Effect of modified atmosphere packaging and temperature abuse on flavor related volatile compounds of rocket leaves (*Diplotaxis tenuifolia* L.)

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Revised: 3 April 2017 / Accepted: 3 May 2017 / Published online: 30 May 2017
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Abstract The effect of storage conditions on flavor-related volatile composition of wild rocket (*Diplotaxis tenuifolia*) was investigated on Modified Atmosphere packed (MAP) leaves stored under isothermal and non-isothermal conditions. In a first experiment the effect of MAP was compared to the storage in air at 5 °C; a second experiment aimed to study the effect of non isothermal conditions, with two temperature abuses (at 13 °C for 24 h) during a 5 °C. Twenty-four volatiles were detected, including C6, C5, isothiocyanate, lipid-derived and sulfur compounds. In the first experiment, MAP-stored rocket showed a slower loss of typical flavour volatiles (thiocyanates and isothiocyanates) and a slower production of off-flavors until 6 days of storage, compared to leaves stored in air. After this time, dimethyl sulfide and acetaldehyde dramatically increased in MAP-stored rocket samples. In the second experiment, samples stored under non-isothermal conditions showed lower O₂ and higher CO₂ concentrations than samples stored under isothermal conditions. Rocket leaves stored under non-isothermal conditions showed an increased production of volatiles responsible of off-flavors (acetaldehyde and dimethyl sulfide) following temperature abuse comparing to storage in isothermal condition. Thus, dimethyl sulfide and acetaldehyde could be effective markers for tracking the effect of temperature fluctuations on rocket during storage.

Keywords *Diplotaxis tenuifolia* · Modified atmosphere · Volatile compounds · Off-flavors · MAP

Introduction

Wild rocket (*Diplotaxis tenuifolia*) is a popular leafy vegetable in Europe that is often used raw in salads and is therefore one of the most represented ready-to-eat fresh products on the market. Packaging of leafy vegetables in polypropylene pouches or in sealed trays, besides facilitating produce handling, creates an atmosphere modification within the package, which reduces loss of humidity, prevents spoilage, and prolongs shelf life (Kim et al. 2004; Sandhya 2010). Modified atmospheres have been shown to preserve quality and phytonutrients of *D. tenuifolia* (Martinez-Sanchez et al. 2006). However, the beneficial effects of modified atmosphere packaging (MAP) are lost if too low O₂ and/or too high CO₂ concentrations develop within the package, eventually inducing the development of off-odors, which are very pronounced in the presence of O₂ levels below 1 kPa (Nielsen et al. 2008). The storage temperature is the main critical factor for the maintenance of the rocket quality and for prolonging its shelf life (Koukounaras et al. 2007; Watada et al. 1996); low temperatures reduce respiration rate and slow down quality degradation (Koukounaras et al. 2007; Lokke et al. 2012). Amodio et al. (2015) used a non-linear model to estimate the shelf life of fresh rocket leaves and to describe the loss of quality attributes during storage. They found different degradation patterns as a function of storage temperature and described different factors that limited shelf life when the cold chain was not ensured.

Recently, Luca et al. (2016) investigated the volatile organic compounds (VOCs) of intact wild rocket leaves

Electronic supplementary material The online version of this article (doi:10.1007/s13197-017-2685-6) contains supplementary material, which is available to authorized users.

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during modified atmosphere storage and described their changes in relation to O₂ and CO₂ partial pressures, temperature, and storage time. VOCs are directly involved in the formation of both odor and flavor sensations, which influence the consumer acceptability (Apréa et al. 2012; Besada et al. 2013; Cozzolino et al. 2016), but flavor is the combination of diverse non-volatile and volatile compounds released during eating. It results by a combination of tastes, trigeminal sensations and olfactory sensations (Apréa et al. 2012; Deibler and Delwiche 2004).

The characteristic pungent flavor of fresh rocket is formed immediately after tissue disruption when enzymatic reactions cause the formation of a number of volatiles (Bones and Rossiter 2006) and is mainly a result of glucosinolates and their breakdown products isothiocyanates (Bennett et al. 2002, 2006; D'Antuono et al. 2009; Pasini et al. 2011). Other several compounds were also found to be of critically importance (Jirovetz et al. 2002). Hexanal, *trans*-2-hexenal, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol are reportedly responsible for the green aroma in Austrian *Eruca sativa* salad, while benzaldehyde and *trans*, *trans*-2,4-heptadienal are responsible for its nutty and almond-like notes (Jirovetz et al. 2002). However, volatiles also contribute to the formation of off-flavors, depending on their concentration (Poll et al. 2006). Therefore, volatiles extracted from the headspace of intact leaves, may be poorly represented by some of the flavor-related compounds. This is the case of Luca et al. (2016) and also of Nielsen et al. (2008) who studied the effect of the packaging atmosphere on the development of off-odors, focusing the investigation on dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) compounds.

The number and proportion of rocket volatiles vary mainly in accordance with isolation methods (Blažević and Mastelić 2008; Jirovetz et al. 2002; Miyazawa et al. 2002). In particular, solid-phase microextraction SPME has proven to be a simple, rapid, solvent-free, and an effective method for purposes of volatile profile characterization. Luca et al. (2015) developed a method for detection of VOCs in wild rocket (*D. tenuifolia*) using SPME and also selected the carboxen/polydimethylsiloxane (CAR/PDMS) fiber as the most efficient.

There is currently no information available on the development of the volatiles responsible for both flavor and off-flavors during storage of packaged 'Diplotaxis' rocket and under temperature abuse conditions. In their recent study, Luca et al. (2016) described changes in volatile profile of wild rocket stored 14 days in closed jars, thus not simulating realistic MAP conditions, conducting their analysis at two different storage temperatures (5 and 10 °C). Moreover, available studies on MAP rocket leaves only focused on off-odors (Nielsen et al. 2008), and not on the compounds positively affecting flavor and/or developed

after tissue rupture, and without accounting for temperature abuse. Therefore, this work aimed to track changes in volatiles responsible for flavor in wild rocket stored in realistic MAP conditions under isothermal (5 °C) and non-isothermal conditions (to simulate thermal abuse in transit) in order to understand the impact of temporary temperature changes and to identify a suitable marker of improper storage conditions.

Materials and methods

Plant material and minimal processing

Fresh rocket leaves (*D. tenuifolia*) were harvested in Guagnano (Apulia, Italy) and washed in chlorinated water (0.01% v/v) before being dried, portioned into 50 g samples and packaged in PP bags (17.5 × 17.5 cm², OTR = 1800 cm³ m² d⁻¹, WVTR = 6 gm² d⁻¹) in air (passive atmosphere) simulating the most common commercial conditions. In the first experiment, 15 bags (3 replicates × 5 sampling times) were stored at a constant temperature of 5 °C. Volatile analysis was carried out after 0, 1, 2, 3, 6, and 8 days of storage. The effects of the packaging treatment (T) on volatiles were compared with control rocket packaged in an unsealed bag (C). In a second experiment, non-isothermal conditions (NI) consisting of nine days of storage at 5 °C with 2 abuse periods of 24 h at 13 °C (at days 2 and 6) were compared with isothermal storage (I) at 5 °C carried out simultaneously. Nine bags were stored under these conditions to assess gas concentration and volatile profiles after 2, 5, and 9 days of storage (three replicates per time point). The effect of the thermal abuse during rocket storage under MAP conditions was compared with control samples stored in PP bags under isothermal conditions at 5 °C.

Gas composition in the packages

O₂ and CO₂ concentrations inside the packages were monitored with a WITT Mapy 4.0 gas analyzer (Witten, Germany). The gas analyzer test probe was inserted into each package through an adhesive rubber septum to prevent any air from leaking out of the package. After determining the gas composition, packages were used for sensory and volatile analysis.

Volatile extraction and headspace SPME GC–MS analysis

Volatile compounds were evaluated in three different bags (replicates) for each temperature and gas condition on each sampling day. Thirty grams of rocket leaves for each

replicate were added with 0.6 g of CaCl_2 and 6 g of NaCl, and homogenized with 30 mL of distilled water for 6 min using a commercial blender. For a single analysis, 8 g of this mixture, added to 2 μL of internal standard solution (IS, 2-methyl-1-pentanol, 100 ppm), were introduced into a 20 mL capped solid-phase microextraction vial and incubated for 20 min at 40 °C. Afterwards, an 85 μm CAR/PDMS fiber was exposed for 30 min to the vial headspace and introduced into the GC injector port for desorption at 250 °C for 4 min in the split injection mode (1:20). An Agilent 6890 Series gas chromatograph coupled to an Agilent 5975 C network mass selective detector (Agilent Technologies, Palo Alto, CA, USA) was used to analyze the samples. Analytes were separated on a DB-WAX capillary column (60 m $\times$ 250 μm $\times$ 0.25 μm ; J&W Scientific Inc., Folsom, CA, USA) by applying the following temperature program: 40 °C for 4 min and 40–140 °C at 3 °C/min with a final holding time of 10 min. The transfer line temperature was 280 °C. Mass detector conditions were as follows: electronic impact mode at 70 eV, source temperature at 230 °C, scanning rate of 2.88 scan/s and a mass scanning range of m/z 30–400. The carrier gas, helium, was used at a rate of 1.0 mL/min. Compounds were identified by comparing their retention times with those of pure compounds (Sigma-Aldrich, Milan, Italy) analysed under the same conditions. A comparison of MS fragmentation pattern with mass spectrum database was performed using the data system library (NIST 02, $p > 80$). Confirmation was also reached using laboratory-built MS spectral database, obtained from chromatographic runs of pure compounds carried out with the same conditions.

Statistical analysis

Data on the gas composition and the volatile compounds represent the mean of three replicates for each treatment (standard deviation was calculated). Data were subjected to analysis of variance (one-way anova), using Statgraphics Centurion XVI software. Linear regression among volatiles of the same group, principal component analysis (PCA) and Linear Discriminant Analysis (LDA) in the forward stepwise mode of the volatile data were carried out using Statistica software (ver.7, StatSoft, Tulsa, OK, USA).

Results and discussion

Volatile profile of fresh rocket leaves

The volatile compounds detected at days 0 and 8 of storage, together with the relevant odor descriptors, are summarized in Table 1. Twenty-four compounds were

identified, representing the major polar volatile compounds in the ground rocket leaves.

Volatile compounds reported at time zero depict the flavor fingerprint of fresh rocket, as they were present in all samples analyzed early on when product quality was at its maximum. These volatile compounds included sulfur, C6 and C5 compounds, lipid-derived compounds, acetaldehyde, and ethanol. Isothiocyanate and thiocyanate derivatives were found, including erucin, 3-butenyl isothiocyanate (3-Bu-ITC), methyl thiocyanate (Me-TC), n-pentyl, and 4-methylpentyl (n-pentyl-ITC and 4-MP-ITC) isothiocyanates, which originated from myrosinase activity on glucosinolates following tissue disruption (Halkier and Gershenzon 2006). These compounds give the odor and flavor notes typical of fresh rocket (Bennett et al. 2002, 2006; Jirovetz et al. 2002; Pasini et al. 2011) and were reported to be biologically active (Yehuda et al. 2009).

Luca et al. (2016) after a preliminary screening made with total ion count (TIC) mode, selected the ions of 23 compounds to be monitored over storage of intact and wounded leaves, preferring the selected ion monitoring (SIM) mode. Among these compounds, only ethanol, and dimethyl sulfide, were detected on grounded leaves in the present study, whereas as example, these authors excluded acetaldehyde and hexanal from the selected volatiles for sensitivity problems. Furthermore, they found only 9 volatiles of the selected group in wounded leaves, in which dimethyl sulfide and ethanol were not detected. The differences found with this work may be therefore due, other than to the extraction method (VOC from the headspace of intact leaves versus the headspace of grounded leaves), also to the fact that not all compounds were included using the SIM mode.

C6 and C5 compounds (esters, alcohols, aldehydes, and ketones), generated via the lipoxygenase pathway from fatty acids, are responsible for the green-leaf flavor of the leaves (Jirovetz et al. 2002; Sigma-Aldrich 2001;) and constitute a plant defense mechanism when leaves are mechanically damaged (Hatanaka 1999; Reineccius 2006). In all samples, (E)-2-hexenal was the most abundant C6 aldehyde. Hexanal, also present but in much lower quantities, is derived from either lipoxygenase action or from chemical oxidation, and has been associated with green grass and green apple odors. With regards to C6 compounds, the rocket at time zero also contained (Z)-3-hexen-1-ol, which has been associated with a fresh green taste, and (Z)-3-hexen-1-ol acetate, responsible for green sweet notes. These volatiles are common compounds found also in '*Eruca sativa*' paste by Jirovetz et al. (2002) but in different amount when compared to our samples of '*Diplotaxis tenuifolia*'. For example, (Z)-3-hexen-1-ol resulted the most abundant C6 compound in salad rocket, while (E)-

Table 1 Volatile compounds detected in control and MAP-stored wild rocket at t_0 (fresh rocket) and t_8

Compound	t_r	t_0^c	t_8 air ^c	t_8 MAP ^c	Odor descriptor ^d
<i>Anaerobic respiration compounds</i>					
Acetaldehyde ^b	5.13	0.25 ± 0.09	0.69 ± 0.14	1.64 ± 0.17	Ethereal, pungent
Ethanol ^b	9.5	0.25 ± 0.04	0.14 ± 0.10	0.28 ± 0.10	Alcoholic, ethereal
<i>C6 and C5 compounds</i>					
Pentan-3-one ^b	10.84	0.63 ± 0.11	0.97 ± 0.34	1.25 ± 0.06	Ethereal
1-Penten-3-one ^b	12.49	4.88 ± 0.88	3.4 ± 1.0	14.3 ± 4.7	Spicy
Hexanal ^b	15.12	14.9 ± 2.8	2.54 ± 0.20	8.6 ± 5.8	Fatty, green, grassy
1-Penten-3-ol ^b	18.81	8.11 ± 0.11	8.7 ± 4.6	18.0 ± 4.4	Pungent, green
(E)-2-hexenal ^b	21.58	484 ± 170	126 ± 21	355 ± 200	Almond-like, green, herbal
(Z)-3-hexen-1-ol acetate ^b	26.15	46 ± 32	5.89 ± 0.96	5.50 ± 0.58	Green sweet
(Z)-2-penten-1-ol ^b	26.38	6.70 ± 0.06	7.5 ± 3.4	12.9 ± 1.9	Fruity, green
(Z)-3-hexen-1-ol ^b	29.31	99 ± 26	52 ± 12	68.6 ± 4.6	Fresh, green grass-like
<i>Lipid derived compounds</i>					
Heptanal ^b	19.93	0.85 ± 0.11	0.16 ± 0.13	nd	Fresh aldehydic, fatty acid
2-Pentylfuran ^b	22.08	0.65 ± 0.06	0.09 ± 0.05	0.006 ± 0.005	Green, bean-like
6-Methyl-2-heptanone ^b	22.45	0.56 ± 0.07	0.10 ± 0.06	nd	Camphoreous
Octanal ^b	24.84	0.47 ± 0.18	0.09 ± 0.05	0.006 ± 0.005	Aldehydic, waxy
(E)-2-heptenal ^b	26.53	0.65 ± 0.16	0.19 ± 0.14	nd	Green
6-Methyl-5-hepten-2-one ^b	27.11	0.66 ± 0.05	0.20 ± 0.16	0.006 ± 0.005	Citrus
(E,E)-2,4-heptadienal ^b	32.79	5.5 ± 2.0	1.78 ± 0.37	3.9 ± 1.9	Fatty
3,5-Octadien-2-one ^a	35.15	0.91 ± 0.29	0.24 ± 0.03	0.14 ± 0.13	Fatty
<i>Sulfur compounds</i>					
Dimethyl sulfide ^b	5.58	0.11 ± 0.06	0.61 ± 0.07	0.71 ± 0.01	Sulfurous
Methyl thiocyanate ^b	24.03	3.00 ± 0.78	2.10 ± 0.52	4.5 ± 1.7	Sulfury onion
4-Methylthiobutyl isothiocyanate ^c	28.58	523 ± 130	81 ± 60	310 ± 190	Cabbage
3-Butenyl isothiocyanate ^a	32.38	2.24 ± 0.17	0.97 ± 0.16	1.36 ± 0.57	Pungent
n-Pentyl isothiocyanate ^a	33.73	11.20 ± 0.59	2.83 ± 0.65	4.6 ± 2.3	Green
4-Methylpentyl isothiocyanate ^a	35.98	22.40 ± 0.31	6.4 ± 2.1	7.9 ± 3.4	Pungent, horseradish

Mean values of three replicates ± standard deviation

 t_r = retention time^a Compounds tentatively identified by mass spectra comparison with Wiley library^b Compounds identified by comparison with standard^c Concentrations, means of 3 replicates, are expressed as µg internal standard equivalent/kg of rocket^d Odor descriptors using published data (Jirovetz et al. 2002; Sigma-Aldrich 2001)^e Compound identified by mass spectra comparison with literature values (Blažević and Mastelić 2008; Vaughn and Berhow 2005). MS spectral data, m/z (relative intensity): 161 (M+, 20%), 146 (10%), 115 (75%), 85 (45%), 72 (28%), 61 (100%)

2-hexenal was more abundant than the other C6 compounds in our samples. These differences may be depended on the rocket genera. C5 compounds included 1-penten-3-one, associated with spicy, pungent, and ethereal scents, 1-penten-3-ol, reported to have pungent, green vegetable nuances, (Z)-2-penten-1-ol, found to be correlated with fruity notes, and pentan-3-one, typically described as having ethereal acetone notes. C5 compounds were not detected in salad rocket leaves by Jirovetz et al. (2002) and in their essential oils by Miyazawa et al. (2002). Other

minor compounds have been reported as a result of lipid autoxidation such as C7, C8 aldehydes (an aldehydic odor type) and 2-pentylfuran (Nawar 1999).

Effects of MAP on the gas composition and volatile profile of rocket leaves

Storage time, the main parameter analyzed in this study, caused dimethyl sulfide accumulation, which is reportedly formed from both (+)-S-methyl-L-cysteine sulfoxide

(Marks et al. 1992) and subsequent degradation of certain volatiles derived from glucosinolates (Jin et al. 1999). Furthermore, acetaldehyde (ethereal notes) and ethanol were monitored as indicators of anaerobic respiration because they have been demonstrated to accumulate in response to low O_2 and/or high CO_2 treatment during controlled atmosphere storage of broccoli florets and fresh-cut peach (Colantuono et al. 2015; Hansen et al. 2001) and modified atmosphere packaged fresh-cut artichoke (La Zazzera et al. 2015). The composition of the gases inside the packages during storage is reported in Fig. 1a. The O_2 and CO_2 levels accumulated within the package at equilibrium are the result of the balance of rocket leaves respiration rate and film permeability at that storage temperature. Storage of rocket in PP bags at 5 °C ensured an aerobic condition until the last day of storage; after eight days the O_2 level inside the packages was less than 8 kPa and the CO_2 level was approximately 9 kPa. To visualize differences between storage under MAP conditions and in air control, a multivariate analysis (PCA) was carried out, considering all volatile compounds, CO_2 and O_2 as variables in the first step. All variables but erucin, octanal, ethanol and 2-pentylfuran received a score higher than 0.9, therefore erucin, octanal, ethanol and 2-pentylfuran were excluded from PCA (Online Resource 1). The occurrence of correlations between variables within the same family was investigated to explore the possibility of using some compounds as representatives in PCA analysis, minimizing data redundancy. Linear correlation coefficients are reported in Table 2. Within each compound family, a good linear correlation was observed for (Z)-3-hexen-1-ol acetate, (Z)-2-penten-1-ol, heptanal and 4-methylpentyl-isothiocyanate. These variables were therefore selected as representatives of C6, C5 and other lipid- and glucosinolate-derived compounds, respectively. Additionally,

acetaldehyde and dimethyl sulfide were used in PCA as known compounds with off-flavor notes (Kim et al. 2005). Ethanol did not accumulate with time, likely due to the residual presence of oxygen in the packages (Fig. 1a, Table 1).

Figure 2 shows the score and loading plots of the data; PC1 and PC2 accounted for 63.60 and 23.96% of the total variance, respectively. Variables and cases spread out similarly as in the PCA including all variables (Online Resource 1) suggesting that the selected variables were effectively representative of respective families. The first principal component (PC1) discriminated rocket degradation over time (loss of freshness) with samples at t0, representing product freshness, in the positive part of the axis and the other samples moving to the left (negative part of the axis) over time. Storage in MAP caused a slower shift from the initial point, showing samples in the negative axis only after 6 days, when CO_2 accumulation and O_2 in MAP reached values of about 10 and 7.7 kPa respectively (Fig. 1a), in comparison with air storage, whose samples were in the negative axis already after 2 days (C2). The variable plot (Fig. 2b) suggests that the slower shift is due to a less pronounced loss of some compounds (heptanal, (Z)-3-hexen-1-ol acetate, 4-methylpentyl isothiocyanate and all the represented compounds) together with a general reduced generation of off-flavors indicating the slower flavor degradation. DMS and acetaldehyde accumulated over time in both storage conditions and therefore can be considered markers of the product degradation. Indeed, DMS, responsible for off-odors in salad rocket (Nielsen et al. 2008) increased in wild rocket during the storage in MAP simulated conditions when O_2 level are above 2.1 kPa (Luca et al. 2016). Furthermore acetaldehyde is well known to increase when fermentative reactions occur and it confers unpleasant notes.

Finally, PC2 discriminated between storage in air and MAP conditions at eight days of storage, as the oxygen

Fig. 1 Gas composition inside the packages during storage under isothermal MAP conditions, experiment one (a) and storage under isothermal and non-isothermal conditions, experiment two (b). Mean values of three replicates $\pm$ standard deviation

Table 2 Linear correlation coefficients between heptanal and 2-pentylfuran, 6-methyl-2-heptanone, (E)-2-heptenal, 6-methyl-5-hepten-2-one, (E,E)-2,4-heptadienal and 3,5-octadien-2-one; between (Z)-3-hexen-1-ol acetate and hexanal, (E)-2-hexenal and (Z)-3-hexen-

1-ol; between (Z)-2-penten-1-ol and pentan-3-one, 1-penten-3-one and 1-penten-3-ol; between 4-MP-ITC and n-pentyl ITC, 3-butenyl ITC and ME-TC

Lipid derived compounds	2-Pentylfuran	6-methyl-2-heptanone	Octanal	(E)-2-heptenal	6-Methyl-5-hepten-2-one	(E,E)-2,4-heptadienal	3,5-Octadien-2-one
Linear correlations (R)							
Heptanal	0.6	0.93	0.7	0.89	0.83	0.59	0.76
C6 compounds	hexanal	(E)-2-hexenal	(Z)-3-hexen-1-ol				
(Z)-3-hexen-1-ol acetate	0.77	0.8	0.73				
C5 compounds	pentan-3-one	1-penten-3-one	1-penten-3-ol				
(Z)-2-penten-1-ol	0.59	0.61	0.9				
thiocyanic compounds	n-pentyl ITC	3-butenyl ITC	ME-TC				
4-MP-ITC	0.99	0.93	0.62

Fig. 2 PCA on the volatile compounds of the rocket stored under isothermal conditions in MAP (t labels) and in air (C labels). Three replicates for each sampling day (the numbers refer to the days of storage). **a** score plot; **b** variable plot

reduction and the accumulation of CO₂ in MAP contributed to the formation of C5 compounds.

As an example, dimethyl sulfide, acetaldehyde and 4-methylpentyl isothiocyanate changes overtime are shown in Fig. 3a–c. The trends are in accordance with PCA results, suggesting both a slower production of off-flavors in MAP-stored rocket and a slower degradation of flavor compounds like isothiocyanates. Indeed, DMS production was lower for samples stored in MAP than for air-stored samples until six storage days, then, dramatically increased in MAP-stored rocket likely due to the increased CO₂ and decreased O₂ in the PP bags, resulting six and five times higher than in the fresh samples for MAP and air-stored samples, respectively. Also acetaldehyde showed a similar behavior, its accumulation after 8 days being about seven and three times in MAP-stored samples and in unsealed

bags, respectively. A similar non-linear behavior for dimethyl sulfide has been also observed by Luca et al. (2016) during the modified atmosphere storage of wild rocket in presence of O₂ levels ≥ 2.1 kPa. With regards to flavor compounds like isothiocyanates, their content decreased more slowly in MAP-stored rocket until they reached values comparable with air-stored samples at day 6. A similar behavior was observed for C6 compounds and lipid-derived compounds (data not shown).

Effects of thermal abuse on volatile profiles of packaged rocket leaves

Volatiles were monitored under non-isothermal conditions, where temperatures fluctuated between 5 and 13 °C on days 2 and 6 to simulate improper management of the cold

Fig. 3 Changes of **a** dimethyl sulfide, **b** acetaldehyde and **c** 4-methylpentyl isothiocyanate contents during storage under isothermal MAP and of **d** dimethyl sulfide, **e** acetaldehyde and **f** 4-methylpentyl isothiocyanate under non-isothermal conditions. The gray areas marked the exposition of the product at 13 °C (24 h). Mean values of three replicates

chain, for comparison with MAP-storage under isothermal conditions (5 °C), carried out simultaneously. To the best of our knowledge, no study under non-isothermal conditions has been previously reported in the literature. The volatile compounds detected at days 0 and 9 of storage are summarized in Table 3. In Fig. 1b, the development of gases in the packages under the two different conditions is shown. When bags were kept under isothermal conditions, O₂ and CO₂ levels reached equilibrium after 5 days (reaching about 11 and 6 kPa, respectively). When samples were stored under non-isothermal conditions, O₂ and CO₂ levels showed greater variation following the two temperature abuses, ending at almost 1 kPa O₂ and 11 kPa CO₂ after nine days of storage. Amodio et al. (2015) highlight the fact that thermal abuse strongly affected the qualitative attributes of the rocket leaves, which varied depending on the temperature conditions during storage.

Therefore, PCA was carried out to get an overview of the data and visualize the effect of temperature fluctuations

on the volatile profile. Using all volatile compounds as variables, no clear groupings was visible in the PCA, suggesting that differentiation between thermal abused and control samples, using all data with an unsupervised classification method, was difficult, likely due to the similarity of samples (data not shown). Therefore, a Linear Discriminant Analysis, which is a supervised classification method maximizing the variance between the classes and minimizing the one within the individual classes, was carried out. The aim was to use the prior class knowledge not as tool to classify samples, but to highlight differences determined by NI and I storage conditions by reducing data dimensionality. The used forward stepwise model builds a discrimination model step by step entering the predictors sequentially. The model first reviews all the variables and include, step by steps, the ones that have the biggest weight for the discrimination between groups. In this way, the analysis gave back a model with three variables, showing a significant discrimination (Wilks' Lambda 0.06740,

Table 3 Volatile compounds detected wild rocket stored under isothermal (I) and non-isothermal (NI) conditions at t_0 and t_9

Compound	t_r	t_0^c	t_9 I storage ^c	t_9 NI storage ^c
<i>Anaerobic respiration compounds</i>				
Acetaldehyde ^b	5.13	0.84 ± 0.34	0.33 ± 0.06	1.35 ± 0.12
Ethanol ^b	9.50	1.03 ± 0.64	0.27 ± 0.13	4.72 ± 0.72
<i>C6 and C5 compounds</i>				
Pentan-3-one ^b	10.84	2.97 ± 0.22	1.7 ± 0.1	1.27 ± 0.13
1-Penten-3-one ^b	12.49	27.17 ± 2.57	16.89 ± 7.54	7.4 ± 1.5
Hexanal ^b	15.12	20.8 ± 7.0	26.2 ± 9.0	18.9 ± 6.3
1-Penten-3-ol ^b	18.81	28.79 ± 0.76	14.76 ± 5.77	12.0 ± 1.8
(E)-2-hexenal ^b	21.58	448 ± 125	409 ± 63	332 ± 129
(Z)-3-hexen-1-ol acetate ^b	26.15	0.65 ± 0.39	0.38 ± 0.04	0.05 ± 0.03
(Z)-2-penten-1-ol ^b	26.38	13.5 ± 1.4	10.3 ± 2.4	7.2 ± 1.4
(Z)-3-hexen-1-ol ^b	29.31	88 ± 16	125 ± 53	42.9 ± 6.9
<i>Lipid derived compounds</i>				
Heptanal ^b	19.93	0.34 ± 0.15	0.24 ± 0.08	0.34 ± 0.18
2-Pentylfuran ^b	22.08	nd	nd	nd
6-Methyl-2-heptanone ^b	22.45	0.26 ± 0.06	0.14 ± 0.06	0.13 ± 0.08
Octanal ^b	24.84	nd	nd	nd
(E)-2-heptenal ^b	26.53	0.3 ± 0.1	nd	0.07 ± 0.03
6-Methyl-5-hepten-2-one ^b	27.11	4.0 ± 2.1	1.04 ± 0.56	0.86 ± 0.48
(E,E)-2,4-heptadienal ^b	32.79	1.24 ± 0.17	1.38 ± 0.59	1.3 ± 0.6
3,5-Octadien-2-one ^a	35.15	0.15 ± 0.06	0.13 ± 0.03	0.11 ± 0.02
<i>Sulfur compounds</i>				
Dimethyl sulfide ^b	5.58	0.27 ± 0.01	0.46 ± 0.03	0.82 ± 0.03
Methyl thiocyanate ^b	24.03	20 ± 12	5 ± 1	7 ± 5
4-Methylthiobutyl isothiocyanate ^d	28.58	14 ± 9	1.42 ± 0.21	0.92 ± 0.19
3-Butenyl isothiocyanate ^a	32.38	9 ± 4	1.35 ± 0.02	2.7 ± 0.3
n-Pentyl isothiocyanate ^a	33.73	9.6 ± 1.0	4.61 ± 0.27	7.34 ± 0.77
4-Methylpentyl isothiocyanate ^a	35.98	31 ± 6	18.9 ± 1.6	20.2 ± 9.9

t_r = retention time. Mean values of three replicates $\pm$ standard deviation

^a Compounds identified by mass spectra comparison with Wiley library

^b Compounds identified by comparison with standard

^c Concentrations, means of 3 replicates, are expressed as μg internal standard equivalent/kg of rocket

^d Compound identified by mass spectra comparison with literature values (Blažević and Mastelić 2008; Jirovetz et al. 2002). MS spectral data, m/z (relative intensity): 161 (M+, 20%), 146 (10%), 115 (75%), 85 (45%), 72 (28%), 61 (100%)

$p < 0.00001$) between the 2 classes. The Pearson correlation coefficients between linear discriminant function and the variables considered in the analysis (structure matrix) obtained for dimethyl sulfide (Z)-3-hexen-1-ol and (Z)-3-hexen-1-ol acetate were respectively -0.685 , 0.0721 and -0.1478 (data not shown). The Pearson correlations are normalized indicators ranging from -1 to $+1$; the higher is their absolute value, the higher is the variable contribution to the discrimination. By convention, correlation higher than 0.33 considering the absolute value may be considered eligible while lower ones are not (Tabachnick and Fidell 2007). Therefore, results indicate that DMS contributed almost entirely to the discrimination between NI and I. The

changes of dimethyl sulfide, acetaldehyde and 4-methylpentyl isothiocyanate over time under isothermal and non-isothermal conditions are shown in Fig. 3d–f. Results suggest the rocket deterioration under thermal abuse was due to the production of dimethyl sulfide and acetaldehyde, which in turn was caused by the CO_2 increase and O_2 decrease in the PP bags. Indeed, DMS production was higher for samples stored in NI condition than for I-samples until 9 storage days, when values were approximately two times higher than samples stored in I condition. Also acetaldehyde showed a similar behavior, but after return to cold storage, at day 5, its production decreased, likely due to its involvement in other reactions; then, at day 9, after

the thermal abuse of the 6th day, its production raised again up to four times in NI-samples compared with I-stored ones. This not linear trend likely caused its lack of relevance in LDA. As regard to flavor compounds like isothiocyanates, their content was similar in NI and I-rocket, suggesting that NI and I conditions were not differentiated by typical aromatic compounds but by off-flavors. A similar behavior was observed for C6 compounds and lipid-derived compounds.

Conclusion

This work provided useful information on changes in volatile profile of packaged rocket salad under isothermal and non-isothermal conditions. Storage in MAP, which maintained aerobic conditions, had a beneficial effect on the quality of the rocket leaves, preserving the retention of typical flavors up to 6 days. At the last day of storage, increased production of dimethyl sulfide was detected, which could confer off-odors and off-flavors to the produce. Nevertheless, the important role of temperature during storage is confirmed by the results of the rocket storage under non-isothermal conditions. Temperature fluctuation induced changes in the rocket volatile profile under these conditions compared with isothermally stored rocket, which persisted even when the cold chain was restored. The production of dimethyl sulfide and acetaldehyde was the greatest modification to the volatile profile inside the packages, suggesting that these compounds can be effective markers for tracking temperature fluctuations in the product's thermal history.

Acknowledgements The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) under Grant agreement No. 289719 (Project QUAFETY).

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