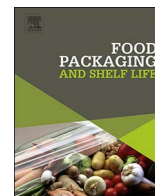




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Effect of temperature abuse and improper atmosphere packaging on volatile profile and quality of rocket leaves

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ABSTRACT

This study aimed to investigate the effect of temperature abuse and improper packaging on volatiles profile, vitamin C and sensorial attributes of rocket leaves packaged in modified atmosphere. Leaves packed in sub-optimal conditions (high ratio of product weight/bag surface) were stored for 10 days at 0 and 5 °C, and for 8 days at 15 °C. Rocket leaves were kept in macroperforated bags in order to prevent modification of atmosphere within the headspace (as control). The packed rockets at 0 °C retained ascorbic acid content while it decreased during storage at higher temperatures. The main losses in the appearance and vitamin C content were observed when the O₂ level reached about 0 kPa corresponding to the highest CO₂ accumulation in the bag (25 kPa). The off-odors from dimethyl sulfide (DMS), dimethyl disulfides (DMDS) and other volatiles were produced at 5° and 15 °C, changing the initial headspace fingerprint, which was best preserved at 0 °C. Results of this work showed that improper packaging condition may decrease the shelf-life of rocket leaves compared to storage in air, inducing loss of appearance score, the production of off-odors and the degradation of Vitamin C. No additional benefit was obtained by optimal gas composition when bags were stored at 0 °C, indicating that the use of low temperature was effective to slowing down degradation reactions.

1. Introduction

Wild rocket (*Diplotaxis tenuifolia*) is one of the most popular leafy green vegetables in Europe. It is most appreciated for its sulfurous odor and characteristic bitter and pungent taste due to the presence of glucosinolates, typical compounds of Brassicaceae family (Blažević & Mastelić, 2008), and their breakdown products isothiocyanates (Bennett, Rosa, Mellon, & Kroon, 2006; Bennett et al., 2002; D'Antuono, Elementi, & Neri, 2009; Pasini, Verardo, Cerratani, Caboni, & D'Antuono, 2011). Rocket is available in the market as raw produce or washed and packed in polypropylene (PP) film bags (Løkke, Seefeldt, & Edelenbos, 2012). The respiration rate (RR) of the product determines the extent of the changes in the internal gas composition of a package, depending also on the barrier properties of the packaging material, and the temperature under which the product is stored. Optimal conditions occur when MAP design results in the optimal gas concentrations at the equilibrium. On the contrary, improper MAP design and temperature abuse may lead to loss of quality, production of off-odor and increased product metabolism, which will in turn affect final gas composition inside the package. For MAP design, once the bag material and dimensions are fixed the atmosphere at the equilibrium

will be strictly related to produce RR (Sivertsvik, Rosnes, & Bergslien, 2002). In rocket leaves, RR has been shown to be highly variable according to the cultivar, the season, and the number of cutting (i.e. first, second, third, etc.). A variation from 6.95 to 3.92O₂ mmol kg⁻¹ h⁻¹ at 20 °C was reported by Seefeldt, Løkke, and Edelenbos (2012) passing from spring to late summer, and some differences are due to the number of plant cuttings (Martínez-Sánchez, Allende, Cortes-Galera, & Gil, 2008). Koukounaras et al. (2007) reported a decrease in RR with increase in maturity, from 500 to 300 mg CO₂ kg⁻¹h⁻¹ at 10 °C. The same authors indicated a potential storage of 16 days for fresh rocket leaves stored in air at 0 °C, and 13 days at 5 °C, whereas, Amodio et al. (2015) reported a shelf-life of about 6 days for packed rocket stored at 5 °C, limited by appearance degradation, when an estimated atmosphere with about 8.5% of O₂ and 8 of CO₂ was reached. Martínez-Sánchez, Marin, Llorach, Ferreres, and Gil (2006) showed that a controlled atmosphere with 5 kPa O₂ and 10 kPa CO₂ was effective in preserving a good appearance of the leaves (still commercially acceptable after 14 days of storage) if compared to the storage in air (not commercially acceptable after 10 days) at 4 °C. A more recent work suggested that an atmosphere with O₂ higher than 2 kPa and CO₂ lower than 15 kPa can be optimal (Rux, Caleb, Geyer, & Mahajan, 2017). As

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for volatiles, an accumulation of ethanol, dimethyl disulfide, aromatic aldehydes (benzaldehyde and benzeneacetaldehyde) and isopropyl isothiocyanate was observed when oxygen dropped below 2 kPa and particularly after washing; volatiles were extracted using the static headspace sampling technique after homogenization and pestling with a mortar for 60 s. According to various studies, extraction methods of volatile organic compounds (VOCs) including sample homogenization, manipulation, the eventual sample heating, and sampling technique, i.e. static (Rux et al., 2017) or dynamic headspace (Spadafora et al., 2016), SPME (Luca, Mahajan, & Edelenbos, 2016), hydrodistillation (Blažević & Mastelić, 2008), significantly affect the volatile composition since they may enhance enzymatic and oxidation reactions yielding to odor formation and degradation. Luca et al. (2016) investigated VOCs of wild rocket by SPME technique, from both intact and wounded leaves, stored in MA in closed jars to mimic packaging in low and high OTR films, at two different storage temperatures (5 and 10 °C). It was concluded that acetone, carbon disulfide, Dimethyl sulfide (DMS), dimethyl disulfide (DMDS), nitromethane, pentane, 3-methylfuran, 2-ethylfuran, and DMDS were released in high concentrations under moderate O₂ conditions (O₂ ≥ 2.1 kPa). Spadafora et al. (2016) recorded VOC profile in fresh rocket packed in PP bags by using thermal desorption as VOC sampling technique and discriminated well between days and storage temperature. In this work, leaves were lightly manipulated (soft crushing) in order to enhance volatile release. The research found a close correlation of a group of aldehydes to the drop in vitamin C content, the fall in isothiocyanates and the increase in DMS and DMDS at 5° and 10 °C, compared with conditions at 0 °C. Loss of Vitamin C has also been associated with gas and temperature conditions over storage. Martínez-Sánchez et al. (2006) reported that ascorbic acid was converted into dehydroascorbic acid during storage and that vitamin C content was higher in rocket leaves stored in controlled atmosphere with the composition of 5 kPa O₂ + 5 kPa CO₂ and 5 kPa O₂ + 10 kPa CO₂, compared to air-stored samples. Amodio, Derossi, Mastrandrea, and Colelli (2015) found that ascorbic acid degradation was a critical factor in non-isothermal storage.

Beneficial effect of MAP on rocket are amply discussed in literature, but the information on detrimental effect of suboptimal storage condition on shelf-life are seldom reported. Objective of this work was to assess the effect of improper MAP on ascorbic acid, appearance and volatile profile of washed rocket packed in PP bags and stored at constant temperature of 0, 5 and 15 °C. Moreover a second objective was to discriminate the effect of gas composition at 5 °C, by sampling volatiles in rocket leaves stored on macroperforated bags, without any sample manipulation.

2. Materials and methods

2.1. Plant material and processing

Fresh rocket leaves (*Diplotaxis tenuifolia*) were harvested in Salento (Apulia, Italy). To ensure minimal processing phase the leaves were washed in chlorine solution (0.01% v/v) before being drained, portioned into 50 g samples and packaged in PP bags (17.5 × 17.5 cm², OTR = 1800 cm³m²d⁻¹, WVTR = 6gm²d⁻¹ at standard temperate conditions). Fifty-four bags (3 replicates × 3 temperatures × 6 sampling times) were stored at 0, 5 and 15 °C for vitamin C determination and sensorial analysis and 54 bags were also arranged for the analysis of headspace volatiles. Similarly, 72 samples were placed in macroperforated bags of the same material, in order to prevent the modification of the atmosphere (control samples). Vitamin C and sensorial attributes were evaluated at day 0 and after 1, 2, 3, 6, 8 and 10 storage days for samples at 0 and 5 °C, while for samples kept at 15 °C sampling was interrupted on day eight. Volatiles analysis was carried out at day 0 and after 2, 3, 6, 7, 8 and 10 storage days for samples at 0 and 5 °C and after 8 storage days for samples at 15 °C.

2.2. Packaging gas composition

Concentrations of O₂ and CO₂ inside the packages were monitored with a gas analyzer WITT Mapy 4.0 (Witten, Germany). Test probe of gas analyzer was inserted into each package through an adhesive rubber septum to prevent air leaking from the package.

2.3. Volatile extraction and headspace SPME GC–MS analysis

Before sampling, bags were equilibrated at 15 °C for 30 min. Before sampling the control samples stored in macroperforated bags in air at 5 °C were transferred in non-perforated bags before sampling. Volatiles were collected in the bag headspace, introducing SPME fibre inside the package through a rubber septum. A carboxen/polydimethylsiloxane (CAR/PDMS) fibre of 85 µm was exposed for 30 min to the bag headspace and introduced into the GC injector port at 250 °C, for a desorption time of 4 min, using the split injection mode (1:20). An Agilent gas chromatograph model 6890 Series coupled to an Agilent 5975C network mass selective detector was used. Analytes were separated on a HP-5 ms capillary column (60 m × 250 µm × 0.25 µm) by applying the following temperature program: 40 °C for 4 min, up to 140 °C at 3 °C/min, with a final holding time of 10 min. The temperature program was optimized selecting temperature and time conditions providing the shortest run and the maximum number of volatile compounds. Transfer line temperature was 280 °C. Mass detector conditions were: electronic impact mode at 70 eV; source temperature at 230 °C; scanning rate at 2.88 scan/s; mass scanning range of *m/z* 30–400. The carrier gas was helium at 1.0 mL/min. Compounds were identified by comparing their retention times and mass spectra with those of pure compounds (Sigma-Aldrich, Milan, Italy), when available, or putatively assigned by comparing the mass spectra with the data of a system library (NIST 02, *p* > 80).

2.4. Vitamin C content

Five grams of fresh rocket tissue were homogenized with 10 mL of MeOH/H₂O (5:95) plus citric acid (21 g L⁻¹) with EDTA (0.5 g L⁻¹). The homogenate was filtered through cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata and Dufour (1992), with some modifications. The HPLC analysis was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of 20 µL were analyzed with an Agilent 1200 Series HPLC. The HPLC system consisted of a G1312A binary pump, a G1329A autosampler, a G1315 B photodiode array detector from Agilent Technologies (Waldbronn, Germany). Separations of DFQ and AA were achieved on a Zorbax Eclipse XDB- C18 column (150 mm × 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH/H₂O (5:95 v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 1 mL/min. AA and DHAA contents were expressed as mg of ascorbic or dehydroascorbic acid per 100 g of fresh weight (mg 100 g⁻¹).

2.5. Sensorial analysis

The appearance and off-odor scores of all samples were evaluated by a 5-member trained panel. Appearance was subjectively scored on a 5 to 1 scale, where 5 = excellent (fresh and turgid appearance, bright and uniform green color), 4 = good (slight loss of turgidity and fresh appearance), 3 = fair (noticeable loss of turgidity and possible slight loss of green color), 2 = poor (severe loss of turgidity, wrinkling and yellowing of leafy blades), 1 = very poor (severe yellowing of leafy blades and wilting, possible appearance of decay). A score of 3 was considered as the limit of marketability. Off-odor was scored on a 5 to 1

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