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Influence of Temperature, Modified Atmosphere Packaging, and Heat Treatment on Aroma Compounds in Broccoli

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The aroma compounds in broccoli stored in different modified atmospheres were studied. The packaging materials used were oriented polypropylene (OPP), poly(vinyl chloride) (PVC), and low-density polyethylene (LDPE) containing an ethylene-absorbing sachet. All samples were stored for either 1 week at a constant temperature of 10 °C or for 3 days at 4 °C, followed by 4 days at 10 °C. The atmospheres that developed inside the packaging materials differed significantly. The broccoli samples were analyzed raw and after cooking, with regard to volatile compounds, using gas-phase (headspace) extraction followed by gas chromatography−mass spectrometry (GC-MS) and GC−olfactometry. Dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), hexanal, 3-*cis*-hexen-1-ol, nonanal, ethanol, and a group of thiocyanates were selected for a detailed study because these compounds cause off-odor and can be used as indicators of stress. Significant differences were found in the aroma profiles of the broccoli samples relative to the packaging materials used for storage. Storage in OPP (14% O_2, 10.5% CO_2) resulted in most of the off-odors, while storage in LDPE (6% O_2, 7% CO_2) and PVC (17.9% O_2, 4% CO_2) was found to maintain the concentration of DMS, DMDS, and DMTS during storage. Heat treatment of the broccoli increased the content of aroma compounds as well as the number of compounds containing sulfur.

KEYWORDS: Broccoli; modified atmosphere packaging; storage; volatile aroma compounds; GC-MS; headspace analysis; heat treatment

INTRODUCTION

Broccoli (*Brassica oleracea* L.) is a highly perishable produce and is best stored at a low temperature and a high relative humidity, ideally 0 °C and >95%. It can be difficult to maintain such a low temperature during postharvest handling. Consequently, modified atmosphere packaging (MAP) has been found to extend the shelf life of broccoli (*1*). Reduced O_2 levels and elevated CO_2 levels have been shown to reduce the respiration rate, decrease the weight loss, and inhibit the yellowing of broccoli (*1*−*5*). The recommended atmosphere for broccoli storage is 1−2% O_2 and 5−10% CO_2. However, if the O_2 concentration inside the package falls below 1%, and if the CO_2 concentration rises above 10%, offensive odors may develop, reducing the shelf life and consumer acceptance of the broccoli (*1*, *4*, *6*, *7*). Temperature fluctuations during storage, transportation, and retail display may also generate unfavorable atmospheres inside the package (*8*). An increased storage temperature enhances the metabolism and respiration rate, which may lead to a decreased O_2 concentration and an increase in the CO_2 level inside the package if the gas permeability of the package material is insufficient. This change in atmosphere inside the package may result in the accelerated deterioration of plant tissue and may induce off-odors (*2*, *6*, *9*, *10*).

Broccoli is characterized by sulfurous aroma compounds. Sulfur-containing volatiles are formed because of deterioration of the cells in the lipid membranes as well as the loss of intracellular compartmentalization, which allows enzyme−substrate reactions to occur (*11*, *12*). The strong off-odors produced by broccoli have mainly been associated with volatile sulfur compounds, such as methanethiol (MT), hydrogen sulfide, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) (*13*−*15*). Other volatile compounds, such as ethanol, ethyl acetate, acetaldehyde, methyl acetate, and acetone (*12*, *13*), have also been found to be responsible for off-odors during anaerobic respiration. In addition to the effects of the storage conditions, mechanical disruptions of the tissues (*12*, *16*) and heat treatment (*17*) have been shown to induce the development of off-odors. Heat treatment results in the increased production of DMDS, which has a sulfur odor that is said to be reminiscent of rotting cabbage, and DMTS, which is described as having a penetrating odor similar to that of fresh onions (*17*).

The objective of this study was to characterize the volatile compounds that develop in broccoli kept in different modified atmospheres, created by storage in commercially available packaging solutions under two defined temperature conditions. Another aim was to study the effect of cooking on the volatiles

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Table 1. Properties of the Packaging Types Used in the Study

packaging type	polymer	thickness, μm	area, m^2	void volume, dm^3	transmission rate (TR) O_2,[a] L/(m^2·day·atm)	CO_2,[b] L/(m^2·day·atm)	H_2O,[c] g/m^2·day
A	OPP[d]	35	0.15	3.3	1.3	4.9	1.6
B	LDPE[e]	23	0.16	2.8	11.0	>46	5.5
C	PVC[f]	10	0.16	1.0	56.1	>173	108.6

[a] Measured at 23 °C and 50% relative humidity (Oxtran 1000, Mocon). [b] Measured at 25 °C and 0% relative humidity (Permatran C, Mocon). [c] Measured at 25 °C and 90% relative humidity (Permatran W 3/31, Mocon). [d−f] The polymer films were manufactured by [d]Amcor flexibles, [e]Gripen AB, and [f]AEP Resinite Group Europe, MX-X.

produced after storing the fresh product in modified atmospheres. A previous study reported results from the sensory analysis of broccoli stored in the investigated packages under the same temperature conditions (*18*). Differences were observed between the cooked broccoli samples due to the storage conditions of the fresh produce. The present study was undertaken in order to find a chemical explanation for the detected sensory differences.

MATERIALS AND METHODS

Materials. *Plant Material*. Broccoli *(Brassica oleracea* L. var. Italica cv. 'Marathon'), freshly harvested, was obtained from a grower in southern Sweden. The broccoli was cooled to 5 °C unpacked overnight before being subjected to the experiments the next morning, i.e., 1 day after harvest. Broccoli heads of uniform size (200−300 g) and free from decay were used.

Volatile Compounds. The compounds used to determine the response factors were hexanal, 3-*cis*-hexen-1-ol, and nonane from Merck AB (Stockholm, Sweden), ethanol from Kemetyl AB (Haninge, Sweden), nonanal, dimethyl sulfide, and dimethyl disulfide from Sigma-Aldrich Co., Inc. (Stockholm, Sweden), and dimethyl trisulfide from Acros Organics, Labora-Chemicon (Stockholm, Sweden).

Packaging Material. The investigated materials were commercially available polymeric films/bags. The materials were oriented polypropylene (OPP) especially designed for broccoli, low-density polyethylene (LDPE) containing an ethylene-absorbing sachet, and poly(vinyl chloride) (PVC). The different materials are referred to as A, B, and C, respectively, and some significant properties of the packaging materials are summarized in **Table 1**. The PVC film, referred to as C, is currently the most commonly used packaging material for broccoli in Sweden. The packages were heat-sealed, except for the PVC film, which is a cling film and not suitable for heat-sealing.

Storage Conditions. The packaged broccoli, one head in each package, was stored in dark, humidified (RH > 95%) cold-storage rooms for 7 days. Two different temperature conditions were used: (I) 7 days at a constant temperature of 10 °C, and (II) 3 days at 4 °C, followed by 4 days at 10 °C. The first condition was chosen to approximate the temperature of the produce in retail displays. The second condition was chosen to investigate how a change in temperature might affect the aroma compounds in the broccoli, as broccoli may experience temperature fluctuations during handling and distribution.

Collection of Volatiles. Volatile compounds were collected from the broccoli packaged in the different materials during 7 days of storage. Sampling was done at days 0, 2, 4, and 7 for broccoli stored under condition I and at days 0, 3, and 7 for broccoli stored under condition II. Samples were taken from three packages of each packaging material. Three broccoli heads from each material were divided into smaller parts and mixed raw or after cooking for 5 min in 1 L of boiling water. Out of this mix, samples were withdrawn and without delay placed into three separate 500-mL glass bottles sealed with plastic screw tops, 50 g going into each bottle. Each sample was allowed to equilibrate for 30 min at room temperature. Subsequently, 1 L of helium was fed through the bottle at a rate of 40 mL/min and allowed to pass a Tenax trap (Tenax TA 60−80 mesh, 150 mg), where the volatiles were trapped.

Gas Chromatography−Mass Spectrometry (GC-MS). The volatile compounds absorbed on the Tenax TA were thermally desorbed for 5 min at 250 °C by means of an ATD 400 automatic injector (Perkin-Elmer, Norwalk, CT) and subsequently injected into the GC-MS system. The gas chromatograph used was a ThermoQuest Trace GC 2000 (ThermoQuest CE Instruments, Milan, Italy) equipped with a 30-m × 0.32-mm capillary column with a 1.0-μm-thick film of DB-1 (J&W Scientific Inc., Folsom, CA). The mass spectrometer used was an Automass Solo (ThermoQuest). The initial temperature of the GC oven was 20 °C, and the oven was kept at that temperature for 2 min. Subsequently, the temperature was increased by 6 °C/min until a final temperature of 220 °C was reached, which was then held at 220 °C for 20 min. Helium was used as a carrier gas at a flow rate of 35 mL/min. The integration and identification of the GC peaks was carried out using Xcalibur computer software (Thermoquest). The compounds were identified on the basis of their mass spectra and a comparison of the retention times from the chromatographic runs of the corresponding reference chemicals. Quantification of the compounds was undertaken by using an external standard (nonane) and performing GC-MS on the pure compounds in question. The concentrations of seven specific compounds were calculated by the means of a response factor, i.e., the relationship between the external standard and the volatile.

GC−Olfactometry (GC-O). To characterize the aroma compounds of importance to the flavor of broccoli, samples which were stored for 7 days under storage condition I, i.e., 10 °C, as well as freshly harvested broccoli, were evaluated using GC-O after the broccoli was cooked for 5 min. The volatiles were thermally desorbed the same way as for GC-MS, described above. However, the effluent from the capillary column was split 1:1 between a flame ionization detector (FID) and a sniffer-port. In the latter, the column effluent was mixed with humidified air in order to facilitate sensory evaluation. Each sample was evaluated by three assessors during a 30-min sniffing session. The signal from the FID was split so that the signal could be registered by the Xcalibur computer software (Thermoquest) and a printer. The assessors were instructed to describe the odor of each compound detected, as well as the intensity, on a scale from 1 to 5, with 5 being the maximum, and to make a note beside the GC peak on the paper from the printer. The result was an aromagram used to select the "impact compounds" of broccoli aroma.

Atmosphere Analysis. The concentration of oxygen and carbon dioxide in the headspace inside the packaging was monitored using a Gaspace 2 v3.3 (Systech Instruments Ltd., Thame, Oxfordshire, UK) at each occasion of sampling. A syringe was inserted into the package through a rubber seal (Toray Engineering Co. Ltd., Osaka, Japan) placed on the film. The instrument was calibrated toward air.

Weight Loss Evaluation. The broccoli heads were weighed prior to packaging and again on each occasion of sampling. Weight losses were expressed as the percentage of the weight on the day of packaging, i.e., 1 day after harvest.

Statistical Analysis. Statistical evaluation was performed using analysis of variance (ANOVA)—the sources of variance were packaging material, storage temperature, and storage time—followed by Tukey HSD (honestly significant difference) multiple comparison of means, using Systat 10 (SPSS Inc., Chicago, IL). Mean values were considered significantly different at $p \leq 0.05$. Tukey's multiple comparison test was performed on the variables that were significantly different in ANOVA.

Principal component analysis (PCA) was performed (Unscrambler v7.6, CAMO ASA, Oslo, Norway) on the averages of subjects and replicates to describe the main variation in the data of the volatile

Table 2. O_2 and CO_2 Concentrations inside the Package plus Weight Loss after 7 Days of Storage[a]

packaging type	storage condition	O_2 (%)	CO_2 (%)	weight loss (%)
A	I	14.0a	10.5a	0.6a
B	I	6.0b	6.8b	1.1a
C	I	17.9c	3.8c	1.6a
A	II	12.9a	11.1a	0.6a
B	II	4.2b	7.2b	0.9a
C	II	16.0ac	4.7c	1.2a

[a] Values represent the mean of five replicate samples. Means in a column followed by a different letter were significantly different at $p \leq 0.05$, according to Tukey's test.

compounds. The model was validated using full cross validation, where each sample was used to test the model estimation by all the other samples.

RESULTS AND DISCUSSION

Atmosphere Analysis. The oxygen and carbon dioxide concentrations created inside the packaging differed significantly ($p \leq 0.05$) among the packaging materials (**Table 2**). The O_2 levels had decreased to 2−18% after 2 days of storage, dependent on the packaging material and storage condition (**Figures 1** and **2**). The CO_2 level rose in all the packages stored at 10 °C, followed by a slight decline to 10.5, 6.8, and 3.8% by day 7, declining to 11.1, 7.2, and 4.7% in the respective packages stored under storage condition II, i.e., 3 days at 4 °C, followed by 4 days at 10 °C. The difference in atmosphere inside the packages was most probably due to the material difference in O_2 and CO_2 transmission rates (**Table 1**), creating different atmospheres due to broccoli respiration. As an example, a low CO_2 transmission rate resulted in a high CO_2 level inside the bag. Other material properties that could affect the atmosphere inside the package were the film thickness and the void volume in the package. A thicker film would probably result in a decreased transport of gases through the film. A large void volume could result in a longer time for the equilibrium inside the package to be reached. To avoid anaerobic respiration and the production of off-odors, the O_2 should not be below 1% (*6*, *7*), and the CO_2 level should be kept below 10% (*4*, *6*). The O_2 and CO_2 levels inside the packages were above 2% O_2 and below 10% CO_2, with two exceptions. The CO_2 level in material A under storage conditions I and II was above 10% at the time of all the measurements during the 7 days of storage, which might have induced the development of off-odors (*1*, *4*). The low O_2 level inside material B under storage condition II after 2 days of storage was also able to result in the production of off-odors (*6*). The first measurement was made after 2 days of storage, and thus there is a possibility that the O_2 and CO_2 concentrations were above or below acceptable levels prior to the first analysis and prior to the equilibrium being reached. As a consequence, the development of the aroma compounds might have been affected during this period.

Weight Loss. No significant differences in weight loss were found between the two storage conditions for broccoli packaged in materials A−C (**Table 2**). Weight losses were between 0.6 and 1.6% of the original fresh weight after 7 days of storage. Water stress, i.e., water deficit, has been found to induce the generation of volatiles (*19*). Toivonen (*20*) reported that water loss could also increase the loss of disulfides in diced onions. The weight losses found in the present study were relatively minor and were not expected to have any significant effect on aroma development.

Volatiles Found in Broccoli. Between 30 and 40 different compounds were identified using GC-MS in the headspace from the broccoli samples. During the GC-O analysis, 28 odor compounds were perceived by at least two of the three panelists. Odors were described as smelling of cabbage, boiled potato, mushroom, onion, garlic, garbage, burnt food, compost, baking, cut grass, floral, citrus, sour, laundry, and vegetation. Eleven compounds were selected as indicators in order to compare the influence of the different storage conditions on the aroma profile of the samples. The investigated volatiles were dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS),

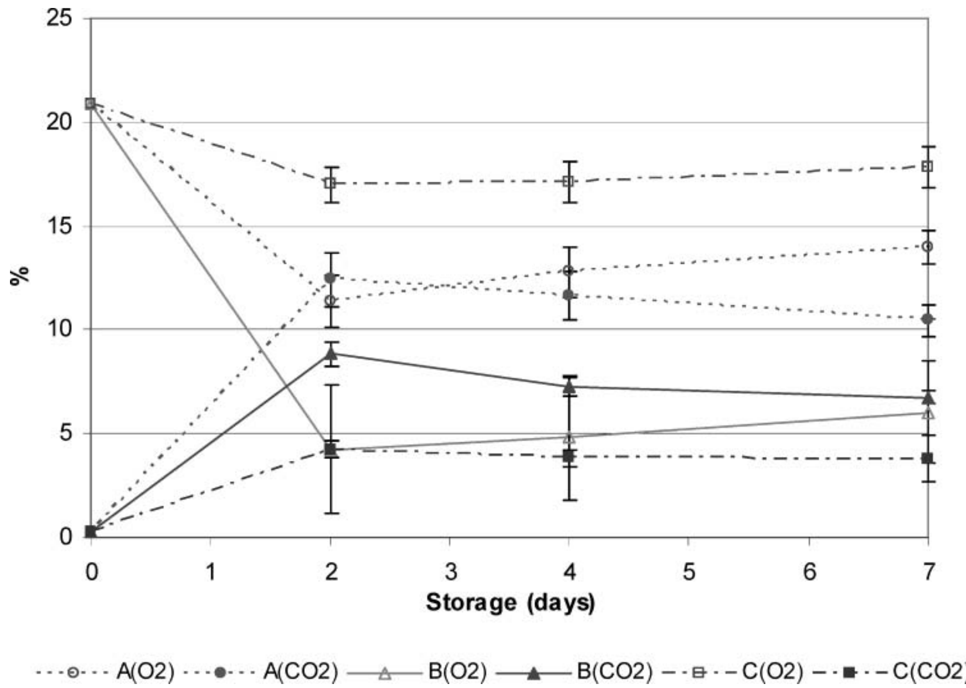

Figure 1. O_2 and CO_2 concentrations inside the packages under storage condition I, i.e., 7 days at 10 °C. Values represent the mean of five replicate samples with standard error of means.

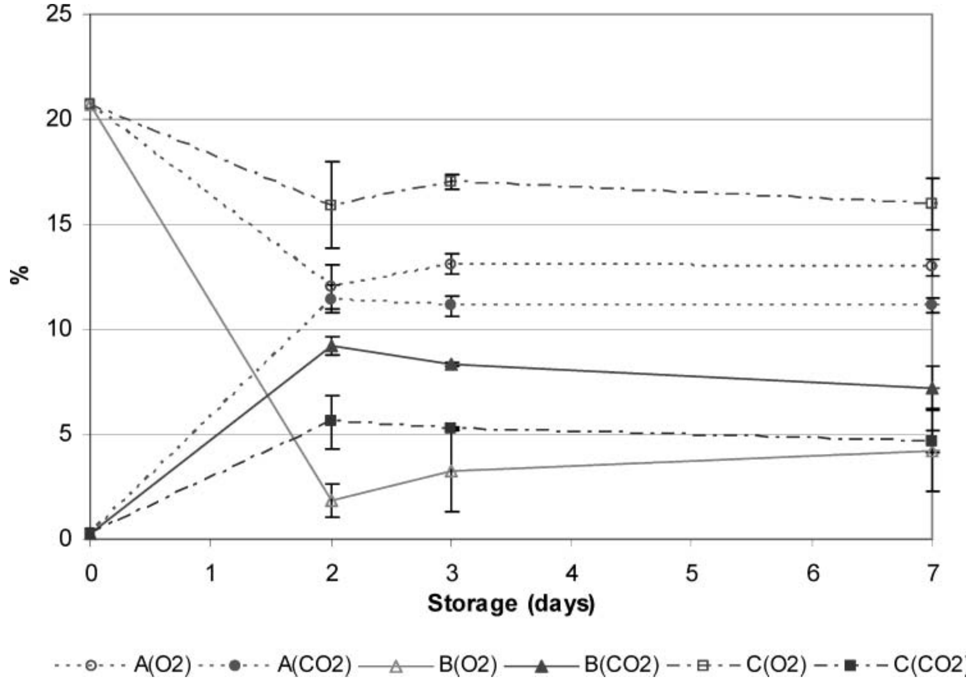

Figure 2. O_2 and CO_2 concentrations inside the packages under storage condition II, i.e., 3 days at 4 °C, followed by 4 days at 10 °C. Values represent the mean of five replicate samples with standard error of means.

Table 3. Amount of Volatile Compounds Found in Raw Broccoli 1 Day after Harvest and after 7 Days of Storage in Different Packaging Materials under Storage Conditions I and II[a]

		storage condition I				storage condition II			
volatile compounds		fresh broccoli	A[b]	B[c]	C[d]	fresh broccoli	A[b]	B[c]	C[d]
dimethyl sulfide	(ppb)	0.2(0.2)	–	–	–	0.2(0.2)a	8.1(2.0)b	2.4(2.4)a	4.8(2.5)ab
dimethyl disulfide	(ppb)	5.9(0.7)a	18(6.2)b	5(1.6)a	4(0.5)a	5.9(0.7)a	26.6 (8.6)b	10.3(10.3)ab	15.5(7.5)ab
dimethyl trisulfide	(ppb)	0.04(0.01)a	0.20(0.01)b	0.06(0.01)a	0.06(0.01)a	0.04(0.01)a	0.16(0.02)b	0.05(0.004)a	0.07(0.01)a
hexanal	(ppb)	0.31(0.01)ns	0.30(0.07)ns	0.28(0.09)ns	0.20(0.04)ns	0.31(0.01)ns	0.38(0.04)ns	0.27(0.17)ns	0.28(0.07)ns
3-*cis*-hexen-1-ol	(ppb)	0.37(0.06)a	0.85(0.22)ab	1.02(0.42)b	0.82(0.06)ab	0.37(0.06)ns	0.15(0.16)ns	0.12(0.07)ns	0.15(0.06)ns
nonanal	(ppb)	0.22(0.03)a	0.13(0.01)a	–	–	0.22(0.03)ns	0.01(0.005)ns	0.17(0.07)ns	0.29(0.13)ns
ethanol	(ppb)	1.5(0.46)a	7.5(2.70)b	3.7(0.02)b	6.0(0.39)b	1.5(0.5)ns	1.3(0.6)ns	7.9(6.3)ns	4.1(2.6)ns
methanethiol	(%)	100a	36(7)b	–	–	100ns	100(45)ns	–	81(47)ns
methyl thiocyanate	(%)	100ab	123(12)a	76(20)b	98(4)ab	100a	154(13)b	99(22)a	119(26)ab
butyl isothiocyanate	(%)	100a	32(16)b	58(11)b	99(12)a	100a	28(7)b	29(22)b	72(8)a
2-methylbutyl isothiocyanate	(%)	–	–	–	–	–	–	–	–

[a] Values are based on GC-MS analysis of three replicates; standard deviations are presented within parentheses. Values within a row followed by a different letter (a–d) were significantly different at $p \leq 0.05$, according to Tukey's test. ns, nonsignificant differences; –, not detected. [b] A = OPP. [c] B = LDPE. [d] C = PVC.

hexanal, 3-*cis*-hexen-1-ol, nonanal, ethanol, methanethiol (MT), methyl thiocyanate (MTC), butyl isothiocyanate (BITC), and 2-methylbutyl isothiocyanate (MBITC). The selected volatiles have been reported as important to broccoli aroma and odor (*13*, *14*, *21*), and DMS, DMDS, DMTS, hexanal, nonanal, and 3-*cis*-hexen-1-ol were the substances given the highest scores by the sniffers in the GC-O study.

Quantification of seven of the selected compounds was undertaken with the aid of a response factor. In the case of the isothiocyanates, the pure substances were not available. Therefore, the original concentration was given a value of 100, and the levels in the stored samples are presented in relation to this.

In this investigation, the main objective was the comparison of the packaging materials' influence on volatile development. Therefore, volatiles collected from 1 L of headspace were quantified. This was deemed to be sufficient in order to carry out a comparison among the samples. No effort was made to extract the full amount of volatiles from the broccoli. Consequently, the reported volatiles levels should not be considered as a total concentration of the substances in broccoli. Longer extraction times and larger extraction volumes would probably result in higher levels of the analyzed compounds.

The variations in the measurements may be due to individual differences between the different broccoli heads, depending on maturity at harvest and growing conditions. Furthermore, there is no guarantee that the mixes of florets and stems were perfectly homogeneous, although care was taken to obtain replicates that were as identical as possible.

Any statistical differences found between the storage conditions are presented below.

Raw Broccoli—The Influence of Storage Temperature on the Aroma Profile. Introducing a change in temperature during storage resulted in changes in the aroma profiles. DMS was detected in raw broccoli stored under condition II, but not in broccoli stored under condition I (**Table 3**). DMS has previously been found in low concentrations in freshly minced cabbage (*22*) and as a major component of cooked cabbage (*23*) but could not be detected in raw broccoli by Hansen et al. (*14*) in

Table 4. Amount of Volatile Compounds Found in Cooked Broccoli 1 Day after Harvest and after 7 Days of Storage Raw in Different Packaging Materials under Storage Conditions I and II[a]

		storage condition I				storage condition II			
volatile compounds		fresh broccoli	A[b]	B[c]	C[d]	fresh broccoli	A[b]	B[c]	C[d]
dimethyl sulfide	(ppb)	31(15)ns	48(8)ns	29(10)ns	46(8)ns	31(15)ns	28(8)ns	20(5)ns	33(18)ns
dimethyl disulfide	(ppb)	19(6)a	73(3)b	27(3)a	23(4)a	19(6)a	56(7)b	36(5)ab	33(10)a
dimethyl trisulfide	(ppb)	1.8(0.6)a	4.6(1.3)b	3.5(0.2)ab	4.2(0.6)b	1.8(0.6)a	4.8(0.3)b	3.3(0.9)ab	3.5(0.05)b
hexanal	(ppb)	2.52(0.89)a	0.24(0.05)b	0.42(0.05)b	0.39(0.14)b	2.52(0.89)a	0.25(0.02)b	0.30(0.07)b	0.47(0.19)b
3-*cis*-hexen-1-ol	(ppb)	–a	–a	0.41(0.27)b	0.03(0.02)c	–a	–a	0.11(0.08)b	–a
nonanal	(ppb)	0.98(0.42)ns	0.38(0.10)ns	0.51(0.06)ns	0.46(0.09)ns	0.98(0.42)a	0.25(0.03)b	0.24(0.05)b	0.38(0.09)b
ethanol	(ppb)	0.8(0.5)ns	1.5(0.4)ns	1.8(0.4)ns	1.3(0.7)ns	0.8(0.5)a	8.1(1.2)b	8.2(4.1)b	2.6(3.1)a
methanethiol	(ppb)	100a	13(5)b	7.6(0.2)bc	5.5(0.3)c	100ns	129(87)ns	126(66)ns	75(43)ns
methyl thiocyanate	(%)	100a	558(182)b	670(3)b	520(60)b	100ns	228(39)ns	179(46)ns	118(95)ns
butyl isothiocyanate	(%)	100ns	205(88)ns	272(150)ns	110(33)ns	100a	18(2)bc	21(5)b	12(0.6)c
2-methylbutyl isothiocyanate	(%)	100ns	315(148)ns	259(172)ns	161(45)ns	100a	6.8(2)bc	11(3)b	6(0.6)c

[a] Values are based on GC-MS analysis of three replicates; standard deviations are presented within parentheses. Values within a row followed by a different letter (a–d) were significantly different at $p \leq 0.05$, according to Tukey's test. ns, nonsignificant differences; –, not detected. [b] A = OPP. [c] B = LDPE. [d] C = PVC.

low-oxygen storage. The *S*-methylmethionine sulfonium salt has been suggested to be the precursor of DMS (*22*, *24*, *25*), in most cases in combination with heat. The presence of DMS in raw broccoli and in freshly minced cabbage (*22*) indicates a decomposition of the salt without heat. Lewis et al. (*26*) reported that the cleavage of *S*-methylmethionine sulfonium salt by a cabbage enzyme yielded DMS, also stating that the enzyme was pH dependent. The presence of DMS in broccoli subjected to storage condition II indicated that the induced temperature change might have affected the production of DMS in raw broccoli. After 3 days of storage at 4 °C, no DMS could be detected, indicating that production occurred after the temperature rise. However, further studies are required in order to verify the influence of temperature.

Significant differences ($p \leq 0.05$) were found between the 3-*cis*-hexen-1-ol and BITC in broccoli stored under conditions I and II. The amount of 3-*cis*-hexen-1-ol as well as BITC increased if the broccoli was stored under condition I, i.e., 7 days at 10 °C (**Tables 3** and **4**). The higher level in broccoli stored at 10 °C indicated a higher degree of deterioration, since 3-*cis*-hexen-1-ol and isothiocyanates are a result of membrane breakdown and oxidation during broccoli senescence (*27*−*29*). Low temperatures decrease the respiration rate (*30*), and Deschene et al. (*31*) reported that membrane deterioration was temperature-dependent; i.e., storage at 10 °C and above resulted in a higher level of deterioration. Aroma development has been reported to be enhanced by tissue disruption and cellular deterioration (*16*, *32*), which is in agreement with the findings.

Storage at the higher temperature, i.e., 10 °C, resulted in the highest concentrations of the investigated aromas. However, the number of aroma compounds found in broccoli stored under storage condition II was somewhat higher than that found in broccoli stored at a constantly higher temperature. One explanation for this could be that the broccoli has different metabolisms at 4 and 10 °C, due to the temperature dependence of membrane deterioration (*31*). This might result in production of different volatiles, resulting in a larger number of volatiles in broccoli stored under storage condition II.

Raw Broccoli—*The Influence of Packaging Material on the Aroma Profile*. The atmosphere was shown to have an impact on the aroma compounds produced during storage, which was well in accordance with previous observations (*4*, *33*, *34*). **Table 3** presents the concentration of the selected aroma compounds in broccoli stored in different materials. Different aroma profiles for the broccoli were found, depending on the packaging material used for storage, and significant differences were found between the packaging materials regardless of the storage condition.

When analyzed raw, broccoli stored in material A (14% O_2, 10.5% CO_2) under storage condition I, i.e., 7 days at 10 °C, differed significantly ($p \leq 0.05$) in DMDS and DMTS concentration compared to broccoli stored in materials B (6% O_2, 6.8% CO_2) and C (17.9% O_2, 3.8% CO_2) (**Table 3**). Storage in material A resulted in a significant increase of DMDS and DMTS compared to the fresh broccoli sample which was not seen in broccoli stored in materials B and C. No significant differences were found with regard to hexanal and 3-*cis*-hexen-1-ol between the packaging materials, although storage in material B resulted in a significant increase of 3-*cis*-hexen-1-ol after 7 days of storage compared to the fresh broccoli. Storage in all packaging materials resulted in increased ethanol in the broccoli compared to the fresh sample. Stress has been found to induce a more rapid deterioration of vegetables (*35*). An increase in ethanol production is usually considered to be a stress indicator in plants (*14*, *17*, *19*) and a result of anaerobic respiration. However, the O_2 and CO_2 levels measured inside the packages did not indicate anaerobic respiration (**Figure 1**), although the metabolic changes during storage could have induced the increase in the ethanol concentration.

Samples kept under storage condition II, i.e., 3 days at 4 °C, followed by 4 days at 10 °C, differed significantly ($p \leq 0.05$), when raw, in DMS, DMTS, MTC, and BITC, depending on the packaging material used (**Table 3**). Storage in material A (12.9% O_2, 11.1% CO_2) resulted in a significantly higher amount of DMS than that found in broccoli stored in material B (4.2% O_2, 7.2% CO_2), and a higher DMTS concentration than that found in broccoli stored in material B or C (16% O_2, 4.7% CO_2). Broccoli stored in materials A and B resulted in a decrease in BITC compared to that stored in material C and the fresh sample.

The difference in sulfur compounds in broccoli stored in material A might be due to the somewhat higher CO_2 concentration inside the package compared to the concentrations in broccoli stored in the other materials. Off-odors have been reported by several authors when broccoli was stored in atmospheres containing in excess of 10% CO_2 in combination with O_2 levels below 2.5% (*1*, *4*, *6*, *7*). Makhlouf et al. (*4*) reported undesirable odors as a result of storage in 20% O_2 and 10% CO_2; the lower the O_2 concentration, the more intense the odors developing. DMDS (*16*), and possibly also DMTS (*22*),

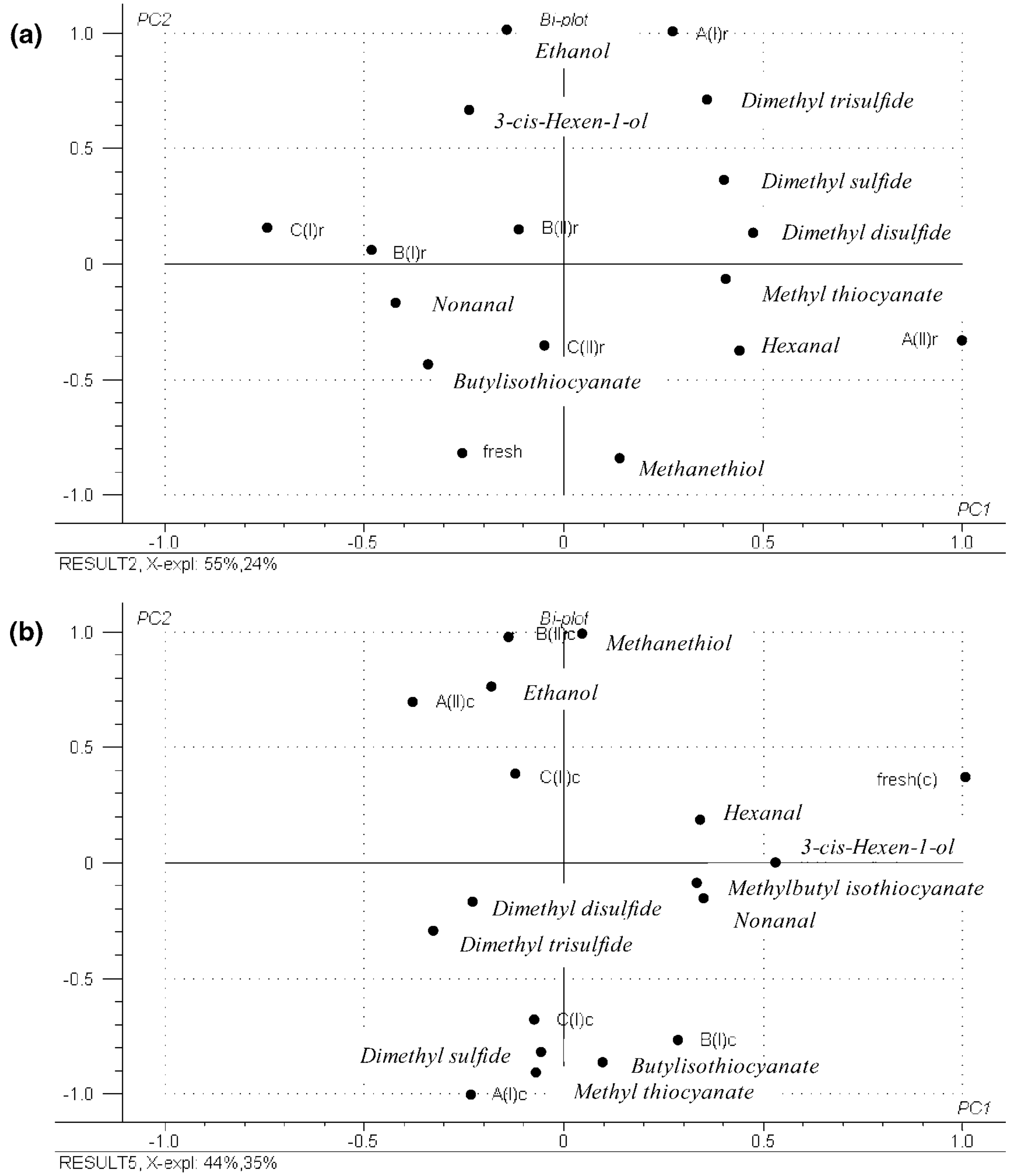

Figure 3. PCA of the GC-MS analysis of the raw broccoli (a) and cooked broccoli (b), including the 11 selected aroma compounds. Bi-plots of the scores and loadings for principal components 1 and 2 are shown. The letters A, B, and C represent the packaging materials used: A = OPP; B = LDPE; C = PVC. r and c represent raw and cooked broccoli, respectively, while (I) and (II) indicate the different storage conditions, (I) 7 days at 10 °C, and (II) 3 days at 4 °C, followed by 4 days at 10 °C.

is formed as a result of the degradation of *S*-methyl-L-cysteine sulfoxide by cysteine sulfoxide lyase (C−S lyase) upon tissue disruption in the presence or in the absence of air (*11*). Dan et al. (*12*) suggested that the formation of volatile compounds under modified atmosphere conditions, and specifically under anaerobic conditions, occurred as a result of membrane deterioration and the loss of cellular compartmentalization, which allowed enzyme reactions to occur. The high CO_2 concentration in material A probably induced the cellular deterioration, thereby increasing the development of DMDS and DMTS.

Cooked Broccoli—The Influence of Storage Temperature on the Aroma Profile. The isothiocyanates in cooked broccoli were all found to differ significantly under the different storage conditions. Storage condition I, i.e., 10 °C, resulted in an increase in MTC, BITC, and MBITC compared to those found after storage under condition II (**Table 4**). This was similar to the findings for raw broccoli, although the differences were more pronounced. Isothiocyantes are generally considered to be a product of membrane deterioration (*29*), as previously mentioned, indicating a higher rate of deterioration in broccoli stored at a higher temperature. MT, however, was found to be higher in broccoli that was cooked after being stored under storage condition II, i.e., 3 days at 4 °C, followed by 4 days at 10 °C, regardless of the packaging material used during storage. DMDS and MT have been found to have the same precursor, i.e., *S*-methyl-L-cysteine sulfoxide (*16*, *36*). However, DMDS may also be formed by the oxidation of MT in the presence of air, following the action of cysteine sulfoxide lyase (C−S lyase) on *S*-methyl-L-cysteine sulfoxide (*11*, *16*). One contributing factor to the difference in MT between the two storage conditions could be the oxidation of MT to DMDS in broccoli stored under condition I. The concentrations of both DMDS and DMTS after 3 days of storage at 4 °C started to decrease, while the concentrations of DMDS and DMTS in broccoli stored under condition I were found to increase throughout the storage period for broccoli stored in materials A and C. This could be an indication of the oxidation of MT in broccoli stored under condition I. However, a comparison of the concentrations could not be made because the amount of MT was not determined. Changes in atmosphere and temperature during storage have previously been found to affect the physiology and biochemistry of vegetables, e.g., increased membrane degradation and lipid peroxidation (*30*, *35*). Heat treatment results in tissue disruption and has also been found to increase the production of volatiles (*17*). The temperature change from 4 to 10 °C, together with the induced change of atmosphere inside the packages, could have affected the development of the aroma compounds in broccoli differently than storage at a high, constant temperature, i.e., storage condition I. The effect of the storage condition of the raw broccoli resulted in differences between the cooked products discovered after heat treatment. However, further studies are needed in order to investigate, for instance, the difference in MT between the storage conditions.

Cooked Broccoli—The Influence of Packaging Material on the Aroma Profile. The aroma profiles of the cooked broccoli changed with the packaging material used to store it. Heat treatment appeared to diminish the differences between the selected compounds in the broccoli stored in the different packaging materials.

After 7 days of storage at 10 °C, broccoli stored in the different materials showed similar differences vis-à-vis the fresh broccoli sample when cooked as found when analyzed raw (**Tables 3** and **4**). Material A resulted in a significantly higher DMDS than materials B and C and the broccoli cooked fresh. However, no significant differences could be found between the packaging materials with regard to DMS and DMTS (**Tables 3** and **4**). Materials A and C did, however, result in a significantly higher amount of DMTS than the broccoli cooked fresh. Similar observations were made for broccoli stored in the different packaging materials under storage condition II, i.e., 3 days at 4 °C, followed by 4 days at 10 °C. The DMDS concentration in broccoli stored in material A was, however, significantly higher than that in broccoli stored in material C. A decrease in 3-*cis*-hexen-1-ol was found in the cooked broccoli samples. This was not expected since 3-*cis*-hexen-1-ol is usually a product of oxidation and membrane damage (*27*, *28*), the latter being a consequence of heat treatment.

Principal Component Analysis. A principal component analysis (PCA) was performed on both the raw and the cooked broccoli. The analysis included the 11 selected aroma compounds and was undertaken in order to obtain an overview of the results.

In the case of the raw broccoli, the first two principal components (PCs) explained the main variation—79% of the total variance (**Figure 3a**)—with an additional 9% being explained by PC3 (not shown). The bi-plot, i.e., scores and loadings (**Figure 3a**), gave a visual representation of how the different packaging materials affected the aroma compounds. Broccoli stored in material A deviated from broccoli stored in materials B and C. Material A used under storage condition I, i.e., 7 days at 10 °C, resulted in broccoli with a stronger correlation to DMTS and ethanol than the other samples (**Figure 3a**). Furthermore, broccoli stored in material A under storage condition II, i.e., 3 days at 4 °C, followed by 4 days at 10 °C, was correlated to hexanal and MTC. Broccoli stored in material C under storage condition II was the sample most similar to the fresh broccoli, while broccoli stored in material B seemed to be less influenced by the different temperature conditions compared to the other materials (**Figure 3a**).

The first two principal components of the PCA of the cooked broccoli explained the main variation—79% of the total variance (**Figure 3b**). Minor differences between the packaging materials could be seen, while two groups could be identified depending on the different temperatures used during storage (**Figure 3b**). Broccoli stored under storage condition I had the strongest correlation to DMS, MTC, and BITC, while storage condition II resulted in broccoli with more ethanol and MT. Overall, taking both storage conditions into account, i.e., I and II, materials B and C showed a similar capability to keep raw broccoli fresh, i.e., keeping the concentration of sulfur compounds low, while material A resulted in a high amount of sulfur compounds in the broccoli.

In conclusion, significant differences were found in the aroma profiles of the different broccoli samples related to the packaging materials used for storage, with regard to both raw and cooked broccoli. The atmospheres within the different packaging materials that developed during storage were significantly different. None of the packages provided an anaerobic environment with the levels of O_2 being above 1%. The CO_2 concentrations in the samples were at most 12.5%.

The PCA gave an overview of the importance of both the storage temperature and the packaging material. The effect of the storage condition used for the raw product was also seen to have an effect on the cooked product. Broccoli stored in materials B and C showed similar concentrations of sulfur compounds when raw. However, after cooking, broccoli stored in material C had a lower amount of BITC and MBITC, as well as a lower ethanol level, than did broccoli stored in material B. Storage in material A was found to result in the highest concentrations of sulfur compounds in the broccoli, while materials B and C kept the aroma compounds at a lower level. Material A is especially designed for the storage of broccoli; nevertheless, an atmosphere was created which induced off-odors.

Further studies are necessary regarding the mechanism of aroma production in broccoli, under different temperature conditions, in order to understand the differences in DMS and MT concentration found during this study, as well as the effects of temperature fluctuation.

The impact of the differences in aroma compounds found between the materials used in this study could further be explained by a correlation between the aroma concentration in the headspace and the sensory data, i.e., smell and flavor, obtained after cooking broccoli stored under the same atmosphere and temperature conditions reported previously (*18*). The evaluation of the correlation will be undertaken and published later.

ABBREVIATIONS USED

DMS, dimethyl sulfide; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide; MT, methanethiol; MTC, methyl thiocyanate; BITC, butyl isothiocyanate; MBITC, 2-methylbutyl isothiocyanate.

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