Food Chemistry 211 (2016) 114–123

Contents lists available at ScienceDirect

Food Chemistry

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

Multi-trait analysis of post-harvest storage in rocket salad (*Diplotaxis tenuifolia*) links sensorial, volatile and nutritional data

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 14 April 2016
Accepted 21 April 2016
Available online 4 May 2016

Chemical compounds:
Ferulic acid (PubChem CID: 445858)
7-Hydroxycoumarin (PubChem CID: 5281426)
Dimethyl sulphide (PubChem CID: 1068)
Dimethyl sulfoxide (PubChem CID: 679)
Dimethyl disulphide (PubChem CID: 12232)
4-Oxohex-2-enal (PubChem CID: 6365145)
2,4-Hexadienal (PubChem CID: 637564)
E/Z-2-hexenal (PubChem CID: 10460)
E/Z-3-hexenal (PubChem CID: 23234)
E/Z-2-pentenal (PubChem CID: 12993)

Keywords:
Diplotaxis tenuifolia
Functional and nutritional quality
Isothiocyanates
Post-harvest storage
Rocket salad
Volatile organic compounds

ABSTRACT

Rocket salad (*Diplotaxis tenuifolia*; wild rocket) is an important component of ready to eat salads providing a distinct peppery flavour and containing nutritionally relevant compounds. Quality deteriorates during post-harvest, in relation to time and storage temperature amongst other factors. Volatile organic compounds (VOCs) are easily measurable from rocket leaves and may provide useful quality indicators for e.g. changes in isothiocyanates derived from nutritionally important glucosinolates. VOC profiles discriminated storage temperatures (0, 5 and 10 °C) and times (over 14 days). More specifically, concentrations of aldehydes and isothiocyanates decreased with time paralleling a fall in vitamin C and a reduction in sensorial quality at the two higher temperatures. Sulphur containing compounds rise at later timepoints and at higher temperatures coincident with an increase in microbial titre, mirroring a further drop in sensorial quality thus indicating their contribution to off-odours.

1. Introduction

Rocket salad is of increasing commercial importance as a fresh cut ready to eat (RTE) salad and is valued for its sensory and nutritional characteristics (Pasini, Verardo, Cerretani, Caboni, & D'Antuono, 2011). Known also as roquette, arugola or rucola it comprises species from two genera in the *Brassicaceae* family: *Eruca sativa* Mill. (cultivated rocket) and *Diplotaxis tenuifolia* (wild rocket). Both species are high in biologically active compounds including ascorbic acid, carotenoids, fibres, polyphenols and glucosinolates (Bell & Wagstaff, 2014; Martínez-Sánchez, Allende, Bennett, Ferreres, & Gil, 2006). Glucosinolates contribute to the pungent flavour of rocket leaves (Bennett et al., 2002) by formation of isothyocyanates (ITCs) through the action of myrosinase on cutting and chewing of leaves (Bennett, Rosa, Mellon, & Kroon, 2006). In *E. sativa* the major glucosinolate appears to be glucoerucin (Nitz & Schnitzler, 2002), which is converted by myrosinase to erucin (4-methylthiobutylisothiocyanate) a compound with strong antioxidant properties (Barillari et al., 2005; Jirovetz, Smith, & Buchbauer, 2002; Miyazawa, Maehara, & Kurose, 2002) but other ITCs were also identified (Miyazawa et al., 2002). Both glucosinolates and ITCs are thought to reduce the risk of carcinogenesis or heart disease (Traka & Mithen, 2009). Volatile organic compounds

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http://dx.doi.org/10.1016/j.foodchem.2016.04.107
0308-8146/

(VOCs) other than ITCs also contribute to the aroma of rocket salad including, in *E. sativa*, sulphur and or nitrogen compounds, esters, alcohols and carbonyl compounds (Blažević & Mastelić, 2008; Miyazawa et al., 2002). Despite its importance as a cut salad there are even fewer reports of VOC profiles in *D. tenuifolia* compared to *E. sativa*.

Storage conditions affect physiological and visual quality of rocket leaves from harvest to consumption (Kader, 2002; Koukounaras, Siomos, & Sfakiotakis, 2007; Watada, Ko, & Minott, 1996). Low temperature is a key factor in quality maintenance, depressing respiration, reducing overall metabolism and extending shelf life (Spinardi & Ferrante, 2012). A temperature of 0 °C is recommended for storage of rocket salad (Cantwell & Kasmire, 2002), however, it is usually shipped and stored at 10 °C and temperature abuse occurs during transport and retail store display (Koukounaras et al., 2007). Moreover, rocket salad in common with most fresh produce has a short shelf life, showing leaf yellowing within 4–8 days even when stored at low (5 °C) temperatures (Ferrante, Incrocci, Maggini, Serra, & Tognoni, 2004) although some reports (Martínez-Sánchez et al., 2006; Nielsen, Bergström, & Borch, 2008) have described shelf life of 14 days for rocket leaves stored at 4 °C. The use of lower temperatures increased shelf life with an extension of 5–6 days at 0 °C compared to 7 °C (Hall, Jobling, & Rogers, 2013). Degradation kinetics indicated that when stored at constant temperature, shelf life is limited by appearance, reaching the limit of marketability after 7.3, 5.8 and 3.7 days, for samples stored at 0, 5 and 15 °C (Amodio, Derossi, Mastrandrea, & Colelli, 2015) respectively. However, leaf yellowing is accompanied by a loss of ascorbic acid (vitamin C) and glucosinolates (Force, O'Hare, Wong, & Irving, 2007). An increase in temperature affected the loss of ascorbic acid more than the release of off-odours, indicating that ascorbic acid levels should limit the shelf life (Amodio et al., 2015). Other nutritionally relevant compounds such as polyphenols, however, increase in vegetables in response to stresses such as wounding and cold (Cisneros-Zevallos, 2003), hence an extension of post-harvest storage may be positive. Polyphenols have been associated with health benefits that in part are related to their antioxidant activity, which may be associated with complex synergistic interactions between different compounds in the produce (Liu, 2004). Sinapic acid and ferulic acid are phenolic acids frequently found in rocket and other plants with potential anti-cancer effects (Liu, 2004) and 7-hydroxycoumarin (also known as umbelliferone) is a potent antioxidant (Kanimozhi, Prasad, Ramachandran, & Pugalendi, 2011). However, information on the impact of post-harvest conditions on levels of these bioactive compounds in rocket salad is lacking, despite their relevance for nutritional value.

Generally, flavour is compromised by periods of storage and off-odours develop towards the end of shelf life (Peneau, Brockhoff, Escher, & Nuessli, 2007). However, there is a lack of information on the effect of storage temperature on VOCs, as well as on concentration of isothiocyanates and on the chemical identity of off-odours in rocket salad.

Relative concentrations of VOCs are easy to measure, and using thermal desorption can be collected with minimal tissue disruption (Spadafora et al., 2015). Therefore monitoring of VOCs could be of use as a marker for sensorial quality and nutritional content. Here, we tested whether changes in VOCs can be linked to physiological markers, phytochemical content and sensorial quality and whether useful markers for changes in nutritional and functional compounds and development of off-odours can be identified. We applied statistical algorithms for ecological and gene expression analyses in a multi-trait analysis of changes in the whole VOC bouquet during post-harvest storage of rocket leaves at three commercially-relevant temperatures over a 14 day period, which is the maximum time considered relevant.

2. Materials and methods

2.1. Plant Material and treatments

Freshly harvested rocket leaves (*Diplotaxis tenuifolia* L.) at commercial maturity, were obtained from a local grower, immediately transported to the RTE salad processing facilities and stored at low temperature (5 °C). Rocket leaves were then inspected for uniformity and visual defects, washed in cold running water, dipped in 100 μg L^{-1} sodium hypochlorite solution for 1 min, and centrifuged to remove the remaining water. Rocket leaves (ca. 100 *g*) were packed in coextruded bioriented polypropylene film bags (Amcor Flexibles Neocel, Palmela, Portugal), heat sealed with a Multivac packaging machine (Gastrovac, Wolfertschwenden, Germany) and stored at 0, 5 and 10 °C for a total storage period of 14 days. The packaging film used permitted an oxygen transmission rate of 1200 cm^{-3} m^{-2} 24 h^{-1} at 23 °C (Amcor Flexibles Neocel, Palmela, Portugal). Three packages were prepared for each evaluation day (0, 2, 6, 9 and 14 days of storage) at each treatment temperature.

2.2. Package atmosphere composition and respiration rate

Oxygen and carbon dioxide concentrations were monitored with a CheckMate II, (PBI Dansensor, Ringsted, Denmark) via a small needle inserted into the package headspace. For respiration rate determination, a closed system method was used: rocket leaves were weighed (ca. 10 g) and placed in 250 mL sealed glass jars, at 4 °C for 3 h. Carbon dioxide production was determined using a CheckMate II, (PBI Dansensor, Ringsted, Denmark) by inserting a small needle into the glass jar headspace through a rubber septum.

2.3. Chlorophyll content

Leaf chlorophyll content was determined using leaf readings from a portable chlorophyll meter (Konica Minolta SPAD-502 Plus; Minolta, Osaka, Japan). The SPAD-502 measurements were made on each analysis day with 15 readings performed for each replicated package from each temperature treatment. The adaxial side of the leaves was always placed toward the emitting window of the instrument and major veins were avoided.

2.4. Phenolic compounds

Total phenolic compound content was determined as described by Ferrante et al. (2004). Fresh leaf tissue (0.1 g) was ground and homogenized with 10 mL methanol. The homogenate was centrifuged (3000 *g*, 15 min). The supernatant was collected (methanol and polyphenols) and 50 μL of Folin–Ciocalteu reagent and 1 mL of 1 N sodium carbonate (Sigma-Aldrich) were added to 50 μL of the methanol extract. The final volume was made up to 2.5 mL with 1.4 mL of deionized water. Samples were allowed to react in the dark. Absorbance readings were taken after 30 min at 765 nm.

Qualitative and quantitative profiles of phenolics were determined by HPLC-DAD (Waters Series 600, Mildford MA, USA) based on the method by Oliveira, Pintado, and Almeida (2012). Separation was performed on a reverse phase Symmetry® C18 column (250 × 4.6 mm I.D., 5 μm particle size and 125 Å pore size) with a guard column containing the same stationary phase (Symmetry® C18). Chromatographic separation of phenolic compounds was carried out with solvent A consisting of formic acid, water and methanol (92.5:5:2.5 V/V), and solvent B consisting of methanol and