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Volatile compounds of modified atmosphere packaged cut iceberg lettuce: Effect of extremely low O_2, season, cultivar and storage time

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ABSTRACT

This study investigates the changes in volatile compounds in minimally processed iceberg lettuce as a function of season, cultivar, packaging and time. In order to achieve this, iceberg lettuce cultivars Platinas, Diamantinas and Morinas were harvested from June to September 2009. Lettuces were minimally processed and stored under three different treatments: two passive modified atmosphere packagings using films of different permeabilities, F1 (OPALEN 65 AF) and F2 (OPP/PE-L 2040 AF), and storage in air. All packages were stored at 5 °C. Gas composition and volatile compounds were assessed at 1, 5, 8 and 11 days of storage in packaged lettuce, whereas in air stored samples volatiles were analyzed only at 1 and 5 days of storage. Twenty one potent odorants were identified by GC–O. Among the months, August presented a notary increase of elemene, β-selinene and 2,3-butanedione, which likely contribute to off-odor of packaged cut lettuce. The content of O_2 and CO_2 was demonstrated to influence the formation of odorants as storage time increased. Higher amount of cis-3-hexenol was related to aerobic conditions found in the modified atmosphere packages and air stored samples after 1 day of storage, whereas levels of odorants such as 2,3-butanedione, elemene and β-selinene were significantly enhanced under anaerobic conditions after 11 days of storage. Film F2 seems to be the most promising because it kept the concentration of elemene and β-selinene lower than film F1. No clear differences were seen between the cultivars Morinas, Diamantinas and Platinas with regard to production of objectionable odorants under anaerobic conditions.

1. Introduction

Minimally processed or cut vegetables are vegetables that have been cut in small pieces and are packaged and ready-to-eat (Saltveit, 2003). Among them, iceberg lettuce is one of the most popular ready-to-eat vegetables in retail groceries. Over the years, the research on cut lettuce has been focused on reducing enzymatic browning by using a modified atmosphere packaging (Heimdal, Kuhn, Poll, & Larsen, 1995), while little attention has been paid to the formation of volatiles. Previous observations indicate that modified atmosphere packaging with extremely low O_2 successfully reduces the browning. As a result, a product with good appearance is obtained, but there is a risk of development of off-odors, which can be a serious quality limiting factor. The volatiles emitted by lettuce have been shown to include aldehydes, alcohols, terpenes, ketones, acids, sulfur compounds, acetate esters, pyrazine and furan (Deza-Durand & Petersen, 2011). Among these compounds, copaene, 2-ethyl-1-hexanol, caryophyllene, α-longipinene, β-elemene, cis-3-hexenol, trans-2-hexenal and 2-methoxy-3-isobutylpyrazine have been identified as key odorants of cut iceberg lettuce (Arey et al., 1991; Lonchamp, Barry-Ryan, & Devereux, 2009; Nielsen & Poll, 2006). Furthermore, in cut lettuce packaged in modified atmosphere, short-chain methyl-branched alcohols and esters were found to predominate after 10 days at 20 °C, as a result of severe fermentation (Smyth, Song, & Cameron, 1998).

It has been indicated that the type and concentration of volatile compounds in fruits and vegetables generally depend on cultivar, season, packaging and storage time (Hodges & Toivonen, 2008; Smyth et al., 1998). To our knowledge there is little information on volatile formation in cut lettuce as a function of the factors mentioned. The cut lettuce industry uses different cultivars of lettuce depending on availability during the season. This could both quantitatively and qualitatively affect the formation of desirable and objectionable volatiles in the processed lettuce (Forney, Kalt, & Jordan, 2000). Likewise, season could affect the volatile formation in lettuce. For example in *Brassica* species, the changes of sulfur volatiles within a season are caused by variations in the amount of aroma precursors, i.e., glucosinolates, as a result of changes in environmental conditions (Mattheis & Fellman, 1999; Vallejo, Tomás-Barberán, Gonzalez Benavente-García, & García-Viguera, 2003).

This paper aims to investigate the changes in volatile compounds of cut lettuce as a function of season, cultivar, packaging and storage time.

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Storage in air is compared to modified atmosphere packaging in two different films. The atmosphere was not initially modified, but the respiration of the product in the packages decreased O_2 and increased CO_2 concentration over time (passive modified atmosphere packaging). The outcomes of this research can be used by the cut lettuce industry to obtain higher quality products.

2. Materials and methods

2.1. Plant material

Three different cultivars of Iceberg lettuce (*Lactuca sativa* L.), Platinas, Morinas and Diamantinas, were obtained from Rijk Zwaan, Odense, Denmark and sowed on 24th March, 11th May, 10th June and 27th June in 2009 in the commercial facilities of the nursery garden 'Bladgrønt', Denmark. Cultivars were transplanted once lettuces had 4 to 6 leaves per plant to an open field in Bogense, Fyn, Denmark on 20th April, 1st June, 6th July and 15th July. The harvest of lettuce was carried out after 51, 49, 43 and 55 days from transplanting on 10th June, 20th July, 18th August and 8th September, respectively. Lettuces were harvested based on a well formed lettuce's head and on a weight range from 400 to 500 g. After harvest, commercial maturity of lettuce was measured as firmness under hand pressure (Kader, Lipton, & Morris, 1973). Lettuces were held at 1 °C for 24 h in the farm's facilities and transported to a processing factory the next day.

2.2. Minimal processing and packaging of lettuce

Iceberg lettuces were processed in the facilities of the vegetable processing factory 'Gasa Odense', Denmark. Each lettuce cultivar was processed separately. A minimum of 20 lettuce heads of each cultivar were used. At the factory external leaves and core of lettuce were removed manually. Lettuces were cut in pieces around 6 mm wide with an Eillert, belt slicing machine G-1500 (Berkshire, UK). Subsequently the cut lettuce was washed in tap water at 2–5 °C for 3 min and centrifuged at 800 rpm for 10 s.

Two hundred and fifty grams of cut lettuce was weighed and packaged in two different films: film F1 (OPALEN 65 AF (65 μm)) and film F2 (OPP/PE-L 2040 AF (60 μm)). These films were commercially used by the processor. The O_2 and CO_2 transmission rate for film F1 was 35 and 158 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$ at 23 °C and 50% RH, respectively. For film F2 the O_2 transmission rate was 68 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$ at 23 °C and 85% RH, but no data was provided for the CO_2 transmission rate by the manufacturer Bemis Packaging (Horsens, Denmark). The F1 film should be considered an 'extremely low' transmission film while the transmission of F2 can be described as 'low'. The cut lettuce was packaged by the processor without any initial modification of the atmosphere (passive modified atmosphere packaging). The packages were stored overnight at the plant at 5 °C and delivered the next day to the University of Copenhagen, Department of Food Science. At the laboratory, a third treatment consisting of cut lettuce stored in air was set up. Packaged cut lettuce of each variety was unpacked and placed in a glass jar and sealed with a household film with 40 punctured holes of 1 mm in diameter in order to maintain an atmosphere similar to air. Samples packed in films F1 and F2 were stored in duplicates for up to 11 days (in total 74 samples), whereas, air stored samples were stored in duplicates for only 5 days due to excessive browning (in total 48 samples). The storage time was calculated from the packaging of the lettuce at the plant (day 0). All samples were stored at 5 °C in cool chambers in the laboratory (Termaks AS, Norway).

2.3. Gas analysis

CO_2 and O_2 concentrations of F1 and F2 packages were measured after 1, 5, 8 and 11 days of storage and for air stored samples after 1 and 5 days of storage. The measurements were made using a gas analyzer (Gaspace, Systech Instruments Ltd., Texas, USA). A syringe was pressed into the package to take the sample. The concentration was expressed as percentage (%) of CO_2 and O_2.

2.4. Dynamic headspace sampling

Volatiles emitted from cut lettuce packaged in films F1 and F2 were analyzed after 1, 5, 8 and 11 days of storage, whereas the analysis of air stored samples was performed after 1 and 5 days of storage. One hundred grams of cut lettuce was blended with 100 mL of tap water and 2 mL of internal standard (50 μg mL^{-1}, 4 methyl-1-pentanol, Sigma Aldrich) was added to verify the performance of the sampling. The sample was homogenized for 15 s using a blender (Struers Kebo Lab A/S, Denmark) and poured in a 1 L gas washing flask. The blender cup was washed with 50 mL of tap water that was added to the suspension and the flask was closed with a purge head. The sample was equilibrated for 10 min in a water bath of 30 °C under magnetic stirring (200 rpm) and then purged with nitrogen (100 mL/min) for 25 min. The volatiles were trapped in a stainless steel trap containing 250 mg of Tenax-TA, a mesh size of 60/80 and a density of 0.37 g mL^{-1} (Buchem bv, Apeldoorn, The Netherlands).

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

The volatiles collected in the Tenax-TA traps were thermally desorbed using an automatic thermal desorption device (ATD 400, Perkin Elmer, Norwalk, USA). The traps were desorbed by heating to 250 °C with a carrier gas (helium) flow of 60 mL min^{-1} for 15 min and volatiles were focused in a cold trap which subsequently was flash heated to 300 °C and held for 4 min. A split ratio of 1:10 was applied during transfer of the volatiles to GC–MS for separation and identification. The gas chromatograph–mass spectrophotometer used was a G1800 GCD System (Hewlett-Packard, Palo Alto, CA, USA) equipped with a DB-Wax capillary column (30 m × 25 mm × 0.25 μm) (Agilent J&W Scientific, Denmark). The column flow rate was 1.0 mL min^{-1} using helium as a carrier gas. The temperature of the column was held at 45 °C for 10 min and then increased by 6 °C min^{-1} up to 240 °C, which was kept constant for 10 min. The mass spectrometric detector was operated in electron ionization mode and scanned mass/charge (m/z) ratios between 15 and 300. Volatile identification was done by probability based matching of obtained mass spectra with those in the G1035A Wiley library (Hewlett-Packard, Palo Alto, CA, USA) and by comparison of the retention time and mass spectra of corresponding commercial standards. Commercial standards 1, 2, 3, 4, 7, 8, 9, 13, 14, 16, 22, 24, 39, 42, 47, 48 and 49 (numbers refer to Table 1) were obtained from Sigma-Aldrich, Copenhagen, Denmark, standards 5, 12, 17, and 35 were obtained from Merck, Darmstadt, Germany, standards 10, 11 and 34 were from Fluka, Buchs, Switzerland, standard 46 was from Valeant, New Jersey, USA and standard 19 was from B.D.H. Laboratory Supply, UK. Volatile peak areas were calculated on the basis of single mass-to-charge fragments and their concentration was expressed as relative area by dividing the peak area of the volatile by the area of the internal standard.

2.6. Gas chromatography–olfactometry (GC–O)

Volatiles from lettuce samples were trapped as described above. The traps were thermally desorbed using a short-path thermal desorption system (Scientific Instruments Services Inc., NJ, USA). The desorption was done at 250 °C for 4 min with a helium flow of 10 mL min^{-1} and a split ratio of 1:10. A Hewlett-Packard 5890 gas chromatograph equipped with an FID detector was used (Palo Alto, CA, USA). The separation of volatiles was carried out using the same column, carrier gas and temperature program as above. The FID temperature was set at 250 °C using air and hydrogen flow of 345 mL min^{-1} and 35 mL min^{-1},