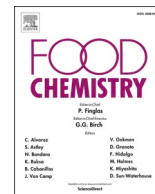


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Integration of non-target metabolomics and sensory analysis unravels vegetable plant metabolite signatures associated with sensory quality: A case study using dill (*Anethum graveolens*)

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ABSTRACT

Using dill (*Anethum graveolens* L.) as a model herb, we reveal novel associations between metabolite profile and sensory quality, by integrating non-target metabolomics with sensory data. Low night temperatures and exposure to UV-enriched light was used to modulate plant metabolism, thereby improving sensory quality. Plant age is a crucial factor associated with accumulation of dill ether and α -phellandrene, volatile compounds associated with dill flavour. However, sensory analysis showed that neither of these compounds has any strong association with dill taste. Rather, amino acids alanine, phenylalanine, glutamic acid, valine, and leucine increased in samples exposed to eustress and were positively associated with dill and sour taste. Increases in amino acids and organic acids changed the taste from lemon/grass to a more bitter/pungent dill-related taste. Our procedure reveals a novel approach to establish links between effects of eustressors on sensory quality and may be applicable to a broad range of crops.

1. Introduction

Dill (*Anethum graveolens* L.) is a culinary herb used worldwide to season foods including sauces, salads and seafoods. Protected cropping in structures such as greenhouses allows dill production to be extended to off-season periods when lack of natural light or low temperatures impair field production, particularly in Eastern Europe and Scandinavia, where dill is extensively consumed. Indeed, greenhouses offer the advantage of year-round production, but when it comes to nutritional or sensory quality, greenhouse products have a poor reputation compared to their outdoor-grown counterparts (Kyriacou & Rouphael, 2018). In the open field, crops are exposed to different growth conditions than in greenhouses, including day-night fluctuations in temperature and

exposure to solar ultraviolet light (UV-A, 315–400 nm; UV-B, 280–315 nm). In greenhouses, the temperature is often kept constant, while UV is blocked by the greenhouse cladding material (Katsoulas, Bari, & Papaioannou, 2020). While greenhouse production protects from extreme temperatures and excess UV that can impair plant growth (Rouphael, Kyriacou, Petropoulos, De Pascale, & Colla, 2018), plants also rely on light and temperature as signals to regulate the synthesis of metabolites that confer environmental acclimation and which, in addition, are responsible for some of the nutritional and sensorial attributes of the horticultural produce (Kyriacou & Rouphael, 2018).

Eustress conditions, including decreased temperatures and supplementary UV light (Hideg, Jansen, & Strid, 2013), are promising pre-harvest tools to improve the nutritional or sensorial quality of

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greenhouse crops by modulating plant metabolism (Kandel et al., 2020). In the case of greenhouse-grown dill, an improvement in sensory quality can be achieved by increasing plant density and irrigation doses (El-Zaaddi, Martínez-Tomé, Calín-Sánchez, Burló, & Carbonell-Barrachina, 2017). Different proportions of red and blue light, emitted by light emitting diodes (LEDs), also modulate the synthesis of volatile compounds, such as dill ether and α -phellandrene (Frąszczak, Gąsecka, Golcz, & Zawirska-Wojtasiak, 2016; Litvin, Currey, & Wilson, 2020), which were shown to be associated with characteristic dill flavour (El-Zaaddi, Martínez-Tomé, Calín-Sánchez, Burló, & Carbonell-Barrachina, 2017). Other strategies aiming to increase dill quality have focused on post-harvest technology, such as drying (Madhava Naidu et al., 2016), modified atmosphere packaging (Koyuncu, Güneşli, Erbas, Onursal, & Secmen, 2018) and cold storage (Ślupski, Lisiewska, & Kmiecik, 2005). Although post-harvest conditions are crucial for maintaining the quality of crops, the management at the pre-harvest stage will undoubtedly be necessary to close the gap between the perceived and the potential quality (Kyriacou & Roupheal, 2018). Dill quality has been defined through association between sensory data and levels of a few specific volatile compounds, particularly dill ether and phellandrenes, while the impact of sugars, amino acids, organic acids and phenolic compounds on dill sensory quality is unknown, but also likely to be important.

Gas chromatography–mass spectrometry (GC–MS)-based metabolomics is considered ideal for identifying small molecular metabolites including small acids, hydroxyl acids (e.g., phenolic acids), amino acids, and sugars, often using chemical derivatisation to make these compounds volatile enough for gas chromatography (Fiehn, 2016). There is an increasing interest in the application of non-targeted analytical approaches to capture the complex regulatory metabolic responses of plants with respect to their surroundings, as they provide data on a wider range of metabolites than traditional targeted MS approaches. In this study, outdoor and greenhouse dill obtained from several sources, and over a period of two consecutive years, were evaluated by using GC–MS-based non-target metabolite analysis, analysis of the levels of known flavour-relevant compounds, and sensory profiling, to gain insights into the relationship between dill metabolism and its sensory quality as perceived by consumers. We also explored whether controlled eustress conditions (Hideg et al., 2013) applied during plant growth can be used to modulate the plant metabolite profile (Schreiner et al., 2012), thereby improving dill sensory quality. By integrating data on plant metabolism with sensory analysis we aimed to define specific metabolic signatures associated with dill quality. We hypothesised that a metabolite profile associated with better sensory quality of greenhouse crops can be achieved through application of eustress conditions during plant growth, particularly using low temperatures or supplementary UV exposure.

2. Materials and methods

2.1. Reagents and chemicals

HPLC–MS-grade methanol and water were from Fisher Scientific (Waltham, MA). All analytical standards (purity $\geq 97\%$) were purchased from Sigma-Aldrich (St Louis, MO) unless stated otherwise.

2.2. Plant material and growth conditions

A total of 18 fresh dill batches were obtained over two consecutive years (nine batches/year). Samples included greenhouse dill batches obtained from an experimental greenhouse as well as dill from various commercial sources (Fig. 1). The same *Anethum graveolens* L. (cv. Lena) seeds (Hild Samen GmbH, Marbach am Neckar, Germany) were used to produce outdoor samples and samples from both experimental greenhouse and local producer. The only sample that was not obtained from dill seeds (cv. Lena) was the commercial shop sample that had been passed through the retail chain (Comm_shop; see also below).

Dill was produced under four conditions at the experimental greenhouse: control condition dill plants (Exp_control) and dill exposed to either UV-A-enriched (Exp_UVA) or UV-B-enriched light (Exp_UVB) or to cold nights (Exp_CN) during the dark hours. Dill seeds were surface-sown in 0.25-L pots filled with peat moss-based substrate (14–7–15 NPK; SW Horto AB, Hammenhög, Sweden) and maintained for one week at 25 °C day/20 °C night and 80% RH under plastic film. After one week the film was removed. All plants, irrespective of further treatment, received natural light through the roof and supplementary light from sodium lamps (Vialox NAV-T Super 4Y; Osram GmbH, Munich, Germany) for 16 h/day. The supplementary light was automatically turned off when total irradiance reached 900 $\mu\text{mol}/\text{m}^2/\text{s}$. Before harvest, plants were maintained for a further three weeks under the control conditions or were exposed daily to UV-A-enriched or UV-B-enriched light or CN as follows: for UV treatments, plants were exposed (4 h/day around the solar noon) to UV-A- or UV-B-enriched radiation using fluorescent UVA 340 (Q-Lab, Cinninnati, OH) or TL40/12 UV lamps (Philips, Eindhoven, The Netherlands), respectively, using the same experimental set-up described in detail by Qian et al. (2019). The irradiance was measured using a double monochromator spectroradiometer (OL756; Gooch & Housego, Ilminster, UK). The UV-A-enriched light contained 3.6 W m^{-2} UV-A and 0.046 W m^{-2} plant-weighted UV-B (Thimijan, Campbell, Carns, Center, & Agency, 1978; Yu & Björn, 1997), the latter corresponding to a total irradiation of plant-weighted UV-B of 0.6 $\text{kJ m}^{-2} \text{day}^{-1}$. The UV-B-enriched light contained 0.083 W m^{-2} plant-weighted UV-B, corresponding to a plant weighted daily UV-B dose of 1.2 kJ m^{-2} (Thimijan et al., 1978; Yu & Björn, 1997). This amount of irradiance is

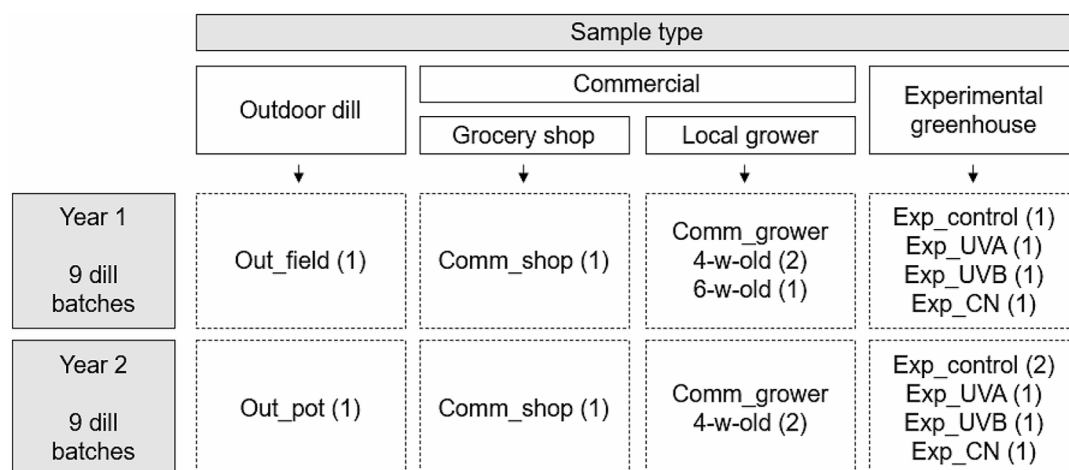


Fig. 1. Samples used in the study. A total of 18 dill batches of fresh dill samples (9 batches/year) were obtained over two consecutive years.

considered environmentally relevant, being around one-quarter of the measured irradiation outdoors in Lund (Sweden) under a cloudless sky at summer solstice (4.8 kJ m^{-2}) (Yu & Björn, 1997). The UV-B-enriched light also contained 0.34 W m^{-2} UV-A. Cellulose acetate was used to filter out any UV-C from the UV-B-enriched radiation and Perspex was used to block all UV produced by both types of fluorescent tubes from control plants (Qian et al., 2019). Exp_CN was produced in the same way as the Exp_control, except that Exp_CN plants were transferred on a daily basis to a refrigerator ($8 \text{ }^{\circ}\text{C}$) for $8 \pm 1 \text{ h}$ during the night.

Dill samples obtained directly from the local producer Svegro AB (Ekerö, Sweden) were named Comm_grower and included in the study to enable comparison with typical dill from a commercial set-up (i.e., 4-week-old dill obtained over consecutive years). Comm_grower also provided an adequate number of samples grown under the same conditions differing only in their developmental stage (i.e., 4- and 6-week-old dill obtained during the first year). Additional commercial samples (bunches obtained from a grocery store), named Comm_shop, were included in this study to check if Exp_control had similar sensory quality to commercially grown plants, that had passed through the retail chain. Two outdoor samples were also included in the study (Out_Y1) and (Out_Y2), sampled in Year 1 and 2, respectively. The outdoor samples were grown in pots for 4 weeks and harvested by the end of May. Each year, harvested samples were directly evaluated for their sensory quality. Three batches from each group (each batch was composed of the upper half of 5–6 randomly selected dill plants) were collected and frozen in liquid nitrogen and stored at $-80 \text{ }^{\circ}\text{C}$ for non-targeted metabolite profiling and analysis of flavour-relevant compounds.

2.3. Non-targeted polar metabolite profiling

2.3.1. Metabolite extraction and two-step derivatisation

Samples ($52 \pm 4 \text{ mg}$) were randomised and vortex-mixed with 1 mL cold methanol containing $1 \text{ } \mu\text{g mL}^{-1}$ valine-d8 (internal standard; IS) and incubated in an ultrasonic bath for 10 min . After centrifugation ($11,000g$, 10 min), $400 \text{ } \mu\text{L}$ of supernatant were collected and vortex-mixed with cold chloroform ($220 \text{ } \mu\text{L}$) and water ($440 \text{ } \mu\text{L}$). Samples were centrifuged again ($2200g$, 15 min) and the aqueous phase transferred to GC vials. Pooled extracts to be used as quality controls (QC) were prepared by mixing equal aliquots of samples. Process/extraction blanks were also prepared by following the same extraction procedure described above except that the actual biological sample (i.e., dill sample) was not included.

For derivatisation, a two-step procedure was performed after solvent evaporation under a stream of nitrogen gas. In the first step, $25 \text{ } \mu\text{L}$ of methoxamine (MEOX) hydrochloride (20 mg/mL in pyridine, $45 \text{ }^{\circ}\text{C}$, 1 h) were added. In the second step, metabolites were converted into trimethylsilyl (TMS) derivatives using $25 \text{ } \mu\text{L}$ of *N*-trimethylsilyl-*N*-methyl trifluoroacetamide ($45 \text{ }^{\circ}\text{C}$, 1 h). Finally, a mixture containing *n*-alkanes (odd C11 to C25, $5 \text{ } \mu\text{g mL}^{-1}$) and 4,4'-dibromooctafluorobiphenyl (DBOBF, injection standard, $1 \text{ } \mu\text{g mL}^{-1}$) was added to the vials to further calculate retention indices (RI) and to evaluate instrument performance, respectively.

2.3.2. Gas-chromatography (GC) Orbitrap high-resolution mass spectrometry analysis

Derivatised samples were analysed using a Q Exactive GC Orbitrap system (Thermo Scientific, Waltham, MA). Samples were randomly separated into three batches for analysis. Each batch also included two quality controls (QC), a process/extraction blank, and a derivatisation control (DC). The latter is a mixture of the derivatisation reagents added following the same derivatisation procedure to that of samples. Analysis of DC allows further exclusion of features related to the derivatisation reagents or formed as a product of the derivatisation procedure. The GC was equipped with an Rxi Guard column ($10 \text{ m} \times 0.37 \text{ mm}$, 0.25 mm i.d. ; Restek, Bellefonte, PA) and an HP-5MS capillary column ($30 \text{ m} \times 0.25 \text{ mm}$, $0.25 \text{ } \mu\text{m i.d.}$; Agilent, Santa Clara, CA). The oven temperature

was held at $70 \text{ }^{\circ}\text{C}$ for 5 min and increased to $260 \text{ }^{\circ}\text{C}$ at $10 \text{ }^{\circ}\text{C min}^{-1}$, then to $300 \text{ }^{\circ}\text{C}$ at $40 \text{ }^{\circ}\text{C min}^{-1}$ and held for 5 min . Helium was used as a carrier gas at 2 mL min^{-1} . The MS detector was operated in EI positive mode (scan range of m/z 50–500, resolution of 60,000). The transfer line and ion source were maintained at $280 \text{ }^{\circ}\text{C}$. Injection ($1 \text{ } \mu\text{L}$) was performed in splitless mode. For performance analysis, calibration curves were also obtained for 38 in-house analytical standards.

2.3.3. GC Orbitrap data pre-processing and analysis performance

The pre-processing steps were performed on MZmine 2.53 using the Automated Data Analysis pipeline (ADAP-GC 4.0) module in order to perform peak deconvolution and alignment of features from samples (Du, Smirnov, Pluskal, Jia, & Sumner, 2020). Firstly, the pre-processing pipeline included raw file import (.mzML data), followed by mass detection and construction of extracted ion chromatograms, to build a separate chromatogram – EIC – to each m/z that was detected by the instrument. Then, peak detection and deconvolution were applied to integrate peaks from EIC and to construct mass spectra of features by combining peaks from different EIC, respectively, thereby resulting in a list of RT- m/z pairs for each sample. Similar RT- m/z pairs in different sample files were then aligned based on similarity between fragmentation mass spectra, to generate a table with all features detected, as well as their intensity values in samples. A filtering step was further applied to remove features associated to extraction blank and derivatisation controls, and gap filling was used to fill missing signals not found by the alignment algorithm. Finally, feature identification was applied to identify peaks by comparing their m/z -RT pairs with those obtained for analytical standards.

The following parameters were applied for the ADAP-GC 4.0 module: (1) mass detection of centroid data, (2) chromatogram building with a minimum group size of 5 scans, group intensity threshold and minimum highest intensity at 1.0×10^6 , and m/z tolerance set at 0.05, (3) chromatogram deconvolution using minimum signal/noise ratio of 5, feature height of 5×10^5 , coefficient/area threshold of 100 and peak duration and RT wavelength range between 0.01 and 0.40 min and 0.00 and 0.20, respectively, (4) spectral deconvolution using the hierarchical clustering method with distance of cluster 0.01 min, minimum intensity of 500, and shape-similarity tolerance of 40, (5) ADAP aligner with minimum confidence of 0.6 (only m/z -RT pairs present in at least 60% of samples were included in the aligned list), (6) manual filter to remove all peaks that match with system suitability blanks, DC and/or process blank (except the RT- m/z pairs 10.24/152.1705 and 16.51/455.8212 relative to IS and injection standard, necessary for further normalisation and analysis performance, respectively), (7) gap filling using the peak finder module with maximum allowed deviation of expected peak shape of 3%, and m/z and RT tolerance of 0.001 and 0.1, respectively, (8) identification of metabolites using a custom in-house database with m/z (base peak) and RT tolerance of 0.005 and 0.1 min, respectively.

The peak list was exported as .mgp file for spectral information and as .csv for metadata (containing RT- m/z pairs of features and their respective intensity levels on samples). Data pre-processing also included calculation of RI based on the retention times of *n*-alkanes (eight peaks containing m/z 71.0855, 85.1012 and 99.1169), as well as normalisation by IS levels and sample weight. A total of 41 features without the characteristic m/z fragment 73.046 (trimethylsilyl moiety) and/or with RSD > 30% on QC samples were also excluded from the final dataset, resulting in a final peak list of 70 features. The relative standard deviation (RSD) of IS in samples, extraction blanks and QC injections was 15.8%, while the inter-batch RSD of DBOBF was 5.0%. Calibration curves constructed for 38 in-house analytical standards also provided good linearity ($r^2 > 0.99$) (Table S1). The entire metabolite dataset (.csv file) comprising the relative levels of 70 features (m/z -RT pairs) in 60 samples (54 samples and six QC) were evaluated for analysis performance. As shown in Fig. S1, QC samples were clustered together with low dissimilarity values between them, while three sample replicates from different treatments (<6% of samples) showed high

dissimilarity compared to the other samples. These three replicates were considered outliers and excluded from further analysis.

2.3.4. Compound annotation

Automated identification of 23 out of the 70 features using the custom database search module on Mzmine was confirmed by comparing the full spectra and RT of peaks with those obtained for in-house analytical standards. Two additional levels of annotation (levels 2 and 3) were defined for the remaining features: level 2 included metabolites ($n = 22$) putatively identified by matching their spectra and RI with those available in Golm Metabolome Database (GMD) using recommended parameters for mass spectral matching – except for substructure prediction and Δ RI that were increased to 90% and 20, respectively; level 3 included the remaining non-identified compounds (NI = 25), whose putative identifications were based on spectra similarity using GMD and NIST databases.

2.4. Target analysis of flavour-relevant compounds

Dill ether and α - and β -phellandrene were extracted from samples (150–200 mg) with 10 mL of cold *n*-hexane: methyl tert-butyl ether (1:1) at 4 °C for 30 min in a sonication bath. After centrifugation (2200g, 15 min), 1000 μ L of the supernatant were transferred to GC vials and analysed using a 6890 GC system coupled to a 5973 MS detector (Hewlett-Packard). The system was equipped with a HP-5MS capillary column (30 m $\times$ 0.25 mm, 0.25 μ m i.d.; Agilent). The oven temperature was held at 70 °C for 2 min and increased to 240 °C at 25 °C/min, then was held for 5 min. The transfer line and detector were maintained at 200 °C and 230 °C, respectively. Helium (1 mL/min) was used as the carrier gas. Injection (1 μ L) was performed in splitless mode. For identification, RT of dill ether (5.6 min) and α - and β -phellandrene (4.1 and 4.3 min), as well as their fragmentation patterns, were compared to those obtained for analytical standards. Three biological replicates were analysed for each sample batch. Results were expressed as relative levels (mean $\pm$ SD) in relation to outdoor-grown samples.

2.5. Sensory analysis

Each year freshly collected batches were evaluated by a sensory panel from the School of Hospitality, Culinary Arts and Meal Science at Örebro University (Sweden). After recruitment, panellists were selected based on availability, motivation, as well as on ability to detect, discriminate and elicit sensory attributes (Meilgaard, Civille, & Carr, 2006). The repertory grid method (RGM) was used to investigate panellist's perception of distinct samples in individual interviews consisting of two parts. First, assessors were provided with triads of samples (two samples associated – same batch – and one different) and asked to describe with their own descriptors how they thought the two associated samples resembled each other, and, likewise, how the associated samples differed from the third. Each assessor performed the analysis of three triads of samples with an interval of approximately 10 min between assessments of triads (panellists consumed dry crackers and water between the triads). All descriptors defined by the panellist with regards to the triads were inserted into the EyeQuestion software. In the second part, panellists were served with the same samples from the first phase in a randomised order and identified with a three-digit code. Panellists were then asked to provide an evaluation of samples using an unstructured scale from 1 (low intensity) to 9 (high intensity) for each attribute. Assessors were also served with water during evaluation of samples. The most frequent descriptors (>15% of frequency) provided by the assessors on each sensory panel were included in the data analysis.

2.6. Statistical analysis

For the metabolomics dataset and for data on flavour-relevant compounds, missing values were replaced with imputed half-minimum

value (i.e., missing values were replaced by half of the minimum value found for each variable). Data were then log-transformed and auto-scaled before data analysis. Analysis of variance (ANOVA) with Tukey HSD was applied ($p < 0.01$) to identify differences in metabolite levels. Flavour-relevant compounds were also explored using ANOVA with Tukey as post hoc test ($p < 0.01$). Student's *t*-test ($p < 0.01$) was applied for group-to-group comparison using GraphPad Prism 6 software. Principal component analysis (PCA) was applied for metabolomics data and sensory data for dimension reduction. The *hclust* function within the R stats package 3.6.1 was applied for hierarchical cluster analysis (HCA). For correlation network analysis, a debiased sparse partial correlation (DSPC) algorithm was applied (Basu et al., 2017). Data were then imported to the MetScape 3.1.3 App into the framework of Cytoscape 3.7.1 to generate network topography plots.

3. Results

3.1. Polar metabolite profiling

A list of 70 polar metabolites including mainly organic acids, carbohydrates and conjugates, and amino acids, were obtained after data alignment and filtering. Of these metabolites, 57 showed significant differences between the different types of samples when the *p*-value threshold was set at 0.01. Although the use of a less stringent *p*-value ($p < 0.05$) would allow the inclusion of five metabolites in the analysis panel (4-aminobutanoic acid, 2-coumaric acid, aspartic acid, ethanolamine, and a non-identified organic acid), these additional metabolites had weak correlation coefficients (<0.20) to other metabolites and sensory attributes, and therefore were excluded from further network analysis. The relative levels of the significant metabolites explored in this study along with their *p*-values are shown in Table 1. Next, PCA and HCA were applied to the metabolomics dataset to explore response patterns. As shown in Fig. 2A, the two main principal components of the PCA model explained 40% of the total variance between samples, allowing discrimination between batches. Comm_shop and Exp_control showed a similar metabolite profile, while Comm_grower was plotted on the opposite side of the PC1 axis. The remaining samples were located more centrally on the PCA plot. Exp_UVA and Exp_CN separated from Exp_UVB, mainly across PC2, while Out_Y1 and Out_Y2 were plotted near to Exp_UVB and Exp_CN, respectively.

HCA (Fig. S2) indicated four main clusters of samples with a similar distribution to that observed in PCA, in which outdoor dill samples were grouped either with Exp_UVB (clustered with Out_Y1) or with Exp_UVA (clustered with Out_Y2). Loading plots from PCA were then explored to identify what chemical classes had the largest effect on the separation of samples across the two principal components. As shown in Fig. 2B, the loading plot depicts different chemical classes as the main discriminants of samples. On the *x*-axis (loadings 1), which separates mainly Comm_shop and Exp_control from Comm_grower, more than 80% of sugars, amino acids, and other metabolites (e.g., hydroxycinnamic acid, GABA) were plotted on the same side of the PCA plot as Comm_grower. On the *y*-axis (loadings 2), which mainly separate Exp_UVB from Exp_UVA and Exp_CN, sugars and other compounds (including phenolic compounds) were plotted near Exp_UVB, while amino acids correlated positively mainly with Exp_UVA and Exp_CN.

3.2. Target analysis of flavour-relevant compounds

As shown in Fig. 3A, Out_Y1, Out_Y2 and the 6-week-old dill from Comm_grower contained higher levels of dill ether, while dill batches from the experimental greenhouse were characterised by reduced (or even non-detectable) levels of dill ether. Among the samples from the experimental greenhouse, only Exp_UVB during the first year had increased levels of dill ether compared with Exp_control. The relative levels of α - and β -phellandrene followed a similar pattern to what was observed for dill ether (Fig. 3B). Again, only Exp_UVB from the first year

Table 1Metabolites with significant differences (ANOVA, Tukey HSD as post hoc, $p < 0.01$).

ID	Compound	p-value	Outdoor ($n = 3, 3$)	Grocery ($n = 2, 3$)	Greenhouse				
					Control ($n = 2, 6$)	UVA ($n = 3, 3$)	UVB ($n = 3, 3$)	CN ($n = 3, 3$)	Local ($n = 8, 6$)
004	Malonic acid ³	6.7E-03	1.0 ± 0.3	1.1 ± 0.1	1.0 ± 0.3	1.6 ± 0.6	1.5 ± 0.4	0.9 ± 0.4	0.8 ± 0.3
014	Oxalic acid ²	7.5E-06	0.8 ± 0.3	0.6 ± 0.1	0.8 ± 0.3	1.2 ± 0.4	1.3 ± 0.2	1.7 ± 0.6	1.4 ± 0.5
017	Valine ¹	1.5E-19	1.0 ± 0.3	0.3 ± 0.0	0.3 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	1.7 ± 0.6	1.5 ± 0.4
019	Serine ¹	8.1E-03	1.2 ± 0.4	0.5 ± 0.1	0.6 ± 0.3	0.9 ± 0.1	0.9 ± 0.1	1.1 ± 0.4	0.8 ± 0.2
022	Leucine ¹	1.6E-12	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.2	0.4 ± 0.0	2.1 ± 1.4	2.3 ± 0.9
023	Phosphoric acid ²	1.8E-04	0.6 ± 0.7	0.2 ± 0.0	0.6 ± 0.2	1.6 ± 0.4	1.8 ± 0.8	1.1 ± 0.6	0.9 ± 0.6
024	Maleic acid ²	5.8E-04	0.6 ± 0.3	2.4 ± 0.4	1.4 ± 0.7	0.6 ± 0.1	0.8 ± 0.1	0.4 ± 0.2	0.4 ± 0.4
027	Glycine ¹	3.7E-03	0.7 ± 0.6	0.8 ± 0.0	1.1 ± 0.6	0.9 ± 0.1	1.7 ± 0.1	1.4 ± 0.6	1.0 ± 0.4
028	Succinic acid ¹	2.1E-04	1.1 ± 0.2	1.4 ± 0.2	1.0 ± 0.2	0.8 ± 0.4	0.5 ± 0.5	0.5 ± 0.5	1.1 ± 0.2
029	Glyceric acid ²	2.1E-05	0.5 ± 0.8	0.9 ± 0.0	1.2 ± 0.9	1.8 ± 0.2	2.5 ± 0.8	0.6 ± 0.4	0.5 ± 0.2
030	Fumaric acid ¹	1.0E-04	0.6 ± 0.3	1.3 ± 0.1	1.2 ± 0.4	1.3 ± 0.4	1.4 ± 0.7	1.0 ± 0.2	0.7 ± 0.3
033	Threonine ¹	7.8E-13	1.7 ± 1.0	0.2 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	0.7 ± 0.1	1.8 ± 0.8	1.7 ± 0.7
034	Alanine ¹	5.0E-10	1.3 ± 0.5	0.3 ± 0.0	0.4 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	1.5 ± 0.7	1.5 ± 0.6
038	Xylose ³	6.4E-03	1.1 ± 0.4	0.8 ± 0.0	1.2 ± 0.4	1.5 ± 0.2	1.1 ± 0.2	1.1 ± 0.6	0.7 ± 0.4
039	Thiamine ³	1.4E-04	0.6 ± 0.3	1.5 ± 0.4	0.9 ± 0.5	1.4 ± 0.3	1.4 ± 0.3	1.2 ± 0.5	1.6 ± 0.6
040	Arabinose ³	1.3E-11	1.2 ± 0.3	0.5 ± 0.1	0.6 ± 0.3	0.4 ± 0.0	1.9 ± 0.2	0.5 ± 0.2	1.4 ± 0.6
041	Malic acid ¹	6.3E-07	0.6 ± 0.5	0.7 ± 0.1	1.0 ± 0.3	1.6 ± 0.3	1.5 ± 0.2	1.1 ± 0.4	0.8 ± 0.2
044	5-Oxoproline ¹	1.4E-09	0.9 ± 0.4	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	1.3 ± 0.2	1.4 ± 0.3	1.7 ± 1.0
048	Threonic acid ²	9.1E-09	3.6 ± 1.4	0.6 ± 0.1	0.8 ± 0.4	0.5 ± 0.1	1.9 ± 0.2	0.2 ± 0.1	0.6 ± 0.6
050	2-OG ^{2, a}	6.5E-07	1.7 ± 2.8	0.4 ± 0.0	0.4 ± 0.7	0.3 ± 0.1	0.4 ± 0.0	0.7 ± 0.3	1.5 ± 0.7
052	Ornithine ¹	1.4E-03	2.5 ± 3.2	0.4 ± 0.2	0.8 ± 0.5	0.5 ± 0.4	1.1 ± 0.5	0.4 ± 0.2	0.4 ± 0.2
053	Xylitol ³	3.2E-16	0.7 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	1.2 ± 0.2	0.8 ± 0.3	1.8 ± 0.8
055	Glutamic acid ¹	1.4E-11	1.1 ± 0.8	0.3 ± 0.0	0.2 ± 0.1	1.1 ± 0.6	0.6 ± 0.2	2.3 ± 0.8	2.0 ± 0.9
056	Phenylalanine ¹	4.3E-17	1.5 ± 1.2	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.3	0.3 ± 0.0	1.9 ± 1.0	2.1 ± 0.7
057	Putrescine ³	1.2E-11	0.3 ± 0.2	0.1 ± 0.1	0.6 ± 0.3	0.5 ± 0.1	1.0 ± 0.6	1.6 ± 0.6	1.9 ± 0.9
059	Arabitol ²	2.5E-03	1.6 ± 0.6	0.8 ± 0.3	0.8 ± 0.2	1.2 ± 0.4	1.9 ± 0.7	1.3 ± 0.6	2.0 ± 1.0
060	4-MGA ^{3, b}	1.1E-09	1.7 ± 0.9	0.7 ± 0.0	0.6 ± 0.2	0.9 ± 0.4	3.4 ± 0.5	0.9 ± 0.2	1.0 ± 0.3
063	Ribitol ³	2.4E-07	0.8 ± 0.5	1.1 ± 0.1	0.5 ± 0.5	0.2 ± 0.1	0.2 ± 0.0	0.9 ± 0.8	1.4 ± 0.6
064	Rhamnose ²	1.0E-04	1.2 ± 0.3	0.4 ± 0.1	0.6 ± 0.3	0.7 ± 0.3	1.5 ± 0.6	0.6 ± 0.2	1.2 ± 0.5
065	Glutamine ¹	9.2E-04	1.5 ± 1.0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.4	1.7 ± 2.0	3.1 ± 2.5
066	Shikimic acid ¹	1.6E-08	1.1 ± 0.1	0.5 ± 0.1	0.6 ± 0.3	0.7 ± 0.1	1.3 ± 0.2	0.8 ± 0.1	1.1 ± 0.3
067	Saccharic acid ³	1.8E-10	0.9 ± 0.7	0.4 ± 0.0	0.7 ± 0.2	0.9 ± 0.2	2.3 ± 0.5	1.1 ± 0.2	1.5 ± 0.4
068	Galactaric acid ³	2.9E-04	2.5 ± 1.2	1.3 ± 0.5	0.9 ± 0.7	0.9 ± 0.4	2.8 ± 1.0	1.1 ± 0.3	2.0 ± 1.0
069	Glucopyranoside ³	4.5E-03	1.3 ± 0.5	0.9 ± 0.1	0.9 ± 0.2	0.6 ± 0.3	1.1 ± 0.6	1.1 ± 0.2	1.6 ± 0.6
070	Citric acid ¹	6.5E-03	2.4 ± 2.4	1.3 ± 0.3	0.7 ± 0.6	0.8 ± 0.1	0.4 ± 0.0	1.7 ± 0.8	0.8 ± 0.4
071	Quinolactone ³	6.5E-06	1.1 ± 0.3	0.3 ± 0.1	1.0 ± 0.5	0.6 ± 0.1	1.8 ± 0.2	1.7 ± 0.8	2.3 ± 1.0
072	Quinic acid ²	1.7E-09	0.6 ± 0.5	0.3 ± 0.0	0.6 ± 0.3	0.2 ± 0.0	0.8 ± 0.3	1.0 ± 0.6	2.2 ± 1.3
073	Fructose ²	1.5E-06	0.3 ± 0.3	1.2 ± 0.2	0.7 ± 0.4	0.2 ± 0.0	0.9 ± 0.3	0.7 ± 0.5	1.5 ± 0.3
076	Ascorbic acid ³	1.7E-04	0.8 ± 0.5	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	1.2 ± 0.5	1.2 ± 0.4	1.8 ± 1.0
077	Galactose ³	3.0E-13	0.2 ± 0.2	1.3 ± 0.2	0.9 ± 0.3	0.2 ± 0.1	0.7 ± 0.4	0.7 ± 0.4	1.5 ± 0.3
081	Glucose ²	3.5E-10	0.6 ± 0.6	0.7 ± 0.1	0.8 ± 0.2	0.3 ± 0.1	1.7 ± 0.2	0.8 ± 0.4	1.4 ± 0.2
086	IAA ^{1, c}	4.8E-08	4.1 ± 3.7	1.0 ± 0.4	0.7 ± 0.3	0.5 ± 0.3	4.3 ± 2.9	1.5 ± 1.0	4.2 ± 2.0
087	scyllo-Inositol ²	5.7E-07	4.6 ± 2.2	1.7 ± 0.3	1.2 ± 0.4	0.8 ± 0.1	1.6 ± 0.7	0.4 ± 0.1	1.6 ± 1.0
088	Gluconic acid ²	3.7E-03	0.4 ± 0.4	0.9 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	1.0 ± 0.4	0.4 ± 0.2	0.7 ± 0.3
089	Hexonic acid ³	8.9E-18	1.1 ± 0.3	0.7 ± 0.1	0.7 ± 0.2	0.2 ± 0.0	1.5 ± 0.3	0.5 ± 0.1	0.9 ± 0.2
092	GHA ^{2, d}	1.9E-05	0.7 ± 0.2	1.3 ± 0.3	1.0 ± 0.3	0.8 ± 0.2	1.4 ± 0.2	0.7 ± 0.1	1.0 ± 0.2
095	6-DOMP ^{3, e}	2.0E-10	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	2.7 ± 1.8
098	Lactose ³	5.1E-13	0.7 ± 0.3	3.0 ± 0.2	1.8 ± 0.9	0.6 ± 0.1	1.0 ± 0.3	0.3 ± 0.1	0.8 ± 0.3
099	G6P ^{2, f}	4.1E-04	0.6 ± 0.3	0.4 ± 0.1	0.6 ± 0.4	0.9 ± 0.5	1.4 ± 0.3	0.6 ± 0.3	1.3 ± 0.5
100	F6P ^{3, g}	2.0E-04	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	0.8 ± 0.1	1.3 ± 0.4
101	Lactitol ³	1.5E-09	1.8 ± 0.7	1.0 ± 0.4	0.9 ± 0.1	0.9 ± 0.1	2.9 ± 0.3	1.0 ± 0.2	1.3 ± 0.4
103	Threolose ³	1.1E-07	2.3 ± 0.6	0.5 ± 0.1	0.5 ± 0.2	0.2 ± 0.2	2.2 ± 0.3	0.5 ± 0.2	0.8 ± 0.6
105	Sucrose ²	2.0E-05	0.8 ± 0.6	1.4 ± 0.2	1.5 ± 0.5	0.8 ± 0.2	1.7 ± 0.3	0.7 ± 0.1	0.9 ± 0.2
108	Maltose ²	7.6E-04	0.6 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	0.7 ± 0.3	1.0 ± 0.3	0.8 ± 0.4	1.6 ± 0.7
109	Maltitol ³	6.9E-03	0.9 ± 0.3	1.2 ± 0.4	0.9 ± 0.4	0.6 ± 0.3	1.1 ± 0.5	0.6 ± 0.4	1.1 ± 0.4
110	Mannobiose ³	1.6E-06	1.0 ± 0.6	1.1 ± 0.3	0.9 ± 0.2	0.3 ± 0.1	1.4 ± 0.5	0.7 ± 0.2	0.9 ± 0.2
111	Galactinol ²	1.9E-09	0.9 ± 0.8	2.3 ± 0.7	1.8 ± 0.8	0.3 ± 0.1	1.5 ± 0.8	0.2 ± 0.1	0.7 ± 0.4

Results represent mean ± SD of relative values in relation to quality control (pooled) samples. n : number of replicates ($n = \text{year 1, year 2}$); CN: cold nights-treated samples; ¹: confirmed with analytical standards (ID level 1); ²: putatively identified (mass spectra and retention index) (ID level 2); ³: putatively identified (mass spectra) (ID level 3); ^a: 2-Oxoglutaric acid; ^b: 4-Methylglutaric acid; ^c: Indole-3-acetic acid; ^d: Glucoheptonic acid; ^e: 6-deoxy-mannopyranose; ^f: Glucose-6-phosphate; ^g: Fructose-6-phosphate.

had increased phellandrene levels among samples from the experimental greenhouse, but these levels were still lower compared to 6-week-old dill from Comm_grower.

3.3. Sensory analysis

In the first year, 29 panellists evaluated the samples. They used a total of 80 descriptors to describe all triads. Of these descriptors, 11 were defined by at least five assessors (descriptor frequency > 15%) and were

included in the data analysis. In the second year, 17 assessors evaluated all samples using a total of 105 descriptors, of which 12 descriptors had frequency > 15%. The list of descriptors defined by at least 15% of the panellists is shown in Table S2. Seven descriptors (sour, grass, sweet, bitter, spicy, dill and lemon) were considered of high frequency, being described by at least 25% of assessors of both sensory panels in Years one and two.

PCA of the data generated by the first sensory panel revealed that the two principal components of the model explained 69% of the total

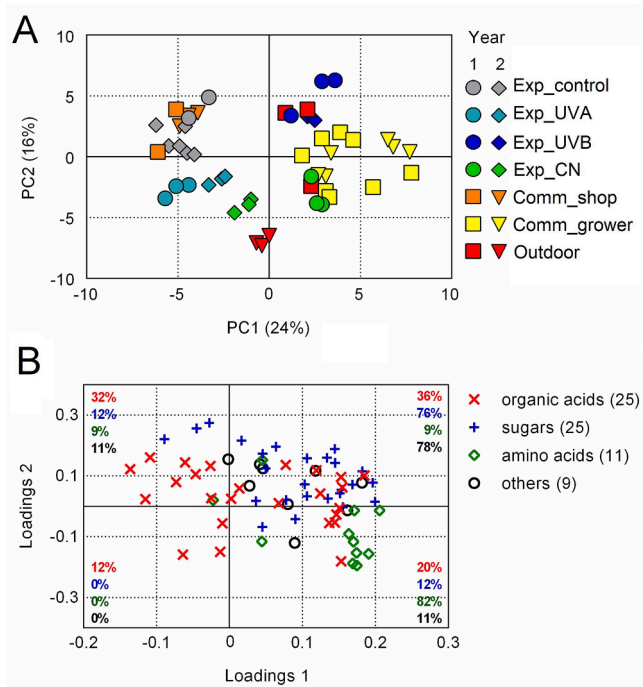


Fig. 2. Metabolite profiling of dill obtained over two consecutive years. (A) PCA scores plot (metabolomics data) of outdoor dill, as well as from greenhouse obtained from a grocery shop (Comm_shop), from a local producer (Comm_grower) and from an experimental greenhouse (Exp_control, Exp_UVA, Exp_UVB and Exp_CN). (B) Features ($n = 70$) were plotted on PCA loadings plot according to their main chemical class. (G): greenhouse samples; Exp_CN: cold night-treated samples. For better legibility of this figure, please refer to the online version.

variance among samples (Fig. 4A). The PCA scores plot shows that the most distinct separation pattern occurs along PC1 and coincides with differences in sample origin. Notably, as observed for the metabolite profile, Exp_control plotted near to Comm_shop, while Exp_UVA, Exp_UVB and Exp_CN plotted near each other and centrally along PC1. Out_Y1 plotted opposite both Exp_control and Comm_shop. On the correlation loadings plot, dill, aniseed, sour, celery, peppery and bitter had positive and strong correlation with PC1, thereby indicating a high association between these sensorial attributes and Out_Y1. On the other hand, grass and sweet had negative values on PC1, which indicates higher association to Exp_control and Comm_shop. Spicy was the main attribute correlated with separation along PC2. These findings suggest that treatment with either UV-A-enriched or UV-B-enriched light or CN appears to improve the characteristic dill flavour compared with control conditions, since the sensory attributes of the dill plants subjected to eustress conditions moved from the situation at outset (Exp_control) towards PC1, i.e. towards the loadings that were related to a characteristic dill taste.

For the data generated by the second sensory panel, the two principal components of the model explained 60% of the variance among samples (Fig. 4B). Exp_control and Comm_shop were plotted on opposite sides across the PC1 axis, while the remaining samples were scattered centrally on the PCA plot. Although the differences with regards to the correlation loadings appear to be reduced compared with the first sensory panel results, PCA also infer distinct patterns of the sensory profile between Exp_control and plants that had been subjected to UV-A-enriched and, UV-B-enriched light and CN conditions. Again, Exp_control was the treatment with least correlation to dill taste but with a higher correlation with lemon taste. Exposure to UV-A-enriched and UV-B-enriched light or CN treatment moved the samples towards the direction of the dill descriptor in PC1.

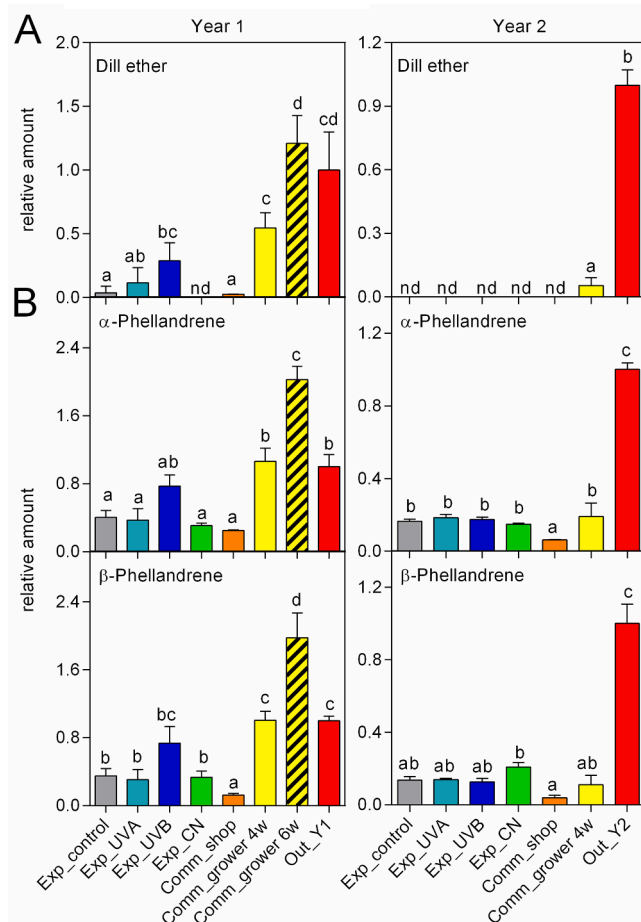


Fig. 3. Flavour-active compounds of dill. (A) Relative levels of dill ether and (B) α - and β -phellandrene in outdoor-grown dill and dill obtained under different greenhouse conditions during two consecutive years. nd: not detected. Results represent mean $\pm$ SD ($n = 3$). Different letters above the bars represent significant differences (ANOVA, Tukey as post hoc, $p < 0.01$).

3.4. Association between metabolic changes induced by UV-A- and UV-B-enriched light or CN treatments and the dill taste

As treatment with either UV-A-enriched or UV-B-enriched light or CN consistently changed the sensory profile from the situation at the outset (i.e. Exp_control) towards a characteristic dill taste, we explored the individual differences at the levels of metabolites and flavour-relevant compounds between the different samples obtained from the experimental greenhouse. As shown in the Venn diagram (Fig. S2), a total of 29 metabolites showed significant differences between Exp_control and at least one type of treated sample (i.e., Exp_UVA, Exp_UVB or Exp_CN). Of these metabolites, 13 showed significant differences between the Exp_control and only one treatment, while 16 metabolites showed significant differences between Exp_control and at least two treatments. No differences were observed with regards to the levels of dill ether and phellandrenes among these samples.

Next, we performed a partial correlation network analysis including the relative levels of the 29 metabolites that showed significantly changed abundance, as well as those high-frequency descriptors defined by at least 25% of panellists of both sensory panels ($n = 7$; sour, grass, sweet, bitter, spicy, dill and lemon). As shown in Fig. 5, network analysis revealed a marked impact of compounds from different chemical classes on dill sensory properties. Amino acids appear to be positively associated with each other and also with dill and sour taste, while total organic acids are positively interconnected with amino acids and with pungency and bitter taste. As expected, sweet taste was positively interconnected

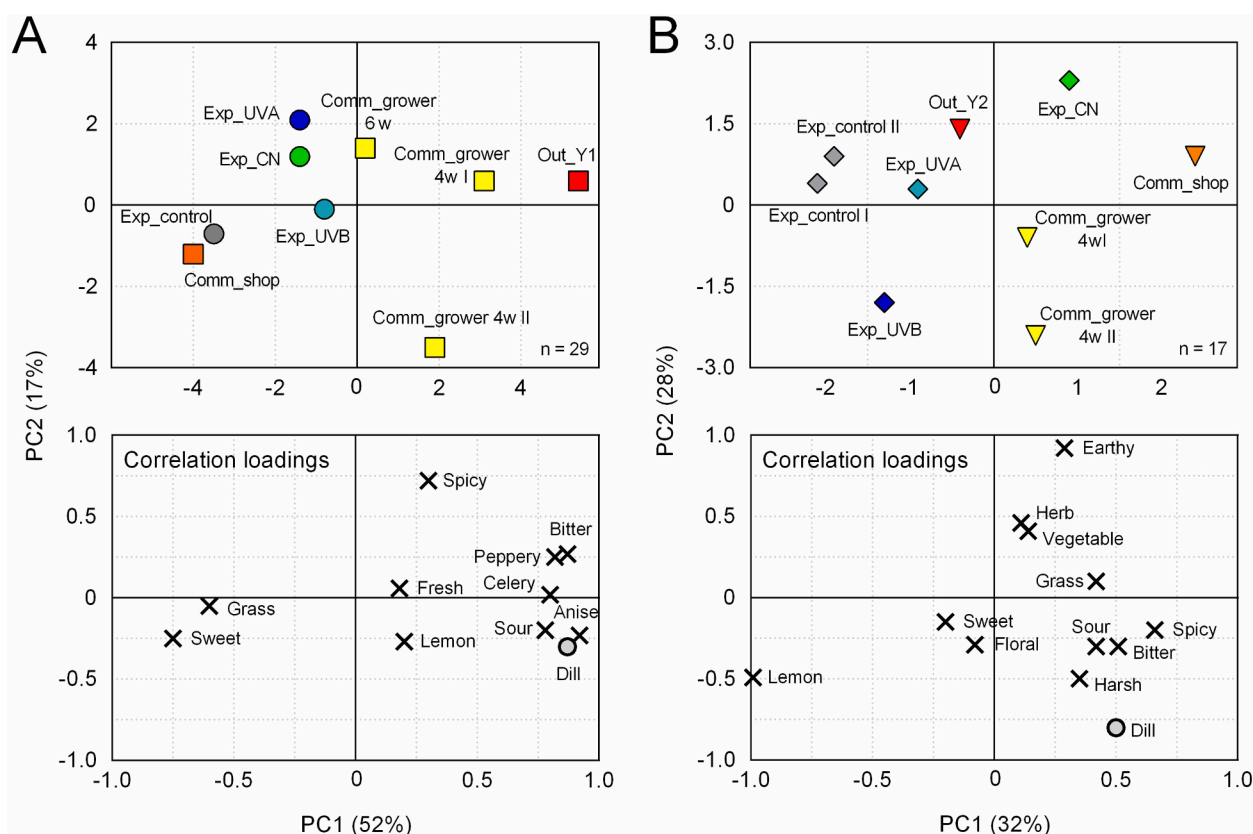


Fig. 4. Sensory profile of dill. PCA scores plot and correlation loadings on the (A) first and (B) second sensory panel.

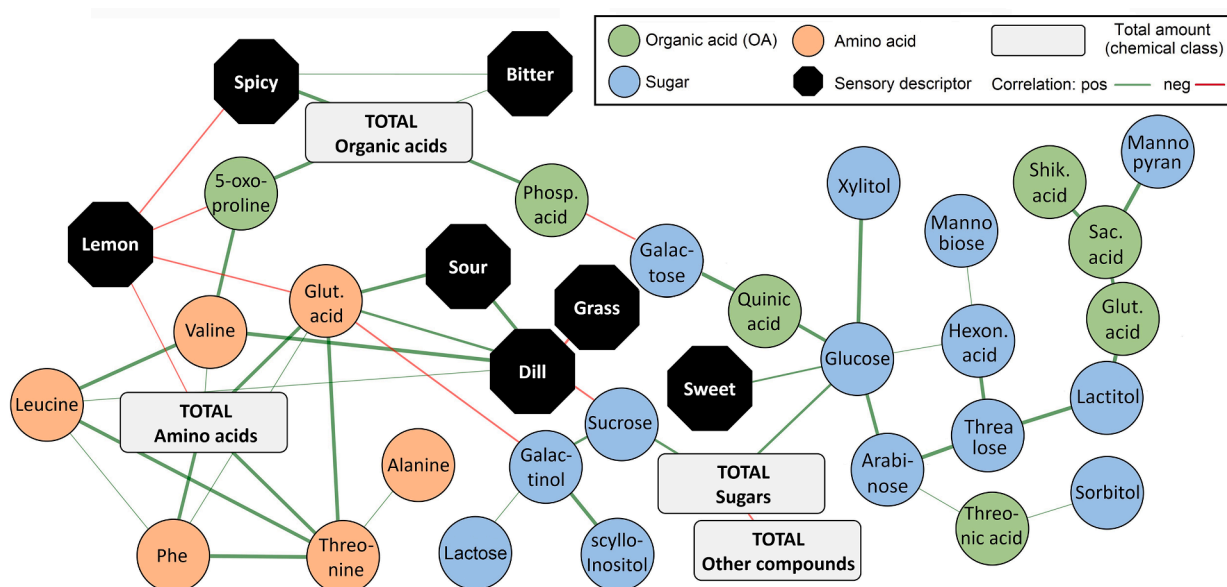


Fig. 5. Partial correlation network showing associations between dill metabolite profile and sensory attributes. Correlation network includes nodes with correlation coefficient > 0.30. Line width was plotted as a function of the strength of the correlation between the variables connected to this particular line, while line colour indicates the direction of the association between the nodes. NI: non-identified (chemical class prediction).

with sugars, but no correlation between sweet taste and a characteristic dill taste was found. Lemon was inversely correlated to pungency and to amino acids levels, and grass was inversely correlated to dill taste. At the individual metabolite levels, valine and glutamic acid were positively associated to dill taste, while sucrose showed an inverse association. Overall, amino acids and related organic acids were positively interconnected between each other and also associated with pungency and

dill, bitter, and sour taste. In addition, they were either not associated, or showed a negative association, with lemon, grass and sweet taste.

4. Discussion

By integrating non-target metabolomics with target analysis of flavour-relevant compounds and sensory data, we were able to provide

new insights linking dill metabolism to its sensory quality. We also revealed that eustress conditions, for instance low temperatures and exposure to UV-A-enriched or UV-B-enriched light, can be applied under indoor production conditions to modulate plant metabolism, thereby improving dill sensory quality. As far as we know, this is the first study that integrates non-targeted metabolomics and sensory data aiming to enhance dill quality.

In order to influence biosynthesis of phytochemicals to produce higher quality vegetable products, knowledge about the plants' metabolome is crucial (Dawid & Hille, 2018). Previous studies have associated dill volatile profile (mainly terpenes, esters and aldehydes) to its sensory quality or to overall consumer preference (Brunke, Hammerschmidt, Koester, & Mair, 1991; El-Zaieddi, Martínez-Tomé, Calín-Sánchez, Burló, & Carbonell-Barrachina, 2017; Madhava Naidu et al., 2016). Here, we applied a GC-MS-based comprehensive non-targeted analysis approach to explore mainly primary metabolites (i.e., sugar, amino acids and organic acids), as well as some phenolic compounds, in outdoor-grown and greenhouse-grown dill. For greenhouse dill, we found that both the sample origin (i.e. obtained from commercial grower or from the experimental greenhouse) and changes in experimental conditions (i.e. in the absence or presence of UV treatment, or in the absence or presence of CN treatment) appear to have a higher impact on plant metabolism compared with the year of production, whilst outdoor-grown dill samples (Out_Y1 and Out_Y2) had pronounced differences in their metabolite pattern. These findings support greenhouse production as a feasible model to replicate metabolite profile of outdoor grown dill. The data also support the concept of application of eustress conditions in greenhouses to modulate plant metabolic profile towards an improvement in quality.

Environmentally relevant doses of UV-A-enriched or UV-B-enriched light induced consistent changes in dill metabolism, while Exp_CN showed larger fluctuations in its metabolite profile between different batches. The variation between Exp_CN batches can be attributed to the protocol applied in the present study, which involved daily/manual transport of plants from the greenhouse to a refrigerator. Although the protocol applied for Exp_CN treatment reduced the reproducibility of results compared to UV treatments, CN had a better correlation with characteristic dill taste compared to Exp_control in both years.

Previous studies have shown that dill ether and α -phellandrene are the main volatile compounds associated with a characteristic dill flavour (Blank, Sen, & Grosch, 1992; Pino, Rosado, Goire, & Roncal, 1995). Our results suggest that plant age is a crucial factor associated with accumulation of these flavour-relevant compounds. The relatively high levels of dill ether and α -phellandrene in outdoor-grown dill are also in line with previous studies showing that a complex combination between biotic and abiotic factors during plant development is associated with the synthesis of specific volatile compounds, as was shown for different plant species (Vivaldo, Masi, Taiti, Caldarelli, & Mancuso, 2017; Wüst, 2018). Surprisingly, sensory analysis revealed that neither dill ether nor α -phellandrene appear to have a strong association with the characteristic dill taste. For example, Exp_UVA, Exp_UVB and Exp_CN had a higher association with dill taste (and a distinct polar metabolite profile) compared with the Exp_control, but no consistent differences were found between the levels of flavour-relevant compounds among samples grown in the experimental greenhouse. A possible interpretation of these data is that metabolites other than dill ether and α -phellandrene have a strong modifying effect on the perception of dill taste by panel members.

Although previous studies focused mainly on the analysis of volatile compounds to explore its sensory quality (Amanpour, Kelebek, & Selli, 2017; Brunke, Hammerschmidt, Koester, & Mair, 1991; El-Zaieddi, Martínez-Tomé, Calín-Sánchez, Burló, & Carbonell-Barrachina, 2017; Madhava Naidu et al., 2016), non-volatile compounds including sugars, organic acids, amino acids, lipids, and phenolic compounds are also present in dill (El-Zaieddi et al., 2017; Saleh, Selim, Jaouni, & Abdelgawad, 2018). To explore whether other metabolites potentially were

associated with improvement of dill sensory quality, individual comparisons were made between metabolite levels of Exp_control and Exp_CN, Exp_UVA and Exp_UVB. Notably, the abundance of five amino acids (alanine, phenylalanine, glutamic acid, valine, and leucine) increased in samples exposed to eustress compared with Exp_control. Integration of analytical and sensory data also revealed a positive and strong association between dill taste and sour taste. These two sensory attributes were also positively associated to glutamic acid levels. Therefore, the development of a characteristic dill taste appears to be associated with an increase in the levels of "sour metabolites", such as glutamic acid (Bachmanov et al., 2016). Our results also suggest organic acids as contributors of dill sensory quality, since positive associations between amino acids and organic acids interconnected dill taste to both pungency and bitter taste, which were more highly correlated to the outdoor-grown samples (as well as to Exp_CN, Exp_UVA and Exp_UVB) than to Exp_control. Valine, which is described as having a predominantly bitter taste (Kawai, Sekine-Hayakawa, Okiyama, & Ninomiya, 2012), was also directly correlated with dill taste. On the other hand, attributes highly correlated with the greenhouse control, such as lemon and grass, were inversely associated to organic acids and amino acids. These findings are in agreement with a recent study showing an association between higher levels of free amino acids and organic acids with a desired bitter taste and pungent characteristic of thyme, sweet basil and cardamon (Duan, Huang, Xiao, Zhang, & Zhang, 2020). Our data also support the notion that increases in amino acids and organic acids are associated with the development of desirable sensory attributes by changing the taste of dill samples from lemon/grass (greenhouse control) to a more bitter/spicy taste (such as after CN-treatment and growth under UV-A-enriched and UV-B-enriched light). The apparent contradictory association between increase in the levels of organic acids and reduction in characteristic lemon taste has also previously been observed for grape-derived products (Dupas de Matos et al., 2017). Apparently, interactions between compounds from a number of different chemical classes determine food sensory quality (Diez-Simon, Mumm, & Hall, 2019), thereby reinforcing the importance of applying non-targeted and comprehensive methods to explore the intricate relationship between plant metabolites and an 'overall sensory experience'.

Interestingly, sugars (which obviously were associated with sweet taste) were mainly interconnected with each other and showed an inverse association with both dill taste and other compounds, such as small phenolic compounds. These findings suggest that sweet taste is not directly associated with improvement in dill sensory quality, even though dill plants grown under UV-B-enriched light led to increased levels of most sugars and to increase in characteristic dill taste. Such contrasting results may be explained by the negative association that was found between sugars and other compounds. Indeed, other compounds – particularly phenolic compounds arising from plant secondary metabolism – not only confer plant protection but also are correlated to characteristic bitterness of plant-derived foods (Soares et al., 2013). As a consequence, sensory characteristics of dill may rely at least partially on these phenolic compounds, which were not fully covered by our analytical approach. Although dill ether and phellandrenes represent more than 50% w/w of dill volatile compounds (Amanpour, Kelebek, & Selli, 2017), other volatiles found at lower concentrations may also contribute to sensory quality. Thus, further studies integrating the analysis of secondary metabolites in the context of dill quality will help to close the knowledge gap with regards to dill metabolism and sensory attributes.

5. Conclusions

Taken together, our results showed that eustress (for instance low temperature during night or supplementary UV-A- or UV-B-enriched exposure) can improve sensory quality of greenhouse dill, increasing its similarity in taste with that of outdoor-grown dill. Such improvements in dill sensory quality are associated with accumulation of organic

acids and free amino acids, which in turn relate to changes in consumer perception in a direction from grass/lemon taste towards a more characteristic bitter/spicy dill-related taste.

Although one of the main foci of this study was to explore how low temperatures and supplementary UV exposure can improve dill sensory quality, it is expected that our approach can be expanded to other greenhouse crops. By further understanding the physiological processes linking eustress conditions to changes in crop metabolism (and its impact on sensory quality), it will be possible not only to optimise agricultural practices with the aim of improving crop quality and/or nutritional value, but also to provide support for breeding programs for development of high-quality agricultural traits.

CRediT authorship contribution statement

Victor Castro-Alves: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Irina Kalbina:** Conceptualization, Methodology, Validation, Investigation, Writing - original draft. **Asgeir Nielsen:** Formal analysis, Investigation. **Mats Aronsson:** Methodology, Investigation. **Eva Rosenqvist:** Conceptualization. **Marcel A.K. Jansen:** Conceptualization. **Minjie Qian:** Methodology, Investigation. **Åsa Öström:** Conceptualization, Methodology, Resources, Funding acquisition. **Tuulia Hyötyläinen:** Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition. **Åke Strid:** Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2020.128714>.

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