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**Volatile organic compounds as markers of quality changes during the storage of wild
rocket**

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Abstract

The quality of leafy green vegetables changes during storage. Leaves become yellow or disintegrate, and an off-odor may develop. In addition, small amounts of volatile organic compounds (VOCs) are released. In this study, the release of acetone, carbon disulfide, dimethyl sulfide, nitromethane, pentane, 3-methylfuran, 2-ethylfuran, and dimethyl disulfide from wild rocket with different **initial** qualities was monitored during 8 d storage at 10 °C and correlated to aerobic bacteria counts, yeast and mold counts, and degree of tissue disintegration. The release of

VOCs, except for 3-methylfuran, was influenced by the initial quality of the leaves. The release of pentane and 2-ethylfuran was related to the degree of tissue disintegration, and the release of dimethyl sulfide and dimethyl disulfide was related to the total aerobic bacteria count. The results demonstrated that VOCs can be used as markers for monitoring the complex quality changes taking place in packaged fresh produce during storage.

Keywords: fresh produce, leaf age and leaf wetness, volatile organic compounds, respiration rate, aerobic bacteria, leaf disintegration

Chemical compounds studied in this article

Acetone (PubChem CID: 180); Carbon disulfide (PubChem CID: 6348); Dimethyl disulfide (PubChem CID: 12232); Dimethyl sulfide (PubChem CID: 1068); 2-Ethylfuran (PubChem CID: 18554); 3-Methylfuran (PubChem CID: 13587); Nitromethane (PubChem CID: 6375); Pentane (PubChem CID: 8003).

1. Introduction

The quality of fresh produce is defined as a combination of attributes related to appearance, texture, odor, flavor, taste, nutritional value, and the safety of a product (Kader, 2002). In combination, these attributes determine the quality and value of a product as a food (Kader, 2002). The loss of a single attribute may lead to loss of overall quality, to termination of the shelf life and to consumer rejection (Barrett, Beaulieu, & Shewfelt, 2010). Such a loss of a single attribute is defined as the shelf life limiting factor. There can be more than one shelf life limiting factor for packaged leafy green vegetables, for instance yellowing of the leaves, loss of vitamin C, excessive growth of microorganisms, and formation of off-odors (Amodio, Derossi, Mastrandrea, & Colelli, 2015; Koukounaras, Siomos, & Sfakiotakis, 2007; Løkke, Seefeldt, & Edelenbos, 2012; Martínez-Sánchez, Marín, Llorach, Ferreres, & Gil, 2006).

Control and monitoring of the quality of leafy green vegetables in the supply chain is challenging due to large number of factors that can affect quality, such as **genotype, growing conditions**, raw material quality, processing, packaging, and storage conditions (Bell, Oruna-Concha, & Wagstaff, 2015; Brosnan & Sun, 2001; Kader & Saltveit, 2003; Løkke, et al., 2012; Nielsen, Bergström, & Borch, 2008). In addition, variability in shelf life limiting factors requires a large number of analytical techniques to monitor separate quality attributes. Instrumental analyses and sensory evaluation can be applied for evaluation of the quality of packaged fresh leafy green vegetables, but these methods are time consuming and require substantial human and financial resources. In addition, the number of samples that can be evaluated in a day is limited. Therefore, there is a need to develop alternative **analytical techniques** for quality control **that can combine measurements of all or the majority of important quality attributes into one analysis**. These techniques can then be

applied at a scientific or commercial level to improve the shelf life of fresh leafy green vegetables in the supply chain and to reduce food waste.

Over the last decade, there has been an increased interest in the usage of volatile organic compounds (VOCs) as markers of quality changes in leafy green vegetables (Cozzolino, Martignetti, Pellicano, Stocchero, Cefola, Pace, et al., 2016; Lonchamp, Barry-Ryan, & Devereux, 2009). The main idea behind this approach is that VOCs can be used as direct markers of sensory quality changes, such as odor and flavor, or as markers of the processes involved in the quality changes. As a part of the development of such a new quality monitoring approach, various sampling methods have been established to collect VOCs released from leafy green vegetables. These methods included non-destructive sampling of VOCs from the headspace of intact leafy green vegetables by solid-phase microextraction (SPME), solid-phase extraction (SPE), and static headspace sampling (Luca, Bach, & Edelenbos, 2015b; Luca, Mahajan, & Edelenbos, 2016; Nielsen, et al., 2008), and sampling of VOCs from the headspace of wounded leaves (Bell, Spadafora, Müller, Wagstaff, & Rogers, 2016; Cozzolino, et al., 2016). After collection, as a general approach, VOCs are separated and measured by means of gas chromatography-mass spectrometry (GC-MS).

VOCs are released from leafy green vegetables in response to genotype, storage time, storage temperature, microbial load, headspace gas composition and O₂ availability (Bell, et al., 2016; Cozzolino, et al., 2016; Luca, et al., 2016; Nielsen, et al., 2008), however, little is known about the generation of VOCs and the relationships between VOCs and quality changes of leafy green vegetables. Nielsen, et al., (2008) reported a strong correlation between the perceived off-odor of rocket (*Eruca sativa* Mill.) and the concentrations of dimethyl sulfide and dimethyl disulfide. In the case of radicchio di Chioggia (*Cichorium intybus* L.), Cozzolino, et al. (2016) found a positive

correlation between ethyl 2-methylpropionate, ethyl 3-methylbutyrate, ethyl pentanoate, 2-ethylfuran, and octane and a sweet taste and a negative correlation between these compounds and freshness, while it was opposite for hexanal, 2-hexenal and benzeneacetaldehyde.

The aim of this study was to investigate the relationship between the release of VOCs from undamaged wild rocket and changes in postharvest quality to verify if VOCs can be used as markers for quality changes in packaged wild rocket during storage. Unwashed 27-d old (young) and 32-d old (old) leaves with differences in surface wetness (dry or wet) were selected for the studies to represent different initial qualities of wild rocket. The leaves were stored under aerobic conditions for 8 d at 10 °C, and the changes in the gas and VOC composition were monitored regularly. At the beginning and end of storage, the aerobic bacteria count, the yeast and mold count, and the index of leaf damage were determined, and the results were correlated with VOC data to identify potential markers of quality changes.

2. Materials and Methods

2.1 Plant material

Seeds of wild rocket (*Diplotaxis tenuifolia* L., ‘Captiva’) were sown on June 6, 2014 (old leaves) and on June 11, 2014 (young leaves) on two nearby fields at a commercial farm in Denmark. The leaves were manually harvested at 10 – 11 AM on July 8, 2014 after 27 and 32 days of production. The age of the leaves reflected different developmental stages in commercial production and was in agreement with previous findings in literature (Hall, Jobling, & Rogers, 2013; Kenigsbuch, Ovadia, Shahr-Ivanova, Chalupowicz, & Maurer, 2014). After harvest the leaves were vacuum-

cooled to 4.5 °C, transported in boxes wrapped with plastic liners to Aarhus University, Årslev, and stored overnight in a walk-in cold room kept at 10 °C and >90% relative humidity (RH).

2.2 Sample preparation and storage conditions

Samples were prepared in the cold room. Leaves were manually sorted to remove wounded and defective leaves, and small leaves, less than 3 cm in length. Samples of sorted young and old leaves were taken for initial quality measurements (dry matter content, respiration rate, color, electrolyte leakage, and microbial load) and for storage experiments in 1-L glass jars. Jars were washed with distilled water, dried, and rinsed with methanol before use. The methanol was allowed to evaporate before the jars were filled with leaves. Samples of approximately 12.5 g of young or old leaves were gently placed in the jars. For studies on wetness, 6.0 mL of sterile ultra-pure water were added to the already filled jars, and lids with a septum were mounted on top of all jars. Water was added to some of the young and old leaves before storage to increase the wetness of the surface of the leaves to favor microbial growth during storage (Huber & Gillespie, 1992). After approximately 1 h, all jars were made air tight by closing the lids. A total of 12 jars were prepared for the storage experiment with three jars containing young dry (no water applied), young wet (water applied), old dry (no water applied) or old wet (water applied) leaves. Jars were randomly placed on a table in the walk-in cold room and stored for 8 d at 10 °C in darkness. Light was switched on periodically during gas and VOC sampling.

2.3 Measurement of gas composition and determination of respiration rate

The gas composition (O₂ and CO₂) of the jars containing leaves for VOC analysis was measured on days 1, 3, 5, 7, and 8 with a CheckMate 9900 Headspace Gas Analyzer (PBI Dansensor, Ringsted, Denmark).

127 The respiration rate was determined at 10 °C on triplicate samples of dry young and old leaves
128 before the beginning of the experiment. Approximately 50 g of leaves were incubated in a 1-L
129 glass jar with a known exact volume and closed with a lid mounted with a septum. The O₂ and
130 CO₂ contents were measured after 0 and 5 h of incubation using a Checkmate gas analyzer. For
131 determination of the respiration rates, the free volume of each jar was determined gravimetrically
132 by filling the jars with water. The respiration rate was expressed as the rate of consumed O₂
133 (RRO₂) and the rate of produced CO₂ (RRCO₂) in mL kg⁻¹ h⁻¹ on a fresh weight basis.

134 2.4 Dry matter content

135 The dry matter content was determined on triplicate samples of young and old leaves by weighing
136 60 g leaves before and after drying at 80 °C for 24 h in a ventilated oven (Lytzen, Herlev,
137 Denmark).

138 2.5 Color analysis

139 The color of the leaves was determined on four samples of young dry and old dry leaves at start of
140 the experiment and of young dry, young wet, old dry, and old wet leaves after 8 d storage at 10
141 °C. A CM-600D spectrophotometer (Minolta, Osaka, Japan) equipped with a F8 mm aperture was
142 used. Spectral data were obtained at a 10° standard observer and a D₆₅ standard illuminant at a 10
143 nm wavelength pitch in the 400 – 700 nm wavelength range. The equipment was calibrated with a
144 standard white tile supplied by the manufacturer. The color of the leaves was measured in the CIE
145 $L^*a^*b^*$ color space. The lightness ranged from $L^* = 0$ (black) to $L^* = 100$ (diffuse white). The
146 greenness of the leaves was determined from the hue value which was calculated as $\text{hue} = 180^\circ +$
147 $\tan^{-1}(b^*/a^*)$ (McGuire, 1992). The higher the hue value, the greener the leaves. The color was
148 determined on four leaves from each triplicate sample taking five readings on each leaf.

2.6 Index of leaf damage

The index of leaf damage (I_{ld}) was determined by measurements of electrolyte leakage. Electrolyte leakage was determined on three samples of the young dry and the old dry leaves at start of the experiment and again after 8 d storage at 10 °C using the samples for gas and VOC analysis. Cleaned jars containing approximately 12.5 g of leaves were prepared for the initial measurement. Five hundred milliliters of sterile, ultra-pure water were added to each jar before the jars were incubated at 23 °C for 30 min in a Tecator Shaking Water Bath 1024 (Hoganas, Sweden). For the measurements after storage, 500 mL of sterile, ultra-pure water were added to the samples with dry leaves and 494 mL to the samples with the wet leaves. After shaking in the water bath, 1.0 mL of liquid was taken for microbiological analyses. The initial electric conductivity ($EC_{initial}$) and the total electric conductivity (EC_{total}) were determined with an electrical conductivity meter (Crison MM40, Crison Instruments S.A., Barcelona, Spain) at room temperature. The total conductivity was determined after heating up the water with wild rocket to 68 °C for 12 h and later bringing it back to ~23 °C. From the obtained values, the electrolyte leakage at day 0 ($EL_{t=0}$) and day 8 ($EL_{t=8}$) was calculated as follows:

$$EL_{t=0} = 100 * \frac{EC_{initial(t=0)}}{EC_{total(t=0)}} \quad and \quad EL_{t=8} = 100 * \frac{EC_{initial(t=8)}}{EC_{total(t=8)}} \quad (1,2)$$

I_{ld} was calculated as reported by Flint, Boyce, and Beattie (1967) with some modifications:

$$I_{ld} = \frac{(EL_{t=8} - EL_{t=0})}{\left(1 - \frac{EL_{t=0}}{100}\right)} \quad (3)$$

Leaves with a high I_{ld} had a high degree of tissue damage and disintegration.

2.7 Microbiological analyses

The number of microorganisms on the leaves was determined on triplicate samples of young dry and old dry leaves at the start of the experiment and on the samples used for gas and VOC analysis after 8 d of storage at 10 °C. Total aerobic bacteria (AE) and yeast and molds (YM) were determined using a 3M™ Petrifilm™ Aerobic Count Plate and a Yeast and Mold Count Plate (3M Health Care, St. Paul, MN, USA), respectively. Liquid samples of 1.0 mL of water containing detached microorganisms were removed from the jars after shaking in the water bath as described in Section 2.6. The samples were then analyzed by making appropriate dilutions by adding sterile, ultra-pure water. Aliquots of 1.0 mL were spread on the respective Petrifilm™ and incubated at 30 °C for 2 d (AE) and at 23 °C for 5 d (YM). The results are expressed as CFU g⁻¹ fresh weight.

2.8 Measurement of volatile organic compounds (VOCs)

VOCs from the headspace of the jars were analyzed on days 1, 3, 5, 6, 7, and 8 as described by Luca, Bach, and Edelenbos (2015a). For the extraction of VOCs, an SPME fiber (85 µm carboxen/polydimethylsiloxane (CAR/PDMS) stableflex/SS) mounted on a manual holder (both obtained from Supelco, Bellefonte, PA, USA) was exposed to the wild rocket headspace for 5 min at 10 °C. Next, the fiber was transferred to a second 1-L glass jar kept at 18 °C for 5 min for external standard (3-methyl-2-pentanone) addition before it was thermally desorbed at 200 °C for 1 min in a gas chromatograph (GC) inlet equipped with a SPME liner (Supelco, Bellefonte, PA, USA). VOCs were separated on an Agilent 7890A GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a HP-PLOT/Q (Agilent Technologies, Palo Alto, CA, USA) column (30 m x 0.32 mm x 20 µm). Compounds were detected on an Agilent 5975C inertXL mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) operating in electron impact mode at 70 eV. The temperature program of the GC oven was 30 °C for 1 min, increasing at 20 °C min⁻¹ to 250 °C and finally held at 250 °C for 10 min. VOCs were detected in the selected ion monitoring mode (Luca,

et al., 2016) and given on fresh weight basis as earlier described (Luca, et al., 2015a). From the data, the changes in the VOC concentrations over time were calculated and given as release rates ($d[\text{VOC}]/dt$) as described by Luca, et al., (2016).

2.9 Statistical analyses

Comparisons of the initial quality (RRO_2 , RRCO_2 , dry matter content, L^* , hue, AE, and YM) of the young dry and old dry leaves were performed by a one-way analysis of variance (ANOVA) at a 5% significance level. This analysis was also used to analyze the effects of leaf quality (young dry, young wet, old dry and old wet leaves) on the partial pressure of O_2 and CO_2 , L^* , hue, AE, YM, I_{ld} , and concentrations of VOCs on day 8. Analysis of covariance (ANCOVA) at a 5% significance level was applied to investigate the effects of leaf age (dry leaves only) and wetness (between leaves of same age) on the VOC release. The response variable was the VOC concentration, the categorical explanatory variables were the leaf age and the leaf wetness, and the quantitative explanatory variable was the storage time. Simple linear regression models of the VOC concentrations relative to the quantitative explanatory variable were applied to calculate the VOC release rates as the slopes of the models. The relationships between AE, YM, I_{ld} and the VOC concentration at day 8 were studied by simple regression analysis. The concentrations of acetone, dimethyl sulfide and dimethyl disulfide were ln-transformed before the statistical analyses as these compounds demonstrated an exponential-like increase in contents with time (Luca, et al., 2016). Tukey's honest significance difference (HSD) test was used for multiple comparisons at $P = 0.05$. All analyses were performed with the R software version 3.1.1 (R Core Team, 2014).

3. Results and Discussion

3.1 Raw material quality

The initial quality and characteristics of the young **dry** and old **dry** wild rocket leaves are summarized in Table 1. The initial respiration rate of the young leaves was higher than that of the old leaves. Young leaves consumed 98.4 mL O₂ kg⁻¹ h⁻¹ and produced 109.2 mL CO₂ kg⁻¹ h⁻¹ while old leaves consumed 61.4 mL O₂ kg⁻¹ h⁻¹ and produced 65.3 mL CO₂ kg⁻¹ h⁻¹ (Table 1). The differences in the respiration rates between young and old leaves correspond to differences in the maturity of the leaves at harvest. Young leaves were harvested after 27 d and old leaves after 32 d and they had different dry matter contents; 9.2 g 100 g⁻¹ for young leaves and 7.0 g 100 g⁻¹ for old leaves. Koukounaras, et al., (2007) reported that young rocket leaves had higher respiration rates than old leaves. In addition, Seefeldt, Løkke, and Edelenbos (2012) found that wild rocket with higher dry matter contents had higher respiration rates than leaves with lower dry matter contents.

The young and old leaves had similar color at harvest as the differences in the L^* and hue values were non-significant (Table 1). Similar results were reported by Koukounaras, et al., (2007), who found no effect of leaf age on leaf color and chlorophyll contents. The hue values of the leaves in the present study was 111.9° for young and 114.2° for old leaves and thus above the 110° level which are associated with high sensory greenness (Løkke, Seefeldt, Skov, and Edelenbos (2013).

The AE counts were higher on old than on young leaves but there were no differences in the YM counts (Table 1). Microorganisms are commonly present on fresh produce at harvest and may cause deterioration during postharvest storage (Martínez-Sánchez, et al., 2006; Nielsen, et al., 2008).

3.2 Change in gas composition during storage

The partial pressure of O₂ dropped to 10.3–13.5 kPa and the partial pressure of CO₂ increased to 8.3–11.7 kPa after 8 d storage at 10 °C (Fig. 1). The leaves were thus kept under aerobic conditions

throughout storage, as the partial pressure of O₂ never fell below 2.1 kPa (Luca et al., 2016). As the quality of wild rocket varies depending on the gas composition (Løkke, et al., 2012; Luca, et al., 2016), it is crucial to store wild rocket under aerobic conditions as in the present experiment. There were differences in the final gas composition between samples (Fig. 1). Due to reduction of free volume of the jars after addition of 6.0 mL of water into wet leaf samples, the partial pressures of O₂ in the headspace of young wet and old dry on day 8 were not significantly different. The same was also observed for the partial pressures of CO₂ in the same samples. However, the partial pressures of both O₂ and CO₂ in the headspaces of young dry and old dry leaves were significantly different. Similarly, the partial pressures of both O₂ and CO₂ were significantly different in wet leaf samples. This was expected because young leaves had higher respiration rates than old leaves (Table 1). The O₂ contents thus decreased faster in the jars filled with young than with old leaves.

3.3 Changes in volatile organic compounds during storage

Acetone, carbon disulfide, dimethyl sulfide, nitromethane, pentane, 3-methylfuran, 2-ethylfuran, and dimethyl disulfide were continuously released into the headspace of wild rocket (Fig. 2) and this result correspond to earlier findings (Luca, et al., 2016). The VOC release changed during storage in response to the raw material quality at start of the experiment except for the concentration of 3-methylfuran, which did not differ between samples. The highest concentrations of pentane, 2-ethylfuran, dimethyl sulfide, and dimethyl disulfide were detected in the headspaces of the young wet and the old wet leaves. The concentrations of these compounds in young wet and old wet leaves were not significantly different ($P>0.05$). However, significantly higher ($P\leq 0.05$) concentrations of carbon disulfide, nitromethane, and acetone were detected in the headspace of young wet leaves when compared to old wet leaves. The highest concentration of acetone and the lowest concentrations of pentane, 2-ethylfuran, dimethyl sulfide, and dimethyl disulfide were

detected in the headspace of the young dry leaves. Based on the final concentrations, there were no differences in the VOC profiles of the old dry and old wet leaves; however, there was a tendency ($P = 0.058$) that the concentration of dimethyl sulfide was higher in the headspace of the old wet than of the old dry leaves.

Interpretation of the results based on final concentrations may lead to misinterpretations as the variations in the initial concentrations and the changes over time are not considered. In addition, lack of significant differences due to high variations between replicate samples may also lead to misinterpretations, as with the data on dimethyl disulfide in the present study (Fig. 2f). Others also report high variations between replicate samples in the VOC analysis (Bell, et al., 2016; Luca, et al., 2016) but the reasons for this result are not well understood. To overcome these challenges in the analysis, the release rates of VOCs over time were studied (Table 2). All compounds, except for acetone, dimethyl sulfide, and dimethyl disulfide, exhibited linear increases with time. In the case of dimethyl sulfide and dimethyl disulfide, linearity was obtained after ln-transformation of the values. As the original and ln-transformed values of acetone were non-linear, acetone was investigated separately. The release rates ($d[\text{VOC}]/dt$) of all VOCs, except for 3-methylfuran, were affected by leaf age (Table 2). Carbon disulfide was released at higher rates from the young dry than from the old dry leaves. There was also a tendency ($P=0.053$) that nitromethane was released at a higher rate from young dry than from old dry leaves. On the contrary, dimethyl sulfide, pentane, 2-ethylfuran and dimethyl disulfide were released at higher rates from the old dry than from the young dry leaves.

A higher release of carbon disulfide and nitromethane from the young than from old leaves (Table 2) corroborated with a higher respiration rate of young than of old leaves (Table 1). This result is in agreement with previous findings that carbon disulfide and nitromethane are released in

response to the respiration of wild rocket (Luca, et al., 2016). Wetness increased the release of all VOCs, except for nitromethane, from the young leaves and dimethyl sulfide, pentane, 2-ethylfuran, and dimethyl disulfide from the old leaves (Table 2). These results indicate that different intrinsic and extrinsic factors have an impact on the release rates of VOCs from wild rocket during storage.

3.4 Quality wild rocket after storage

The quality and characteristics of wild rocket after 8 d storage at 10 °C are shown in Table 3. At the end of storage the partial O₂ pressure was above 10.3 kPa. The L^* value increased from 42.2 to 45.1 in young dry and from 42.2 to 46.6 in old dry leaves during storage, while the hue values decreased to below 110° except for the old wet leaves (Table 1, 3). This result shows that the green color of leaves decreased with storage time in agreement with earlier findings (Løkke, et al., 2013). After 8 d storage at 10 °C, the L^* and hue values were not different between the young dry and the old dry leaves, showing that the leaves' colors were similar. In contrast, the L^* value of the young wet leaves was significantly higher than the L^* value of old wet leaves. However, this observation cannot be directly attributed to difference in the color since the reflective properties of light are altered by presence of water on the surface of the plant tissue (Heusinkveld, Berkowicz, Jacobs, Hillen, & Holtslag, 2008).

The AE and YM values increased during 8 d storage at 10 °C (Table 3). Growth of microorganisms during postharvest storage of rocket at refrigerated conditions (4 – 8 °C) in air or modified atmospheres has also been reported in other studies (Martínez-Sánchez, et al., 2006; Nielsen, et al., 2008). The AE loads were not different between the young wet, the old dry, and the old wet leaves but the young dry leaves had lower AE counts. Parallel, the young wet leaves had higher YM than the other leaves. Wetness had a clear impact on the microbial load of young leaves but

not on the old leaves. There were no differences between the I_{ld} of young dry, young wet, old dry, and old wet leaves after 8 d storage at 10 °C. Generally, the I_{ld} was very low, ranging from 0.6% to 2.8%, and thus the differences in leaf damage were very small.

3.5 Relationship between volatile organic compounds and quality

The correlation coefficients between AE, YM, I_{ld} and the final VOC concentrations are summarized in Table 4. One outlier (pentane in the young wet sample) was removed before analysis on suspicion of faulty instrumentation. High positive correlations were found between AE counts and concentrations of dimethyl sulfide ($r = 0.88$), pentane ($r = 0.82$), 2-ethylfuran ($r = 0.80$), and dimethyl disulfide ($r = 0.95$). In contrast, these VOCs did not correlate well with the YM counts ($r = 0.46 - 0.67$). These findings suggest that dimethyl sulfide, pentane, 2-ethylfuran, and dimethyl disulfide were produced upon growing aerobic bacteria on wild rocket leaves during storage. The AE counts were also positively correlated with the I_{ld} data ($r = 0.80$; results not shown) indicating that **disintegration of the plant tissue took place during growth of AE bacteria**. The I_{ld} was also positively correlated with the concentration of dimethyl sulfide ($r = 0.94$), pentane ($r = 0.89$), 2-ethylfuran ($r = 0.79$), and dimethyl disulfide ($r = 0.85$), and this result indicates that tissue disintegration, growth of AE bacteria and formation of specific VOCs were related. From this result it is suggested that dimethyl sulfide, pentane, 2-ethylfuran, and dimethyl disulfide may be used as markers of plant tissue disintegration caused by growth of AE bacteria.

Release of pentane and 2-ethylfuran with storage time and formation during wounding (Luca, et al., 2016) suggests that these compounds may form from cell damage. Pentane is known as a marker of lipid peroxidation and is suggested to be formed from β -scission of the 13-hydroperoxide of linoleic acid (Belitz, Grosch, & Schieberle, 2009). The mechanism of 2-

ethylfuran formation in plant tissue is not clear although this compound is often reported in the headspace of wounded plant tissues (Cozzolino, et al., 2016; Deza-Durand & Petersen, 2014; Luning, de Rijk, Wichers, & Roozen, 1994; Wang, Xing, Chin, Ho, & Martin, 2001). Formation of 2-ethylfuran in fish is suggested to occur through a mechanism involving β -cleavage of fatty acid hydroperoxides including the 12-hydroperoxide of linolenate followed by a formation of vinyl hydroperoxide in the presence of O₂, loss of a hydroxyl radical, and cyclization of the formed alcoxyl radical (Medina, Satué-Gracia, & Frankel, 1999). The degradation of linolenate hydroperoxides during the oxidation of olive oil has also lead to formation of 2-ethylfuran (Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003). The same mechanisms may take place in plant tissues. In 2001, Wang, et al., reported dramatic increases in the concentration of 2-ethylfuran in transgenic tomato leaves having high contents of fatty acids. Consequently, pentane and 2-ethylfuran seem to be markers of fatty acid oxidation taking place in wild rocket during disintegration of the tissue.

The non-linear release of dimethyl sulfide and dimethyl disulfide in the samples with wet leaves and the positive correlation with AE points to fact that these compounds are of microbial origin which is in agreement with Nielsen, et al., (2008). The microbial origin of these compounds also explains the high variations in the concentrations (Fig. 2).

No correlation was found between AE, I_{ld} , and the final concentration of carbon disulfide (Table 4). However, there was a positive correlation between YM and the carbon disulfide concentration ($r = 0.78$). Based on this finding, higher release rates from young wet than from young dry leaves (Table 2) may relate to the higher YM counts in young wet than in young dry leaves (Table 3) and higher carbon disulfide concentrations from the action of yeast and molds on wild rocket leaves. Carbon disulfide is produced during respiration and from wounded leaves (Luca, et al., 2016).

However, there was no correlation between carbon disulfide and I_{ld} in the present study (Table 4). Overall, there was no correlation ($r = -0.36 - 0.59$) between the concentration of nitromethane and the values for AE, YM, and I_{ld} (Table 4) probably because nitromethane is a marker of product respiration. No correlation ($r = -0.16 - 0.62$) was found between 3-methylfuran and AE, YM, and I_{ld} (Table 4). The release of 3-methylfuran was unaffected by leaf age (Table 2) suggesting that the release of 3-methylfuran was controlled by other processes than respiration. Wetness had a positive effect on the release rates of 3-methylfuran from young leaves but no effect on the release rates from old leaves. The storage temperature influenced the release of 3-methylfuran (Luca, et al., 2016), however, the origin of this compound is unknown and needs further investigations.

Acetone was negatively correlated to the AE counts ($r = -0.76$), while there was no correlations to YM or I_{ld} (Table 4) and the origin of acetone still remains unknown. Based on the non-linear release of acetone and the absence in the VOC profile of wounded wild rocket (Luca, et al., 2016) acetone may originate from microorganisms not encountered in this study.

4. Conclusions

The release rates of VOCs from wild rocket stored for 8 d at 10 °C under aerobic conditions were influenced by the initial quality of the leaves. The release rate of nitromethane was related to the respiration rate of the wild rocket and was primarily influenced by the maturity of the leaves, as young leaves had higher respiration rates than older leaves. The release rate of carbon disulfide was also related to the respiration rate but there was also a positive correlation with the YM counts. The release of pentane and 2-ethylfuran was related to the disintegration of the tissue during storage, and the concentrations of dimethyl sulfide and dimethyl disulfide were related to the AE counts. The study showed that five VOCs were related to different extrinsic and intrinsic quality

processes of wild rocket during storage; nitromethane was a marker of respiration, pentane and 2-ethylfuran were markers of tissue disintegration, and dimethyl sulfide and dimethyl disulfide were markers of bacterial growth on the leaves. The use of the VOC method demonstrates that it is a promising tool for monitoring quality changes in leafy green vegetables. However, more studies are needed to determine appropriate VOC markers of quality changes in leafy green vegetables.

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Conflict of interest and compliance with ethics requirement

Alexandru Luca declares that he has no conflict of interest.

Anders Kjær declares that he has no conflict of interest.

Merete Edelenbos declares that she has no conflict of interest.

This article does not contain any studies with human or animal subjects.

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478 **Tables:**

Table 1.

Initial quality of young dry and old dry wild rocket leaves.

Parameter	Leaf age		N
	Young	Old	
Days from sowing to harvest	27	32	
Respiration rates (mL kg ⁻¹ h ⁻¹)			
RRO ₂	98.4 ^{a1}	61.4 ^b	3
RRCO ₂	109.2 ^a	65.3 ^b	3
Dry matter (%)	9.2 ^a	7.0 ^b	3
Color			
L*	42.2 ^a	42.2 ^a	4
Hue°	111.9 ^a	114.2 ^a	4
Microbial load (log CFU g ⁻¹)			
Aerobic bacteria	4.87 ^b	5.66 ^a	3
Yeast and molds	4.83 ^a	4.90 ^a	3

479 ¹Means followed by different letters in the same row are significantly different at $P = 0.05$.

Table 2.Release rates ($d[\text{VOC}]/dt$)¹ of VOCs from dry (no water added) and wet (water added) young and old leaves of wild rocket ($N = 3$).

VOC	Young leaves				Old leaves				Significance level between young dry and old dry leaves
	Dry		Wet		Dry		Wet		
	Slope (ng g ⁻¹ d ⁻¹)	R ²	Slope (ng g ⁻¹ d ⁻¹)	R ²	Slope (ng g ⁻¹ d ⁻¹)	R ²	Slope (ng g ⁻¹ d ⁻¹)	R ²	
Carbon disulfide	1.439 ^{b2}	0.92	2.057 ^a	0.92	1.178 ^a	0.97	1.143 ^a	0.97	≤ 0.05
Dimethyl sulfide ³	0.225 ^b	0.74	0.830 ^a	0.80	0.484 ^b	0.59	0.971 ^a	0.80	≤ 0.05
Nitromethane	0.041 ^a	0.90	0.047 ^a	0.97	0.034 ^a	0.97	0.032 ^a	0.96	0.053
Pentane	0.056 ^b	0.74	0.282 ^a	0.95	0.105 ^b	0.90	0.195 ^a	0.79	≤ 0.001
3-Methylfuran	0.218 ^b	0.93	0.269 ^a	0.98	0.202 ^a	0.91	0.187 ^a	0.93	ns
2-Ethylfuran	0.001 ^b	0.65	0.005 ^a	0.97	0.002 ^b	0.73	0.005 ^a	0.63	≤ 0.01
Dimethyl disulfide ³	0.093 ^b	0.20	1.173 ^a	0.89	0.753 ^b	0.73	1.106 ^a	0.89	≤ 0.001

¹Calculated by linear regression. ²Means followed by different letters in the same row within leaf age (young or old) are significantlydifferent at $P = 0.05$. ³Ln-transformed concentrations were used.

Table 3.

Color, microbial load, and index of leaf damage of young dry, young wet, old dry, and old wet wild rocket leaves stored for 8 d at 10 °C ($N = 3$).

Parameter	Young leaves		Old leaves	
	Dry	Wet	Dry	Wet
Color				
L^*	45.1 ^{ab1}	48.3 ^a	46.6 ^{ab}	42.6 ^b
Hue°	106.6 ^a	105.5 ^a	109.0 ^a	110.9 ^a
Microbial load (log CFU g ⁻¹)				
Aerobic bacteria	6.15 ^b	8.14 ^a	7.81 ^a	8.41 ^a
Yeast and molds	5.40 ^b	6.04 ^a	5.47 ^b	5.47 ^b
Index of leaf damage (%)	0.59 ^a	2.44 ^a	1.34 ^a	2.76 ^a

¹Means followed by different letters in the same row are significantly different according to Tukey's honest significance test at $P = 0.05$.

Table 4.

Correlation coefficients (*r*) between aerobic bacteria, yeast and mold, and index of leaf damage and concentrations of VOCs measured in the headspace of young dry, young wet, old dry, and old wet wild rocket stored for 8 d at 10 °C.

VOC	Aerobic bacteria	Yeast and mold	Index of leaf damage
Acetone ¹	-0.76	0.19	-0.43
Carbon disulfide	-0.07	0.78	0.18
Dimethyl sulfide ¹	0.88	0.47	0.94
Nitromethane	-0.36	0.59	-0.16
Pentane	0.82	0.67	0.89
3-Methylfuran	-0.16	0.62	-0.03
2-Ethylfuran	0.80	0.55	0.79
Dimethyl disulfide ¹	0.95	0.46	0.85

¹Ln-transformed concentrations were used.

List of Figures:

Figure 1. Changes in the O₂ and CO₂ partial pressures inside jars filled with young dry, young wet, old dry, and old wet wild rocket leaves stored for 8 d at 10 °C. Values are means $\pm$ standard error ($N = 3$). Means followed by different lowercase (O₂) and uppercase (CO₂) letters at d 8 are significantly different according to Tukey's honest significance test at $P = 0.05$.

Figure 2. Change in the concentration of a) carbon disulfide, b) nitromethane, c) pentane, d) 2-ethylfuran, e) dimethyl sulfide, f) dimethyl disulfide, g) acetone, and h) 3-methylfuran in the headspace from young dry (■), young wet (□), old dry (▲), and old wet (△) wild rocket stored for 8 d at 10 °C. Values are means $\pm$ standard error ($N = 3$). Means within compound followed by different letters at d 8 are significantly different according to Tukey's honest significance test at $P = 0.05$.