., et al. (2007). Dietary intakes of flavonols and flavones and coronary heart disease in US women. *American Journal of Epidemiology, 165*, 1305–1313.

Murtaza, I., Beigh, G. M., Shah, T. A., Hussain, A., Khan, A. A., & Kaur, C. (2005). Antioxidant activity and total phenolic content of kale genotypes grown in Kashmir vally. *Journal of Plant Biochemistry and Biotechnology, 14*, 215–217.

Raisanen, J., Hansson, H., Ullerstig, A., Doscher, R., Graham, L. P., Jones, C., et al. (2004). European climate in the late twenty-first century: Regions simulations with two driving global models and two forcing scenarios. *Climate Dynamics, 22*, 113–119.

Reay, P. F. (1999). The role of low temperature in the development of the red blush on apple fruit ('Granny Smith'). *Science Horticulture, 79*, 13–31.

Reifenrath, K., & Müller, C. (2007). Species-specific and leaf-age effects of ultraviolet radiation on two Brassicaceae. *Phytochemistry, 68*, 875–885.

Rochefort, S. J., Imsic, M., Jones, R., Trenerry, V. C., & Thomkins, B. (2006). Characterization of flavonol conjugates in immature leaves of pak choi [*Brassica rapa* L. ssp. *chinensis* L. (Hanelt.)] by HPLC-DAD and LC–MS/MS. *Journal of Agricultural and Food Chemistry, 54*, 4655–4860.

Ryan, K. G., Markham, K. R., Bloor, S. J., Bradley, J. M., Mitchell, K. A., & Jordan, B. R. (1998). UVB radiation induced increase in quercetin: Kaempferol ratio in wild-type and transgenic lines of *Petunia*. *Photochemistry and Photobiology, 68*, 323–330.

Suzuki, T., Honda, Y., & Mukasa, Y. (2005). Effects of UV-B radiation, cold and desiccation stress on rutin concentration and rutin glucosidase activity in tartary buckwheat (*Fagopyrum tataricum*). *Plant Science, 168*, 1303–1307.

Tattini, M., Guidi, L., Morassi-Bonzi, L., Pinelli, P., Remorini, D., Deglínnocenti, E., et al. (2005). On the role of flavonoids in the integrated mechanism of response of *Ligustrum vulgare* and *Phillyrea latifolia* to high solar radiation. *New Phytologist, 167*, 457–470.

Tsormpatsidis, E., Henbest, R. G. C., Davis, F. J., Battey, N. H., Hadley, P., & Wagstaffe, A. (2008). UV irradiance as a major influence on growth, development and secondary products of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. *Environmental and Experimental Botany, 63*, 232–239.

Vogt, T., & Gulz, P. G. (1994). Accumulation of flavonoids during leaf development in Citrus-Laurifolius. *Phytochemistry, 36*, 591–597.

Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). Flavonoids: Antioxidants or signalling molecules? *Free Radical Biology and Medicine, 36*, 838–849.

Winter, T. R., & Rostas, M. (2008). Ambient ultraviolet radiation induces protective responses in soybean but does not attenuate indirect defense. *Environmental Pollution, 155*, 290–297.

Yonekura-Sakakibara, K., Tohge, T., Matsuda, F., Nakabayshi, R., Takayama, H., Niida, R., et al. (2008). Comprehensive flavonol profiling and transcriptome coexpression analysis leading to decoding gene–metabolite correlations in Arabidopsis. *Plant Cell, 20*, 2160–2176.

Zhang, J. M., Satterfield, M. B., Brodbelt, J. S., Britz, S. J., Clevidence, B., & Novotny, J. A. (2003). Structural characterization and detection of kale flavonoids by electrospray ionization mass spectrometry. *Analytical Chemistry, 75*, 6401–6407.