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Effects of photoperiod, growth temperature and cold acclimatisation on glucosinolates, sugars and fatty acids in kale

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ABSTRACT

Curly kale is a robust, cold tolerant plant with a high content of health-promoting compounds, grown at a range of latitudes. To assess the effects of temperature, photoperiod and cold acclimatisation on levels of glucosinolates, fatty acids and soluble sugars in kale, an experiment was set up under controlled conditions. Treatments consisted of combinations of the temperatures 15/9 or 21/15 °C, and photoperiods of 12 or 24 h, followed by a cold acclimatisation period. Levels of glucosinolates and fatty acid types in leaves were affected by growth conditions and cold acclimatisation, being generally highest before acclimatisation. The effects of growth temperature and photoperiod on freezing tolerance were most pronounced in plants grown without cold acclimatisation. The results indicate that cold acclimatisation can increase the content of soluble sugar and can thereby improve the taste, whilst the content of unsaturated fatty and glucosinolates acids may decrease.

1. Introduction

Curly kale (*Brassica oleracea* L. var *acephala*) is a biannual leafy vegetable, of the Brassicaceae family, being grown globally along a wide range of latitudes. Kale plants are robust and can tolerate cold temperatures below freezing. Its foliage is rich in nutrients and bioactive compounds, such as vitamins, minerals, glucosinolates and phenolic compounds (Ayaz et al., 2006). Epidemiological studies have shown an inverse relationship between consumption of *Brassica* vegetables and development of cancer and cardiovascular diseases (Jahangir, Kim, Choi, & Verpoorte, 2009). Anti-cancer attributes have been linked to glucosinolates and their degradation products, in addition to other compounds (Traka & Mithen, 2008; Verkerk et al., 2009). Glucosinolates are characterised by a core structure consisting of a β-D-thioglucose group, a sulfonated oxime moiety, and a variable side chain derived either from an aliphatic, aromatic or indolic amino acid (Moreno, Carvajal, López-Berenguer, & García-Viguera, 2006).

Growth conditions, such as day length and temperature, vary greatly with changing latitudes. Due to a low temperature optimum, kale is often grown in the cool season (late-summer until December) at low latitudes and in the summer season at higher latitudes (Decoteau, 2000). In areas above the Arctic Circle in Fennoscandia (66°N) the growth season (May–September) is characterised by low temperatures (9–14 °C average in Tromsø, Norway 70°N) and photoperiods up to 24 h. However, these 24 h photoperiods still have distinct night periods, with reduced radiation and lower temperatures at night (Nilsen, 1985). Broad latitudinal growing ranges have been reported to result in significant variation in the nutritive value and content of health-promoting components in some berries and vegetables (Hårdh, Persson, & Ottosson, 1977; Zheng, Kallio, & Yang, 2009). Although similar studies have not been conducted on *Brassica* crops, the content of glucosinolates in *Brassica* vegetables is affected not only by genotype variation, but also by environmental growth conditions, such as temperature and light conditions (Charron, Saxton, & Sams, 2005; Farnham, Wilson, Stephenson, & Fahey, 2004; Vallejo, Tomás-Barberán, Benavente-García, & García-Viguera, 2003; Velasco, Cartea, González, Vilar, & Ordás, 2007). In a previous study on broccoli (*B. oleracea* var. *italica*) we found higher content of glucosinolates at 21/15 °C compared to 15/9 °C, and also contents of individual glucosinolates were affected by day length (Steindal, Mølmann, Bengtsson, & Johansen, 2013). Schonhof, Kläring, Krumbein, Claußen, and Schreiner (2007) reported that low temperatures increased the content of aliphatic glucosinolates in broccoli, whilst high temperatures increased the levels of indolic glucosinolates.

Kale is highly tolerant to frost and it is not unusual that these plants remain unharvested in the field late in the growth season,

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exposed to low temperatures and frost. Low temperature activates cold acclimatisation processes in plants, involving many biochemical and physiological changes, leading to enhanced tolerance to freezing (Levitt, 1980). These processes include growth reduction, accumulation of osmolytes, modification of membrane fatty acid composition and alterations in gene expression (Heino & Palva, 2004). Changes in levels of soluble sugars are often correlated to freezing tolerance for various plants (Savitch, Harney, & Huner, 2000). Soluble sugars are key contributors to cold acclimatisation as low molecular weight osmolytes accumulates in cells of frost tolerant plants. The accumulation of soluble sugars may also have an effect on taste in kale by increasing sweetness and tenderness of the leaves (Decoteau, 2000). Exposure of kale to low temperatures increases the content of soluble sugars, and is reported to both reduce (Hagen, Borge, Solhaug, & Bengtsson, 2009) and increase (Neugart et al., 2012) content of health-promoting phenolic compounds. Shifts in membrane fatty acid composition are a way for plants to protect membrane stability, integrity and function by increasing the proportion of unsaturated fatty acids (Steponkus, 1984). These cold-enhanced polyunsaturated fatty acids and their enhanced intake for humans are also linked to a range of health-promoting benefits (de Lorgeril & Salen, 2012).

Even though kale is a frost tolerant plant, there is little research on how low temperatures inducing cold acclimatisation affect the nutritional and health-promoting quality. In this paper we hypothesised that growth conditions associated with latitudinal growth sites can affect the levels of both the primary metabolite, sugars and fatty acids, as well as the secondary plant metabolite, glucosinolates, before and after a cold acclimatisation period. The frost tolerance is also believed to be affected by the conditions the plants have grown under before a cold acclimatisation period, in addition to the cold acclimatisation treatment which is known to increase the frost tolerance. With the increased interest in content of compounds associated with human health, elucidating the effects of environmental growth conditions and cold acclimatisation is of interest to growers and consumers.

In the present study the aim was therefore to see how the levels of glucosinolates, fatty acids and soluble sugars in kale related to temperature, photoperiod during growth and subsequent cold acclimatisation.

2. Material and methods

2.1. Plant material and experimental set-up

Different climatic conditions were simulated in temperature (±0.5 °C) and humidity controlled (0.5 kPa ± 3%) chambers (Phytotron of the University of Tromsø, Norway). Diurnal temperature was used to reflect different mean day and night temperature, in combination with contrasting photoperiods reflecting high and low latitudes. Kale (*B. oleracea* L. var *acephala* 'Reflex') seeds were germinated in a dark chamber (21 °C under 24 h light) in a fertilised peat/perlite soil mixture (1:1 v/v) under plastic cover. After germination (4 days), the plastic cover was removed and temperature was lowered to 15 °C. Two weeks after germination the plants were transferred to 12 cm diameter pots with a mixture of sand/compost and perlite (4:1 v/v). One month after germination, the plants were transferred to 12 l plastic pots with 10 l 70% sand/compost mixture and 30% perlite (8.5 kg) and fertilised 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 re-fertilised with 3 g calcium nitrate (Yara, Bor-Kalksalpeter, 15.4% N) every 6 weeks from the start of treatment until cold acclimatisation.

The experimental treatments were divided into two main parts: a growth period and a subsequent cold acclimatisation period. The experiments were set up in a $2 \times 2 \times 2$ factorial design. Initially plants were exposed to four different treatments; high (21/15 °C, day/night) or low temperature (15/9 °C, day/night) in combination with long (24 h) or short photoperiods (12 h). After the growth period, half of the plants were harvested and the other plants were exposed to cold acclimatised treatment.

During the growth treatments, photosynthetic active radiation was given for 12 h with fluorescent light tubes 165–175 μmol $m^{-2}s^{-1}$ (Phillips TLD 58W/840; Eindhoven, The Netherlands). The long photoperiod treatments were extended to 24 h with low irradiance lamps for 12 h, 6–8 μmol $m^{-2}s^{-1}$ (Osram delux el longlife 2020, Munich, Germany). The 12 h low irradiance extensions were in combination with the reduced night temperature. Eleven weeks after the germination period, 50% of the plants were harvested and the remaining plants exposed to a cold acclimatised treatment. All treatments had similar plant height except low growth temperature/short day which were about ~30% lower. The remaining plants were kept for 2 weeks at 6 °C, then two weeks at 3 °C and finally at 0.5 °C for 1 week. All plants received 12 h of low intensity light (6–8 μmol $m^{-2}s^{-1}$). There was little or no plant growth during the cold acclimatisation.

2.2. Freeze test

The effects of growth temperature, photoperiod and cold acclimatisation on frost tolerance were assessed by electrolyte leakage from leaf discs of kale leaves. Discs (diameter 11 mm) were cut out between main veins of the leaf number eight from the top leaf (the second fully extended leaf). Sixteen leaf discs from each treatment were placed between sheets of moistened paper towels and packed in moist sand in 2 l plastic boxes. The boxes were placed at 0.5 °C overnight before being moved to a temperature-regulated freezer at −3 °C for 17 h. Temperature was lowered at a rate of 1 °C h from −3 to −10 °C and then 3 °C h until 17 °C, and the boxes were removed each second degree. The temperature in the boxes was recorded with a thermocouple probe. After freezing, plants were thawed overnight at 4 °C. Leaf discs were then removed from the sand, and four leaf discs were placed in 5 ml of distilled water for 1.5 h in tubes with a lid. Leaf discs were also placed at continuous 0.5 °C for comparison. The electrical conductivity of the extract was measured by a digital conductivity metre (712 Conductometer, Metrohm, Switzerland) at room temperature. Each treatment had four replicates at each freezing temperature.

2.3. Chemical analysis

The first full expanded leaf, leaf number seven from the top was harvested for chemical analysis. The main vein was removed before being flash frozen in liquid nitrogen and stored at −80 °C. The samples were freeze dried and ground to a fine powder using a steel ball mill (Retsch MM301, Retsch GmbH, Haan, Germany).

2.3.1. Extraction and determination of glucosinolates

Freeze dried plant material (40 mg) was extracted according to Mellon, Bennett, Holst, and Williamson (2002) with some modifications. The glucosinolates were quantified using 0.05 μmol/sample glucotropaeolin as internal standard (AppliChem (A5300.0020), Darmstadt, Germany) applied by an automated pipette (HandyStep electronic repeating pipette, Brandtech Scientific Inc., Essex, CT, USA). Seven biological replicates were extracted per treatment and every tenth sample was extracted in duplicate to verify reproducibility (within 95%). Glucosinolates were analysed in randomised order by UHPLC–HR-MS on a Waters Acquity UPLC (Milford, MA) coupled to Waters LCT-Premier time-of-flight MS with electrospray ionisation. The glucosinolates were separated on a Waters Acquity charged surface hybrid (CSH) C18 column (2.1×50 mm, 1.7 μm) using a gradient of 2–30%