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Off-odor compounds responsible for quality loss of minimally processed baby spinach stored under MA of low O_2 and high CO_2 using GC–MS and olfactometry techniques

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ABSTRACT

Strong off-odor development is still one of the major problems associated with quality loss of baby spinach stored in MA with low O_2 and high CO_2. Freshness has been generally evaluated by sensory techniques that allow the description of aromatic profiles but it does not identify the responsible compound(s). An approach including sensory analysis and Gas Chromatography-Mass Spectrometry (GC–MS)/Gas Chromatography-Olfactometric (GC-O) techniques were applied to elucidate the complex combination of volatile organic compounds (VOCs) responsible for the off-odor perception of intact baby spinach without generation of new volatiles due to tissue manipulation. After 14 days, levels of low O_2 and high CO_2 (0.3 and 9.3 kPa, respectively) were achieved and off-odors development was detected. After GC–MS/GC-O analysis, there were 39 main compounds with olfactory activity, identified as alcohols associated with lipid peroxidation or LOX pathway; sulfur compounds from amino acid degradation; and alkanes from lipid autoxidation processes or carotenoid degradation. Odor-active compounds were grouped into the six odor categories as described by the sensory panel, with rotten and fishy being the strongest odors perceived after storage. The isolated VOCs grouped in the rotten descriptor were the alcohols 1-pentanol, (*Z*)-3-hexen-1-ol and 1-octen-3-ol, and the sulfur compounds methanethiol and dimethyl disulfide. A compound responsible for fishy notes was dimethyl sulfide but some evidence indicated that amine compounds with low odor thresholds could be also implicated. Since sulfur compounds were perceived by all sniffers and easily detected by mass spectrometry, they may be good candidates as biomarkers of off-odors in baby spinach.

1. Introduction

Baby spinach (*Spinacia oleracea* L.) of outstanding nutritional and sensory quality has been developed through the optimization of many pre and postharvest processing operations including the production system (Gutiérrez-Rodríguez et al., 2013), harvest time (Garrido et al., 2015a), cooling (Garrido et al., 2015b), postharvest handling (Medina et al., 2012), packaging and storage (Allende et al., 2004; Garrido et al., 2016) as well as distribution and commercialization (Kou et al., 2014). Strong off-odor development is still one of the major problems associated with its quality loss (Cantwell et al., 1998; Tudela et al., 2013). Volatile organic compounds (VOCs) responsible for the characteristic aroma and off-odors of salad greens have been investigated very little. Most of the studies related to the characteristic flavor attributes, loss of freshness odor and/or accumulation of off-odor have been carried out on baby leaves of *Brasicaceae* family such as rucula (Jirovetz et al., 2002; Endelenbos et al., 2015; Luca et al., 2016; Rux et al., 2017). Only a few of them have addressed the off-odors produced by ammonia and fermentative volatiles as indicators of stressful conditions under storage in modified atmosphere (MA) (Cantwell et al., 2010; Tudela et al., 2013). Generally, off-odors are developed in spite of the visual quality that remains unchanged (Cantwell et al., 1998). This is the case of baby spinach in which off-odors are developed during storage when O_2 and CO_2 concentrations in the headspace of the bags reach certain limits due to the respiration rate and the darkness conditions (Tudela et al., 2013; Garrido et al., 2016). Under darkness the degradation processes are accelerated through anticipated damage of the photosynthetic machinery with respect to the respiratory pathway (Keech et al., 2007).

For most salad leaves, it is difficult to assess the perception of

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freshness, therefore the absence of negative attributes is often more appropriate than the presence of fresh-like attributes (Løkke et al., 2012). Freshness of baby spinach has been generally evaluated by a sensory panel (Allende et al., 2004; Kaur et al., 2011; Medina et al., 2012; Tudela et al., 2013; Kou et al., 2014; Garrido et al., 2016). This approach allows the description of aromatic profiles but it does not identify the compound(s) responsible for consumer preferences or rejections. Several volatiles related to off-odors of spinach have been described as present after treatments such as high-pressure high-temperature (Kebede et al., 2013) as well as after cooking (Näf and Velluz, 2000) or dry processes (Masanets et al., 1998). In our previous study, we observed that the shelf life of baby spinach stored under MA with low O_2 and high CO_2 was reduced because of the development of strong off-odors (Tudela et al., 2013). However, the identification of off-odors responsible for the product rejection and their precursor compounds was still unsolved. This is particularly relevant for processors who need to assure freshness, including the absence of off-odors, as a challenge that affects not only consumers' acceptance but also future purchase intentions.

Gas-Chromatography coupled to Mass Spectrometry (GC–MS) is an analytical technique that can identify the volatile profile even in complex mixtures (Masanets et al., 1998; Näf and Velluz, 2000; Kebede et al., 2013). However, the real role of each component in the aromatic pool must be assessed by the odor activity (Jordán et al., 2001). Olfactometry is a powerful technique to determine essential VOCs responsible for the characteristic aroma of a product (Jirovetz et al., 2002). Applications of GC coupled to a sniffing port detector such as olfactometry (GC-O) include the correlation between sensory responses and VOCs, allowing the identification of essential compounds responsible for the off-odors (Van Ruth, 2004). Furthermore, the increasing accuracy of pressure and flow control has allowed the use of the retention-time locking (RTL) method to assure the same retention time for the same compound in different GC systems (Etxebarria et al., 2009).

With this background, the purpose of this study was to identify the volatile compound(s) responsible for the characteristic off-odor of minimally processed baby spinach stored in MA with low O_2 and high CO_2 using GC–MS and olfactometry. These off-odor VOCs that cause product rejection have not been identified previously in minimally processed baby spinach.

2. Materials and methods

2.1. Plant material

Baby spinach (*Spinacia oleracea* L.) washed and dried by a processor (Florette Iberica, Torre Pacheco, Murcia, Spain) was transported (40 km) in polystyrene boxes under refrigerated conditions to the Quality and Safety Laboratory (CEBAS-CSIC, Murcia, Spain). In the processing room at 7 °C, baby leaves were hand-sorted and damaged leaves discarded. Then, baby spinach was packed using a vertical packaging machine (Etna 280-X model, Ulma, Oñati, Spain) and polypropylene (PP) film with the following characteristics: 35 μm thickness, O_2 permeance of 2.629 E^{-12} mol m^{-2} s^{-1} Pa^{-1}, CO_2 permeance of 9.84 E^{-12} mol m^{-2} s^{-1} Pa^{-1} and H_2O permeance of 5.408 E^{-6} mol m^{-2} d^{-1} at 7 °C and 97% RH. Bag size was 230 mm x 300 mm for each 100 g leaves. Bags were sealed under ambient air conditions. Passive modified atmosphere (MA) was created by the film permeability and the product respiration rate during storage for 14 days at 7 °C under darkness.

2.2. Headspace gas composition

Changes in the headspace gas composition during storage were measured every 2 days in individual bags (n = 10) by sampling 0.5 mL of the headspace gas composition with a plastic syringe through a silicone sealant placed on the bags. Samples were analyzed using an infrared CO_2 detector (Via 510, Horiba Instruments Co., Irvine, CA, USA) and an O_2 analyzer (CG-1000, Ametek, Thermox Instruments Co., Pittsburgh, PA, USA). Results were expressed as kPa of O_2 and CO_2.

2.3. Judge training for olfactometry detection and description

A panel composed by 10 persons was trained on the olfactory sensations in previous experiments. From that group, only 4 persons were nominated as members of the judge panel, 3 women and 1 man, non-smokers and aged between 33 and 42. The judge members, or sniffers, were trained with commercial bags of baby spinach at different quality stages for 4 months previous experiments. An expert on volatile compounds helped to standardize lexicon and descriptors for the olfactometry detection following previous descriptors (Talavera-Bianchi et al., 2010). The same judges performed the sensory evaluation and the GC-O analyses.

2.4. Sensory evaluation

Hedonic tests were performed by 4 trained judges at day 0 and after 14 days of storage. A closed bag was presented to each evaluator for odor rating. Each evaluator assessed the spinach odor just after opening the bag and after placing the baby spinach in a tray at room temperature 5 min later. Odor description tests were assessed with low light intensity. Judges were required to describe the olfactory sensation and the intensity (1 = slight and 5 = severe) after a deep breath. Odor descriptor were grouped into six categories according to previous trials (Talavera-Bianchi et al., 2010): spinach (the brown, green, slightly musty, earthy aromatics associated with fresh spinach), sea breeze (the smell of sea cliffs, beach sand, seaweed and salty aroma), fishy (fresh fish, below slight shellfish-clam, and very slight salty), rotten (humus-like aromatics with damp soil and decaying vegetation), fruity (the aromatic associated with commonly known bananas, mandarins, pineapple and apple) and vegetable (a green aromatic associated with newly cut-grass and leafy plants). Data normalization was performed to present results as Σintensity/number of panelist describing the odor.

2.5. Extraction and separation of volatiles

Headspace solid phase micro extraction (HS-SPME) technique was used to extract and concentrate VOCs of baby spinach as previously described (Cachet, 2010). After each evaluator assessed the odors, all the leaves from each bag (100 g) were placed in a 1000 mL Erlenmeyer flask sealed with PTFE-faced septa and a plastic crimp-cap. Equilibration was achieved by placing the flask at 20 °C for 40 min. Volatiles were then adsorbed for 15 min using a 1 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane Stable Flex SPME fibre (Supelco, Bellefonte, PA, USA). Then, the adsorbed VOCs were subjected to separation by capillary GC. For thermal desorption, the needle was inserted into the splitless injection port (200 °C) of the GC-O and the GC–MS system for 5 min. In both cases VOCs were separated on a DB-WAXTER 30 m × 0.25 mm (i.d.) capillary column (Agilent Technologies) with helium as a carrier gas. Injector and detector temperatures were set at 200 and 250 °C, respectively. The oven temperature was 40 °C for 5 min, then an increment of 10 °C/min was applied until achieving 240 °C, holding for 2 min.

2.6. Gas chromatography – mass spectrometry (GC–MS)

The analysis was carried out using an Agilent 7890A GC equipped with an Agilent 5975C series inert MSD with triple axis detector (Agilent Technologies, Santa Clara, USA). Helium was used as a carrier gas at a flow of 1.0 mL min^{-1}. Mass spectra data were acquired in the full-scan mode ranging from m/z 30 to 350 with a scan rate of 5.0

scans/s. The mass spectrometer was operated in electron impact ionization mode with an ionizing energy of 70 eV. The quadrupole and ion source temperatures were set at 150 °C and 230 °C, respectively. The electron multiplier voltage was maintained at 70 V.

2.7. Gas chromatography- olfactometric (GC-O)

The olfactory detection was carried out on an Agilent 7820A GC equipped with a flame ionization detector (FID) (Agilent Technologies, Santa Clara, USA) and a Gerstel ODP-3 sniffing port (Baltimore, MD). Flow rate was 25 mL min^{-1} with helium as a carrier gas with temperature control (Gerstel C200) set at 300 °C.

Gas chromatography effluent was split 3:1 among sniffing port and FID using a deactivated capillary column. Humidified air at 15 mL min^{-1} was added to avoid condensation and provide better olfactory sensations to sniffers. Data were collected using Gerstel olfactory recorder software that allows recording a verbal description of the perceived odors made by the sniffers and its retention time.

2.8. Identification and data analysis

For the tentative identification in GC–MS, data were processed as previously described (Cozzolino et al., 2016) using MSD ChemStation software (Agilent Technologies, Inc, Santa Clara, CA, USA). First, pure standard compounds were used for comparison of the retention time and mass spectra. Odor standards used for identification were: 1-octen-3-ol, acetone, dimethyl sulfoxide and dimethyl sulphide (HPLC grade from Sigma-Aldrich). The second step was the comparison between the MS spectra for each compound with those provided by a library search of the National Institute of Standards and Technology database (NIST, 2008) and a similarity coefficient (Sim) or reverse similarity coefficient (RSim) > 85%. Then, the experimental retention index using a C7-C40 *n*-alkane series and reference retention index in Chemspider Database (2015) were considered as previously recommended (Delahunty et al., 2006; Molyneux and Schieberle, 2007; Stiles et al., 2007). For odor description, each sniffer evaluated twice the same sample and only odors with similar description in both evaluations were recorded. For frequency response (FR), a modified methodology from Jordán et al. (2003) was used. The retention times measured in the olfactometry runs were compared with the mass spectrometer retention times (Etxebarria et al., 2009).

Bibliography search was made on GC–MS results and only those compounds with similar description in GC-O, Flavornet (2004) and CheBi (2013) databases Flavornet (2004) and CheBi (2013) databases were considered as aromatically active compounds.

3. Results and discussion

3.1. Baby spinach stored under low O_2 and CO_2

Changes in the headspace gas composition were measured during storage. Reduction of O_2 along with the increase of CO_2 concentration was observed during storage of baby spinach at 7 °C (Table 1). After 14 days, levels of 0.3 ± 0.2 kPa O_2 and 9.3 ± 0.5 kPa CO_2 were achieved. Concentrations of low O_2 and high CO_2 generated with storage of baby spinach under passive MA at 7 °C affected the sensory quality, particularly the development of off-odors as previously reported (Medina et al., 2012; Tudela et al., 2013; Garrido et al., 2016). The headspace gas composition was similar to that described by Medina et al. (2012) and the development of off-odors was evident after 14 days of storage, 2 days after steady state. In Tudela et al. (2013) levels of 1 kPa O_2 and 11 kPa CO_2 were reached after 10 days at 7 °C and shelf life was reduced to 7 days because of the off-odors. According to Garrido et al. (2016), bags stored under light conditions slightly modified the composition of the headspace while the highest modification of the headspace gas composition was observed under darkness, with the consequent development of off-odors at levels of 0.2 kPa O_2 and 9.2 kPa CO_2.

Table 1
Headspace partial pressure of O_2 and CO_2 of minimally processed baby spinach stored under MA for 14 days at 7 °C (n = 10 ± standard deviation).

Storage time (days)	O_2 (kPa)	CO_2 (kPa)
1	18.7 ± 1.1	1.3 ± 0.1
3	14.8 ± 0.4	3.7 ± 0.1
6	9.2 ± 0.9	6.9 ± 0.2
8	5.8 ± 0.4	8.7 ± 0.3
10	3.7 ± 1.1	9.6 ± 0.4
13	0.3 ± 0.1	11.0 ± 0.5
14	0.3 ± 0.2	9.3 ± 0.5

3.2. Odor profile by sensory evaluation of baby spinach stored under low O_2 and high CO_2

The odor description of baby spinach at day 0 and at day 14 was as expected, spinach-like with green notes turned to a disgusting fishy odor after storage under MA with low O_2 and high CO_2 (Fig. 1). The descriptors were grouped into six categories. At day 0, the typical spinach odor was perceived by all panelists, along with fruity nuances described as green apple as well as sea breeze scent that included notes of the smell of sea breezes, beach sand, seaweed and salty aroma that disappeared with time. After 14 days, the development of off-odors was noticeable although the visual quality of the product was acceptable (data not shown). The spinach was free of any visual sign of decay and therefore the potential contribution of decay organisms to off-odor production was expected to be very low. Just after opening the bags, other odors rather than saturating fishy ones were barely distinguished at first (Fig. 1). In addition, salty notes and rotten vegetables were also described with high intensities as secondary olfactory sensations. Five min after opening the bags, differences between the rotten and the fishy scents were noticed, both of them described as saturating. Kou et al. (2014) reported that storage at temperature above 8 °C significantly shortened the shelf life of baby spinach due to off-odor development, tissue electrolyte leakage and yellowing. Sensory evaluation was complemented with the perception of individual odor attributes by GC-O from the volatile pool to elucidate the VOCs responsible for off-odors in baby spinach.

3.3. Identification of odor-active compounds of baby spinach stored under low O_2 and high CO_2

By comparing experimental retention index with those reported in Chemspider Database (2015) and the resulting fragmentation spectrum with NIST Database (2008), a total of 62 volatile compounds (33 at day 0 and 47 at day 14) were tentatively identified by GC–MS. Among them, 18 appeared on both sampling days, 15 were exclusive detected at day 0 and 29 at day 14 (data not shown). Most of them were identified as alkane and alcohols, followed by sulfur compounds, terpenes, nitrogenated compounds, benzenes and furans among others. After comparing retention time between GC–MS/GC-O analyses and odor description in databases, from the 39 odor-active volatiles detected (Fig. 2), the identification of 29 of them was possible (Table 2). In MA, the metabolism can change from aerobic to anaerobic fermentation and accumulation of alcohols such as ethanol and acetaldehyde is primarily associated with anaerobic atmospheres or high CO_2 atmospheres (Toivonen, 1997; Nielsen et al., 2008). However, as previously reported in baby spinach, the fermentative volatiles were not responsible for the off-odor developed under low O_2 and high CO_2 (Tudela et al., 2013).

The major chemical reactions for the formation of the characteristic volatiles responsible of off-odors were unsaturated fatty acid degradation, Maillard and consecutive reactions, enzymatic hydrolyses and

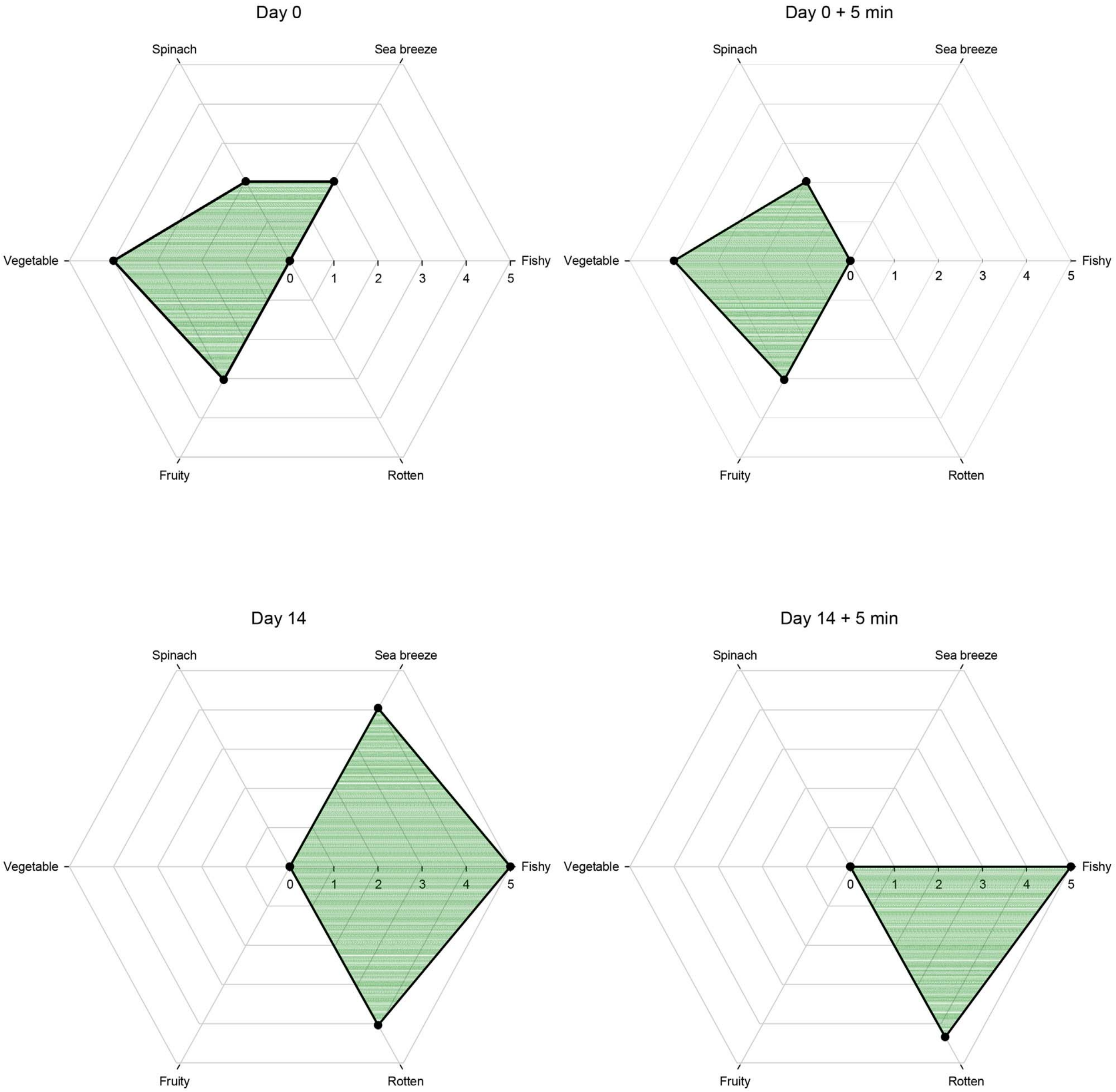

Fig. 1. Odor profile of minimally processed baby spinach under MA with low O_2 and high CO_2 before storage (day 0) and after storage at 7 °C (day 14) after opening the bags and 5 min later.

degradation of sulfur-containing amino acids (Kebede et al., 2013). It is notable that, at day 14 an increase in the variety of compounds as well as in the number of odor-active volatiles was observed, enabling a better description of these perceptions. The number of odors detected by all sniffers (FR = 4) increased from only three at day 0 (27, 34 and 37) to nine at day 14 (2, 3, 4, 5, 11, 27, 33, 37 and 39). In contrast with the results from GC–MS, the importance of the main chemical families was different; the main compounds with olfactory activity being alcohols and sulfur compounds follow by alkanes.

Accumulation of alcohols was primarily associated with anaerobic or high CO_2 concentrations but also with lipid peroxidation (Toivonen, 1997). Among alcohols, four of them were generated after storage (12, 13, 14 and 19), but only 1-pentanol (19) was negatively described. Hexanol (23) and (*Z*)-3-hexen-1-ol (25), which are green leaf volatiles (GLV), described from the linoleic acid pathway formed from the 13-LOX branch in the lipoxygenase pathway (King et al., 2006; Loreto and Schnitzler, 2010). These compounds are usually synthesized in green organs and released immediately after the damage or wounding of cellular structures (Dudareva et al., 2013). Compounds (14), (19) and (25) have been associated with lipid hydroperoxy reactions (Toivonen, 1997). Unsaturated alcohols such as 1-octen-3-ol (27), mainly derived from the hydrogen peroxide degradation of linoleic acid, generally have much a lower odor threshold than the saturated ones and therefore they have greater impact on the overall aroma (Wu et al., 2014).

Regarding sulphur compounds (3, 4, 11 and 33), all of them with negative description, only dimethyl disulphide (11) was detected at both sampling days. This compound together with dimethyl sulphide (4), both with a low odor threshold, was described as the most potent odorant in rucola (Nielsen et al., 2008; Spadafora et al., 2016) with similar descriptors to the present study. Methanetiol (3) production is induced under low O_2 atmospheres and inhibits cytochrome C oxidase and other enzymes involved with protection from peroxidative damage (Toivonen, 1997). Compounds (3) and (4) were previously reported in raw spinach (Masanetz et al., 1998). Previously, thermal induced

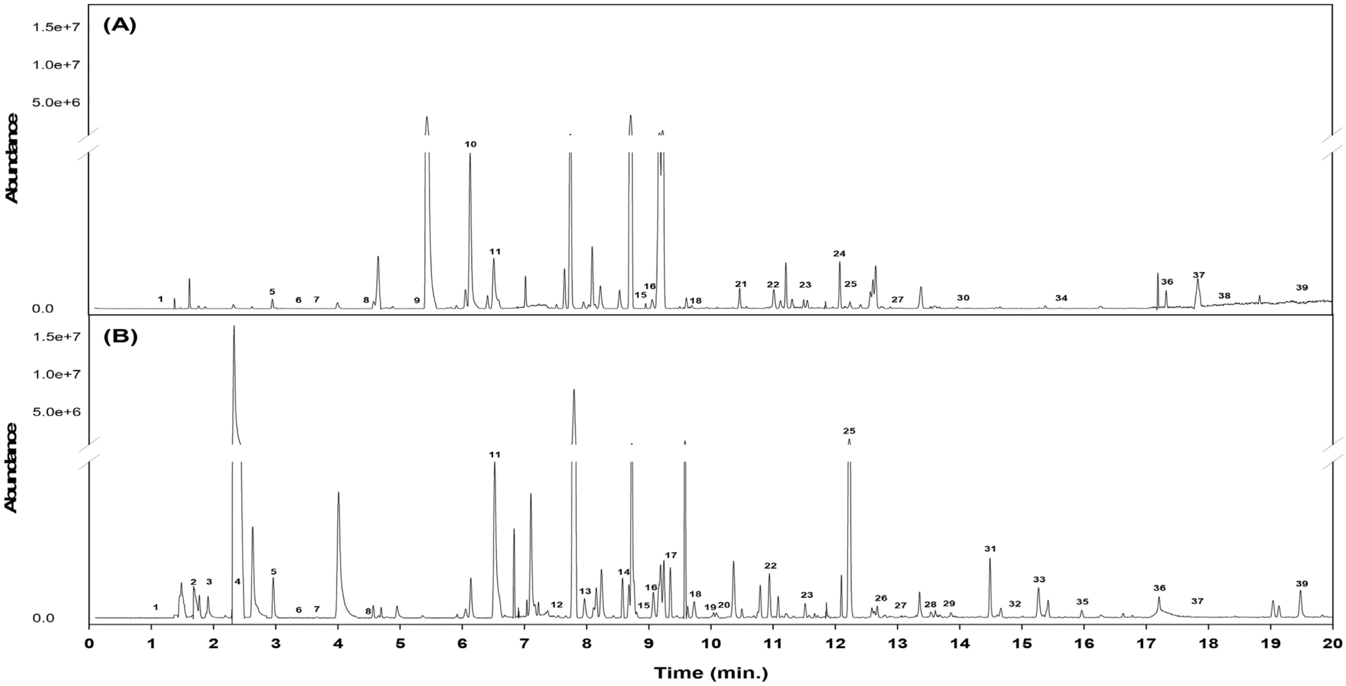

Fig. 2. Chromatogram (GC–MS) of volatile organic compounds of minimally processed baby spinach at day 0 (A) and after storage for 14 days under MA at 7 °C (B). Numbers correspond with the compounds and olfactory descriptions by GC-O included in Table 2.

degradation and enzymatic hydrolyses, ensuing from loss of cellular integrity, were described as the two possible reactions involving sulfur amino acids responsible for the formation of characteristic sulfur volatile compounds of spinach (Kebede et al., 2013). In fresh spinach, it has been reported that the amino acids limiting the quality of protein are methionine and cysteine, both sulfur-containing compounds with an average concentration of 33 and 36 mg/100 g, respectively (Lisiewska et al., 2011).

Although alkanes were the main compounds detected in the volatile pool, only four (1, 7, 17 and 24) were identified by their odors related to chemical sensations, but neither of them with negative descriptions. Alkanes are originated from lipid autoxidation processes or from decomposition of carotenoids and have relatively high thresholds (Wu et al., 2014). This can be the reason why, although alkanes were the main volatiles compounds in GC–MS analysis, they were almost not detected by the sniffers. This fact supports the need for another approach rather than exclusively instrumental to elucidate odor active compounds responsible for product rejection.

Volatile terpenes (31, 35 and 36) were detected, probably formed from the methylerythritol phosphate (MEP) pathway under anaerobic conditions, in which the production of ATP shifted from the mitochondrial to anaerobic glycolysis, pyruvate being one of its products and the main substrate for MEP (Agarwal and Grover, 2006; Dudareva et al., 2013). The release of terpenes is elicited by abiotic stresses such as salt, drought or tissue damage (Niederbacher et al., 2015). Terpene formation requires high levels of photosynthetic carbon but isoprene may also be formed from chloroplastic starch (Loreto and Schnitzler, 2010).

A volatile chlorinated compound (10) was identified only at day 0. This emission could have been produced as a result of washing with chlorine as previously reported in baby spinach (Gómez-López et al., 2013). Only one furan, 2-pentylfuran (18), was confirmed by GC-O although many of them were tentatively identified by GC–MS. Among them, 2-methylfuran and 3-methylfuran were compounds described from Maillard and Stecker degradations between amino acids and reducing sugars (Kebede et al., 2013). Similarly, among all ketones detected in GC–MS analysis, only two of them (5 and 20) were confirmed. These types of compounds have been described after treatment with high pressure that accelerates anaerobic thermal degradation of unsaturated fatty acids leading to the formation of aliphatic aldehydes and ketones (Kebede et al., 2013).

3.4. Relationship between odor profile and GC-O/GC–MS data

It is difficult to determine the relevance of VOCs as compounds responsible for the rejection of baby spinach by GC-O. This is because of the complexity of the matrix and also the specific anosmia, inattention or simply the noncontiguous breathing process that alters the perception (Pollien et al., 1997; Jordán et al., 2001). In order to understand the off-odor development and the precursors, the relationship between odor profile and GC–MS/GC-O was examined. Low O_2 injury associated with ethanol accumulations does not occur until ethanol is oxidized to acetaldehyde via catalase when tissue returned to air (Toivonen, 1997). Moreover, this phenomenon may enhance the quick release of volatiles once the product has been removed from the package (Toivonen, 1997). Since ethanol was tentatively identified on both days, although no odor description was given, it can explain the change in the perception of odors in the bag and once it was exposed to room atmosphere for 5 min.

Odor-active compounds included in Table 2, except 8 VOCs (10, 17, 24, 30, 31, 32, 35 and 38), were grouped into the six odor-profile categories as mentioned in Fig. 1. Even though unpleasant odors were perceived for some compounds (1, 5 and 33), no description was given for them and so none of these volatiles were considered as contributors to off-odors.

The compounds related to the vegetable odor profile at day 0 were alcohols (6, 16, 22, 23 and 25). The green apple note described at secondary odor in bagged baby spinach could be due to the presence of another primary alcohol (29) in low concentration. Among all odor descriptions at day 0 with olfactometric technique, none of them reflect the sea breeze notes described in the sensory analysis. These sea breeze notes disappeared 5 min after opening the bags, and the fact that they did not appear in GC-O analysis supported the theory that the sea breeze scent was due to VOCs with high vapor pressure such as hydrogen sulfide (Du et al., 2014). Surprisingly, the fresh spinach odor was not perceived in GC-O. If multiple odorants coexist, the possible occurrences of synergy, neutralization, antagonistic interference, etc. could make the perception of odor ambiguous (Kim and Park, 2008).

Table 2
Headspace SPME volatile organic compounds (VOCs) and olfactoric descriptions (GC−O) of minimally processed baby spinach at day 0 (D0) and after storage for 14 days under MA at 7 °C (D14).

#	RT	Compound	RI	Odor description D0	FR	Odor description D14	FR	Identification
1	1.10	Propane	638	burning	1	pungent	1	LRI, AD, MS
2	1.50	Unknown		–		fishy, rotten	4	
3	1.91	Methanethiol	719	–		rotten eggs	4	LRI, AD, MS
4	2.33	Dimethyl sulphide	758	–		cockle	4	LRI, AD, Std, MS
5	2.95	Acetone	811	irritating	1	irritating	4	LRI, AD, Std, MS
6	3.40	Unknown		vegetable	2	seashells, sea breeze	2	
7	3.82	Nonane	880	chemical	1	rancid	3	LRI, AD, MS
8	4.40	Unknown		medicinal	1	grass	1	LRI, AD, MS
9	5.20	2-Oxobutanoic acid	976	sweet	2	–		
10	6.14	Chloroform	1035	floral	2	–		
11	7.10	Dimethyl disulfide	1093	burning nose	2	feces, sulfurous	4	LRI, AD, MS
12	7.63	3-Pentanol	1124	–		greenish	2	LRI, AD, MS
13	7.89	2-Hexanol	1139	–		vegetable	3	LRI, AD, MS
14	8.58	1-Penten-3-ol	1179	–		green vegetable	3	LRI, AD, MS
15	8.80	Unknown		meaty, acid	1	mild rotten	2	LRI, AD, MS
16	9.08	o-Xylene	1208	spiced, mustard	3	geranium	2	LRI, AD, MS
17	9.41	Hexadecane	1228	–		petrol, chemical	1	LRI, AD, MS
18	9.73	2-Pentylfuran	1246	cantaloupe	1	green, grass	3	LRI, AD, MS
19	10.04	1-Pentanol	1265	–		fermented	3	LRI, AD, MS
20	10.20	3-Octanone	1274	–		woody, forest	3	LRI, AD, MS
21	10.36	Styrene	1284	green melon	1	–		
22	11.19	(*Z*)-2-Penten-1-ol	1335	ethereal, fresh	1	green, damp	3	LRI, AD, MS
23	11.67	1-Hexanol	1365	fresh cut grass	2	burning, green	2	LRI, AD, MS
24	12.10	Tetradecane	1392	mild butane gas	1	–		
25	12.23	(*Z*)-3-Hexen-1-ol	1401	cauliflower	3	rotten vegetable	3	LRI, AD, MS
26	12.60	Unknown		–		green pepper	3	LRI, AD, MS
27	13.10	1-*O*cten-3-ol	1459	earthy, clay	4	mushrooms, moldy	4	LRI, AD, Std, MS
28	13.35	Acetic acid	1476	–		lettuce	2	LRI, AD, MS
29	13.69	2-*E*thyl-1-hexanol	1500	green apple	1	lettuce	1	LRI, AD, MS
30	14.00	Unknown		creamy, buttery	2	–		
31	14.49	Linalool	1558	–		floral, roses	3	LRI, AD, MS
32	14.90	Unknown		dusty, potato	3	–		
33	15.27	Dimethyl Sulfoxide	1616	–		intense burning	4	LRI, AD, Std, MS
34	15.70	Unknown		sweet	4	–		
35	15.96	β-Cyclocitral	1669	–		saffron	2	LRI, AD, MS
36	17.20	+ Valencene	1768	white flowers	3	sweet, medicinal	1	LRI, AD, MS
37	17.60	Unknown		sweet, floral	4	sweet, melon, floral	4	LRI, AD, MS
38	18.20	Unknown		chemical	2	–		
39	19.40	2-Phenylethanol	1951	candy	2	sweet	4	LRI, AD, MS

Odor description using GC-olfactometry technique. RT: Retention time. RI: Retention Index. FR: Frequency response. Identification: LRI (Linear Retention Index in Chemspider and CheBi databases), AD (Aroma Description), Std (Standard), MS (Mass Spectrum in NIST database).

This suggests that several compounds could be involved in the perception of fresh spinach odor as described previously in other produce (Forney, 2016).

The odor description of two alcohols (25 and 27) changed from vegetable notes to rotten vegetable and moldy respectively. This change from positive to negative perception also occurs for other compounds (7, 11 and 29), presumably due to the change in their concentration, as has been seen in other salad greens such as iceberg and butterhead lettuce (Longchamp et al., 2009), in which the increase in 2-ethyl-1-hexanol (29) was correlated with odor changes. At day 14, a potent sea breeze first impact that was reflected in the olfactometric analysis with dimethyl sulphyde (4) and one unknown compound (6) was perceived, but fishy and rotten were the predominant scents.

The isolated VOCs grouped in the rotten descriptor were mainly alcohols (19, 25 and 27) and sulfur (3 and 11) compounds as well as nonane (7) and an unknown volatile (15). The hidroxyperoxy lyase branch after LOX pathway degradation of fatty acids seems to be the main pathway related to the rancid and rotten descriptor as well as amino acids formed by enzymatic degradation (Dudareva et al., 2013). In addition, fishy notes were perceived but the compound was not identified as responsible (2). According to Masanetz et al. (1998) (Z)-1, 5-octadien-3-one and methional were the compounds responsible for fishy odors in dry spinach, but none of them were found in the present study. Other compounds with low odor threshold and fishy odor were volatile amines, such as trimethylamine (TMA), which has been described previously in spinach by Shim and Hee Baek (2012) after extraction and optimized conditions by GC–MS. TMA can easily be formed from choline, betaine and carnitine via Hofmann elimination reaction, which generates amine compounds at alkaline pH. In fact, the pH of baby spinach stored in MA with low O_2 was previously reported to increase from 6.6 to 7.2 after 12 days at 7 °C (Tudela et al., 2013). Moreover, it is known that high CO_2 concentrations might damage tissues by inducing NH_3 formation from amides and amino acids such as glutamine (Burg, 2004). Another two compounds that may contribute to the strong perception of fishy odors were dimethyl sulphide (4) and an unknown compound (6) described as sea breeze odors.

4. Conclusions

The combined use of headspace SPME analysis and GC–MS/GC-O techniques allowed the identification of 29 characteristic odor-active impact compounds from the 39 odor sensations described when baby spinach was stored under MA. Concentrations of low O_2 and high CO_2 inside the bags during storage at 7 °C in darkness had a high impact on the development of off-odors. The significant off-odor of baby spinach was not the result of one single odor impression. A multidisciplinary approach including the sensory analysis and GC–MS/GC-O was adjusted to determine the complex combination of compounds that contributed to the off-odor perception, mainly sea breeze, salty and fishy odors. Even though compound(s) related to fishy off-odors could not be elucidated, volatile amines seem to be responsible for some off-odor VOCs. Sulfur compounds were the most powerful off-odor compounds identified and played an important role in the negative perception of the product. Since sulfur compounds were perceived by all sniffers and easily detected by GC–MS, they seem to be good candidates for biomarkers of off-odors in baby spinach. Further analyses are needed to determine possible reaction pathways and the potential precursors as well as the relationship with some pre and postharvest strategies to reduce the incidence of off-odor development and quality loss of baby spinach.

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