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Effect of Elevated CO_2 during Low Temperature Storage on the Quality Attributes of Cut Spearmint

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Abstract: The effect of elevated CO_2 in a controlled atmospheric condition (CA) on the quality attributes of fresh-cut spearmint (*Mentha spicata*) during refrigerated storage is investigated in the present study. Cut stems of spearmint were exposed to the continuous flow of humidified air enriched with 0 (as a control), 5, 10 and 20% CO_2 during storage at 5 °C. Weight loss, leaf colour, total phenols, antioxidant activity, aromatic profile, ascorbic acid, ethanol, ammonia and ethanol-acetaldehyde concentrations were measured before and after storage for 5, 10 and 14 days. Over time, CO_2 treatments increased the weight loss, surface colour, L* (from white to black) and b* (from blue to yellow) values, but lowered a* (from green to red). When compared to fresh spearmint, the lowest CO_2 concentration was able to maintain the overall colour variations. The 20% CO_2 treatment showed significant declines in the total phenolic content, antioxidant potential and low appearance score after 10 days, thus its quality assessment was terminated. Vitamin C levels decreased with time in all the treatments, although the 10% and 20% CO_2 treatments had the lowest levels. The toxicity of the cell structures detected by the ammonia content increased and was significantly higher in all CO_2 treatments. Storage in the CA with the gas composition at 5% preserved the aromatic profiles similar to those stored in air. In conclusion, increased CO_2 did not improve the storability of fresh cut spearmint held at low temperatures, and the 20% gas composition had a significant negative impact on the visual quality.

Keywords: atmospheric compositions; herbs; low temperature storage; modified package; shelf life

1. Introduction

Modified atmospheric packaging (MAP) in low temperature storage with an elevated CO_2 level has been shown to improve the shelf life of leafy vegetables and fresh herbs by reducing metabolic activity, i.e., the respiration rates, as well as inhibiting ethylene-induced senescence [1–4]. Increased CO_2 levels in the fresh cut industry can reduce rates of yellowing and browning appearances as well as improve levels of bioactive ingredients, thereby extending shelf life either during transpiration or on the supermarket display shelf [4,5]. However, extremely high CO_2 levels that are not within the tolerated range of the crops could have an adverse effect on the texture and enhance off-flavour development [2,6]. The

mechanism of CO_2 towards postharvest quality alteration has been explained previously: CO_2 inhibits ethylene action by acting as an ethylene competitor at the ethylene receptor site and on some metabolic pathways, as well as acting as an ethylene binding protein (EBP), thereby illustrating its senescence-retarding effect [7–10]. Additionally, a modified atmosphere of a high level of CO_2 is a known-effective alternative to postharvest methyl bromide fumigation for pest control during food production [11].

Nowadays, fresh cut herbs are available on the refrigerated shelf of supermarkets. Among the variety options, mints are one of the most popular as the ingredients or garnishers in food and beverages. Spearmint is the most preferred type that is used in many ethnic foods, which gives a strong aroma, a sweet character and a sharp menthol undertone [12,13]. Fresh spearmint is the key ingredient in modern recipes, such as mojitos, and as a side dish in Asian fusion foods, such as Thai and Vietnamese. Furthermore, the essential oil compositions of spearmint include carvacrol, menthol, carvone, methyl acetate, limonene and menthone, all of which have known biological activities and could potentially open up a market for it as a functional food ingredient [12,14–16]. Fresh herbs, however, are sensitive to low temperature storage (i.e., 3–5 day storability) on the refrigerated shelf (~5 °C) in the supermarket [17–19]. The postharvest defects of mint leaves include leaf abscission, yellowing and epinasty [2]. Additionally, Curutchet et al. [20] and Santos et al. [21] reported that low temperature storage could induce changes in the physical properties, nutritive values as well as bioactive properties.

Fresh fruits and vegetables are able to maintain their quality by storing them at an appropriate temperature during the postharvest and handling chain. However, attaining the products at such optimum temperatures is hardly possible; thus, the controlled atmosphere (CA) storage is able to alleviate the effects of non-optimum temperatures [7,22]. Food commodities are typically kept in an atmosphere with varying gas compositions, as well as proper temperature supplementation and relative humidity control, which, to some extent, inhibits the rates of respiration and metabolic mechanisms [8,9,23]. Gas requirements for the CA of some leafy vegetables range from 1–10% O_2 and 2–20% CO_2, depending usually on factors, such as the duration of storage, temperature, relative humidity and ethylene concentration [24]. Even though the majority of the research on the CA storage of vegetables continues to be studies of modified atmosphere packing (MAP), the suggestion is that the effect of one gas on quality attributes should be studied independently [24]. While modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage, which generate a high CO_2 environment, are used to preserve the quality of fresh cut fruits and vegetables, there is no information on the impact of CO_2 concentrations on the postharvest quality changes of freshly cut spearmint. The objective of this work is to evaluate the effectiveness of elevated CO_2 during cold storage to maintain the quality of cut spearmint. In an attempt to study the storability of this fresh cut produce under the adjusted CO_2 condition, the onsets of physiological and chemical attributes along with the sensorial evaluation were investigated. The research outcome is, therefore, used as the initial information toward the development of innovative packaging for the fresh cut herb industry.

2. Materials and Methods

2.1. Plant Materials

Cut spearmint stems baring healthy leaves of different stages were purchased from Mileti Agelo, Puglia, Italy. Approximately 50 g of the stems were packed in a transparent polystyrene tray (179 × 119 × 60 mm^3) without a lid prior to subjecting them to the CA treatments. The CA was a 10 L plastic container conditioned with the continuous flows of humidified air enriched with CO_2 at a flow rate of 10 mL min^{-1}. Different CO_2 treatments (5%, 10% and 20%) were examined. Into each CA container, 3 mint trays were placed and there were 3 separated CA replications for each treatment. The containers were kept in the dark at 5 °C for 14 days. The containers flushed with air with 0% CO_2 were used as a control treatment (CTRL). To maintain the relative humidity at 95% inside the container, each gas mixture was bubbled through deionised water [25,26]. Samples were withdrawn

periodically at day = 0, 5, 10 and 14, respectively, to evaluate them for their physical qualities, or were otherwise stored at −80 °C for phytochemical analyses.

2.2. Quality Assessments

2.2.1. Weight Loss and Colour Measurement

Wight loss (%) was the change in the total weight of the samples in each tray over time, which was calculated as $100 \times (W_i - W_f)/W_i$, where W_i and W_f were the weight of the tray at the initial day (D0) and the weight of the tray at any sampling dates [27]. The colour alteration was determined from the individual leaf surface (L*a*b* CIE space, Minota, Japan), where L* is the degree of lightness to darkness, a* refers to the degree of redness to greenness and b* refers to the degree of yellowness to blueness. A total of 10 leaves was measured for each replication. The difference (ΔE*) of the colour space to the control (D0) was calculated according to the following formula:

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (1)$$

when the value $0 < \Delta E^* < 1$ is considered similar to the fresh leaves, and when the value >3.5 is an unacceptable appearance for the consumers [28].

2.2.2. Texture Analysis

The texture was as a force (N) required to compress a layer of mint leaves (~50 g) between 2 parallel plates of Ø 10 cm using an Intron Universal Testing machine (model 3343, Canton, MA, USA) at a speed of 50 mm min^{-1} [29].

2.2.3. Total Phenolic Content and Antioxidant Activity

The leaf tissue of spearmint (1.0 g) was homogenised in 10 mL of ice-cold methanol at a high speed for 1 min using an IKA homogeniser (T18 Basic; Wimington, NC, USA). The homogenate was then centrifuged at 10,000 rpm for 10 min, and the supernatant (methanol extract) was separated. Total phenols were determined using the Folin–Ciocalteu reagent and an absorbance reading at 725 nm, according to the method described by Ricci et al. [30]. The content of total phenols was calculated on the basis of the calibration curve of gallic acid and was expressed as milligrams of gallic acid equivalents (GE) per 100 g of fresh weight. The antioxidant activity against the DPPH (diphenylpicrylhydrazyl) radical was performed according to Miliauskas et al. [31]. The same methanol extract (50 µL) was incubated with 950 µL of 0.12 mmol L^{-1} DPPH solution for 24 h. The absorbance at 515 nm was read thereafter. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard and the antioxidant activity was expressed as mg Trolox equivalent (TE) per 100 g of fresh weight.

2.2.4. Ascorbic Acid

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined on the HPLC system according to the adapted method of Cefola et al. [32]. The sample (2.0 g) was homogenised with 10 mL of methanol/water (5:95) with citric acid (21 g L^{-1}) and EDTA (g L^{-1}). The homogenate was filtered through a cheesecloth and then a C18 Bakerbond SPE column (Waters, Milford, MA, USA), prior to HPLC injection. The HPLC system (Agilent 1200) consisted of a G1312A binary pump, a G1329A auto sampling and a G1315B photodiode array detector from Agilent Technologies (Waldbronn, Germany). Separation was achieved on a Zorba Eclipse XDB- C18 column (150 mm Ø 4.6 mm; 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was methanol/water (5:95 v/v) containing 5 mmol L^{-1} cetrimide and 50 mmol L^{-1} potassium dihydrogen phosphate (pH 4.5) with the flow rate of 1 mL min^{-1}. The AA and DHAA contents were expressed as mg of ascorbic or dehydroascorbic acid per 100 g of fresh weight. Total ascorbic acid was the summation between ascorbic and dehydroascorbic contents.

2.3. Volatile Organic Compositions

2.3.1. Ammonia and Ethanol-Acetaldehyde Contents

The cut stem sample (5 g) was homogenised with chilled 15 mL deionised H_2O and centrifuged for 1 min. Then, after 1 h of incubation at 65 °C, headspace gas samples (0.5 mL) were injected into the Agilent 7890 A Series gas chromatograph coupled with the Agilent 5975 C network mass selective detector (Agilent Technologies, Palo Alto, CA, USA). A HP-INNOWax capillary column (60 m × 250 µm × 0.25 µm; Agilent) was employed and GC was operated with the following method: 40 °C for 4 min, then increased from 40 to 60 °C at 3 °C min^{-1} and from 60 to 140 °C at 20 °C min^{-1}. Helium carrier gas was used at a constant flow of 1.0 mL min^{-1}. Compounds were identified by a comparison of ethanol and acetaldehyde standards and expressed as µmol g^{-1}. The ammonium was measured with phenol/hypochlorite, as described by Weatherburn [33] and Cantwell et al. [34]. The amount of ammonium was expressed as µg ammonia g^{-1} sample.

2.3.2. Solid Phase Microextraction (SPME)

The volatile compositions were assessed using a headspace solid phase microextraction (SPME) by using 50/30 µm Divinylbenzene/Carboxen/Polidimethylsiloxane (DVB/CAR/PDMS) fibre, following the method described in Piazzolla, Pati, Amodio and Colelli [29], with some modifications. At each sampling day, the stems bearing leaves (5.0 g) were incubated in a 150 mL capped SPME jar at 40 °C. After 20 min in the temperature, the fibre was exposed to the capped vial headspace for 30 min prior to insertion into the injecting port of an Agilent 6890 N (Palo Alto, CA, USA) chromatograph with an HP-INNOWax capillary column (60 m × 250 µm × 0.25 µm; Agilent, Santa Clara, CA, USA). The running conditions were set to the following: a spitless mode (injection port temperature 280 °C), 50 °C for 1 min, the temperature was increased from 50–100 °C at 3 °C min^{-1} with a holding time of 2 min, from 100–140 °C at 1.5 °C min^{-1} with a holding time of 10 min and finally from 140–180 °C at 6 °C min^{-1} [29]. The identification of detected volatile compounds was achieved by a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and by a comparison of the mass spectra with reference spectra contained from the NIST 02 library.

2.4. Sensory Evaluation

The spearmint stems were evaluated for sensory attributes by 10 panellists. At each sampling day, the panels were asked to compare the (1) odour intensity, (2) off-odour, (3) visual quality, (4) yellowing or degreening and (5) browning using a 7-point scale ranging, where 7 was described as having the sensorial attributes much higher than the reference (CTRL).

2.5. Statistical Analyses

The effects of the durations of the storage and CO_2 concentrations on the quality attributes were evaluated by two-way analysis of variance (ANOVA) using IBM SPSS software. Heat map and principal component analysis (PCA) were used to summarise the visual relationships of the volatile metabolites and the treatments using XLSTAT ver. 2018.5 (Suite NY, New York, NY, USA) [35].

3. Results and Discussion

3.1. Effect of High CO_2 in CA on Physiological Attributes

High water loss through transpiration is the major cause of quality reduction in leafy produce, including culinary herbs such as mints. High-surface-area-to-volume ratios and number of stomata in leaf tissues result in high rates of water loss, which as a consequence, affect marketable weight and visual quality, and may affect the physiology and ability to resist pathogen invasion [1]. During the first 5 day storage, CO_2 treatments significantly maintained weight loss to the levels close to the CTRL (Figure 1A). The highest weight loss was observed in 5% CO_2 at day 10, while that of the CTRL group was the lowest. The relationship between

level of CO_2 and stomata closure has been studied [36]. However, no unequivocal conclusion could be drawn. CO_2 concentration of the air may or may not induce stomatal frequency and the results may vary from one genotype to another [37]. Throughout the respiration process, oxygen is consumed, thus producing by products, i.e., water, carbon dioxide, and energy. The latter is the vital resource for subcellular activity. Low O_2 atmospheric conditions may result in a reduction in the respiration rate due to the reduction in overall metabolic activity [8,38]. The influence of CO_2 concentrations on respiration, however, depends upon the type and developmental stage of the commodity, and time of exposure [38,39]. Based on our results, higher atmospheric percentages of CO_2 may accelerate the respiration and transpiration of freshly cut spearmints, which declined thereafter.

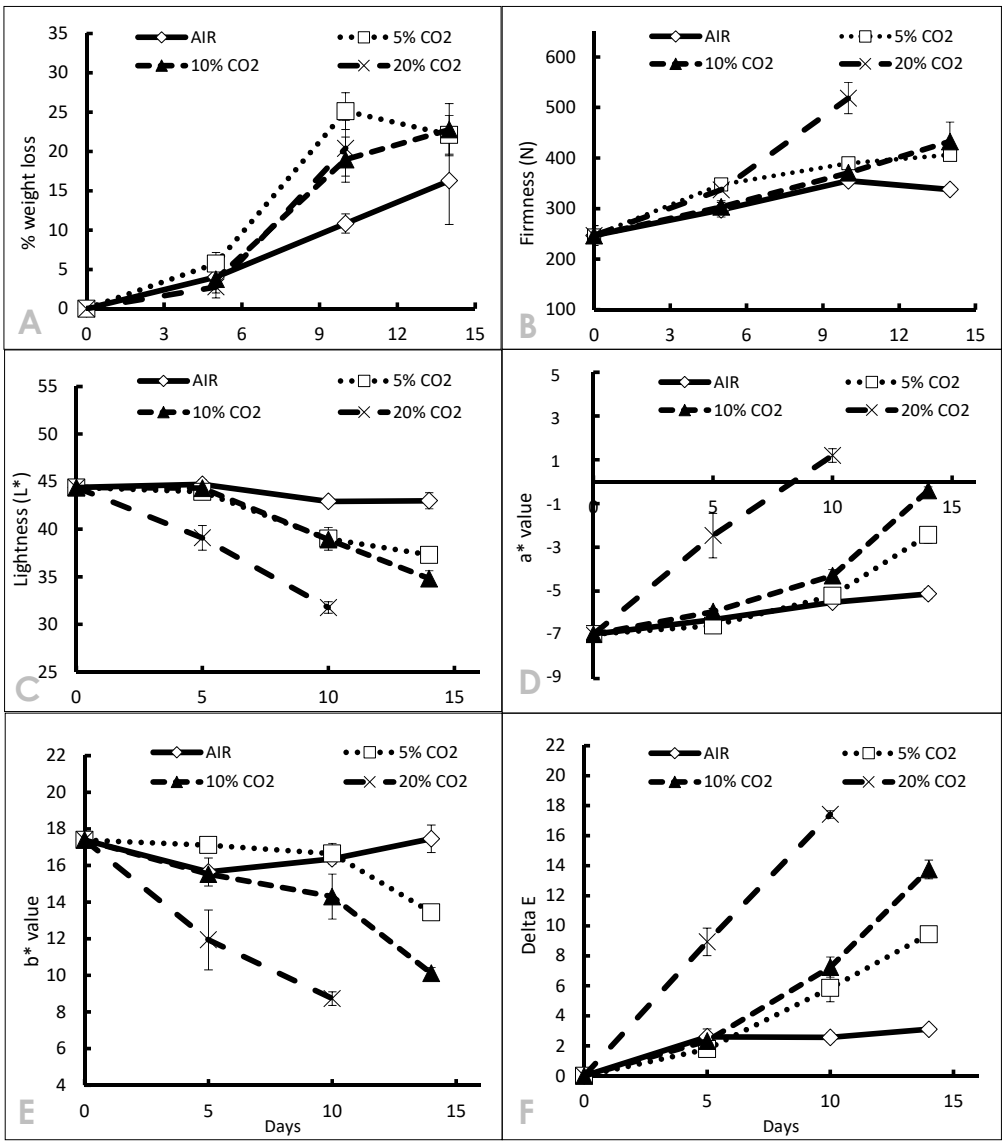

Figure 1. Effect of CO_2 concentrations in the control atmosphere (CA) storage of cut spearmint on weight loss (%, (**A**)), firmness (N, (**B**)) and CIE space L* (**C**), a* (**D**), b* (**E**) and the difference of the colour space or delta E (**F**). Vertical bars represent standard error (n = 3).

The determination of firmness or texture is used to describe the freshness of freshly cut herbs [40]. In our present study, the force (N) used to compress spearmint leaves stored in CA significantly increased with the CO_2 concentration and storage times (Figure 1B and Table 1). This is not in agreement with other studies where the texture attribute was independent from the concentrations of gases [40,41]. Maguire, Sabarez and Tanner [39]

advised that a water loss of the stored commodities greater than 3% may cause the development of a rubbery texture. This is what possibly occurred in our case, as all CO_2 treatments led to an increase in weight loss, thereby leading to the loss of freshness and as a result the texture became tough rather than crispy.

Table 1. Effects of the main factors (high CO_2 treatments, time of storage and their interaction) on the quality attributes of cut spearmint stored at 5 °C for 14 days.

Quality Attributes	Factor Effects		
	Treatment	Storage Time	Treatment * Storage Time
Physiological attribute			
Weight loss (%)	**	***	NS
Firmness (N)	***	***	***
Colour			
Lightness (L)	***	***	***
a *	***	***	***
b *	***	***	***
Delta E	***	***	***
Phytochemical attributes			
Total phenolic content (mg gallic acid equivalent 100 g FW^{-1})	**	***	NS
Antioxidant capacity (DPPH) (mg trolox equivalent 100 g FW^{-1})	NS	***	*
Total ascorbic acid (mg 100 g FW^{-1})	**	***	NS
Off flavour attributes			
Ammonia content (μg g^{-1})	***	***	***
Ethanol (μmoL g^{-1})	**	***	NS
Acetaldehyde (μmoL g^{-1})	***	***	***

The effect of each main factor and of their interactions is significantly different for $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); or not significant (NS).

CO_2 treatments also altered leaf colour surface (Figure 1C–F). L* and b* significantly decreased, while the a* value increased; the results in the CO_2 treatments were apparent from day 5 onwards. The higher percentage of CO_2, the grater the alteration of these values; a reduction in the lightness (L*) and yellowness (b* value) were also observed in the elevated CO_2 packaging of cilantro, possibly illustrating the discolouration (browning) of the leaves [40]. The increase in a* value also reveals the loss of greenness of the leaf, which is as a result of the ongoing loss of fresh weight due to water also leading to discoloration and the degradation of the chlorophyll pigment [1,39,42]. The physical quality of cut spearmint was also evaluated based on the difference in the colour surface values to the fresh sample. It appears that lower levels of CO_2 treatments are able to maintain the overall colour changes in the refrigerated temperature within the consumer acceptable range (<3.5) for 5 days.

3.2. Effect of High CO_2 in CA on Phytochemical Attributes

Mints are rich sources of phenolics, especially phenolic acid and flavonoids, with a long list of biological activities, including those that have antioxidant potential [43,44]. In horticulture produce, antioxidants have been used as key quality characteristics to determine the freshness of produce [45]. It was found that the content of phenolics in minimally processed herbs was maintained during the first 3 days at the refrigerated temperature and, in some cases, such as that of freshly cut celery, the content did not change significantly during 28 days of storage at 0 °C [20,46]. Our results, nonetheless, exhibited significant drops in the total phenolic content in all cases, while the highest CO_2 treatment showed substantial changes (Figure 2A). It was evident that CO_2 treatments did not improve total phenolic content; however, the DPPH antioxidant potential was maintained at least during the first 5 days of storage for the CTRL and slightly improved with CO_2 treatments. This is possibly due to the ability of the CO_2 to inhibit cellular free radical accumulation through the activation of the activity of the antioxidative enzymes SOD, CAT and POX, which are normally present during catabolic respiratory processes [47,48].

Additionally, the modified atmosphere is possibly able to minimise the use of antioxidants to scavenge free radicals [48].

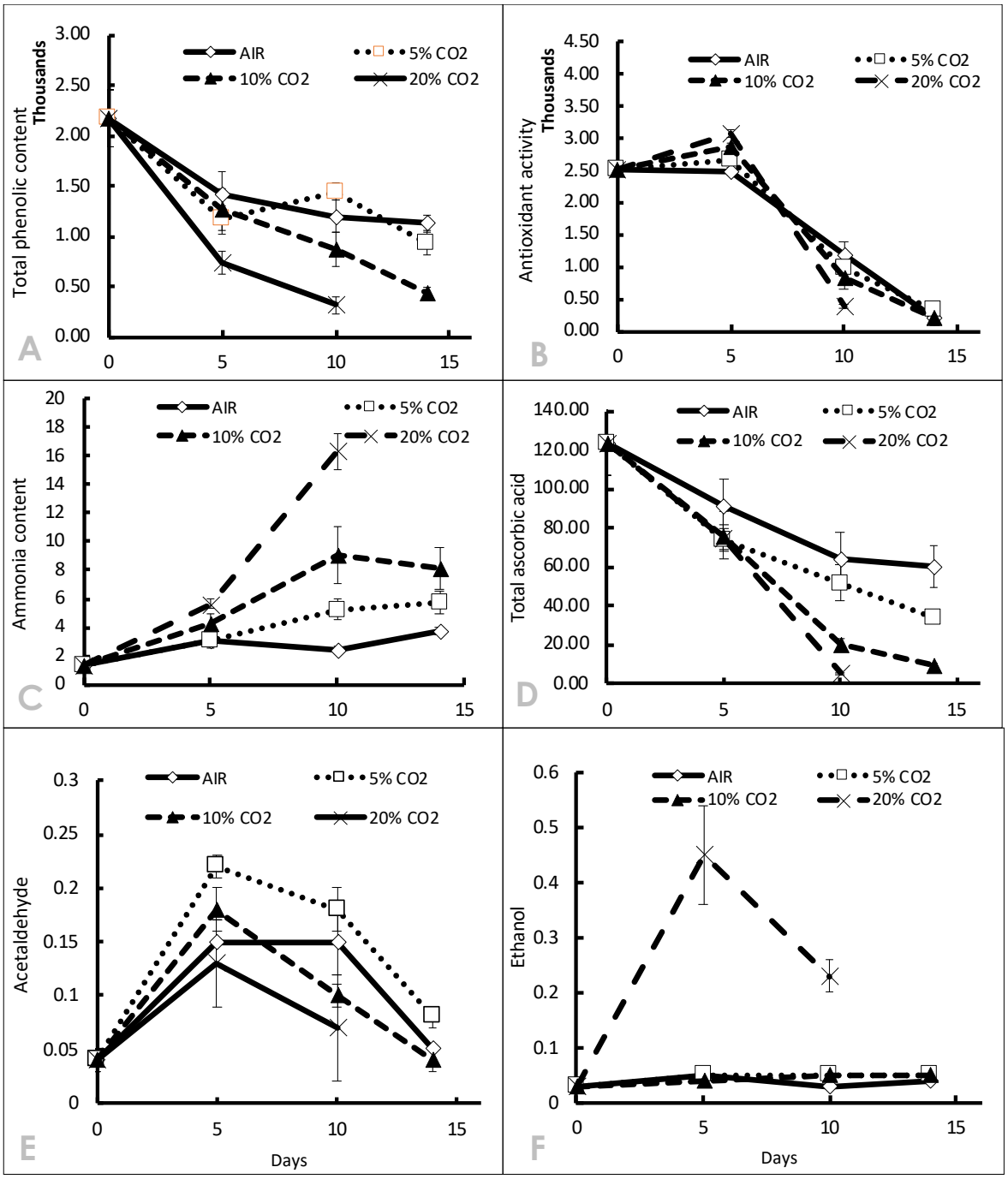

Figure 2. Effect of CO_2 concentrations in the control atmosphere (CA) of cut spearmint on total phenolic content (mg GAE 100 g^{-1}, (**A**)) and DPPH activity (mg TE 100 g^{-1}, (**B**)), ammonia content (µg g^{-1} sample, (**C**)), total ascorbic acid D (mg/100 g^{-1} sample, (**D**)), acetaldehyde (µmoL g^{-1} sample, (**E**)) and ethanol (µmoL g^{-1} sample, (**F**)). Vertical bars represent standard error (n = 3).

The accumulation of ammonia protein catabolism is used to determine the toxicity of the subcellular structures of leafy produce in CA [34,49,50]. During the storage, the toxicity of cell structures increased and was significantly higher in all CO_2 treatments (Figure 2C). Cantin et al. [6] and Ramos et al. [51] suggested that freshly cut produce is less tolerant at high CO_2 concentrations in CA and it should be avoided to prevent the development of off-flavours and the potential stimulation of pathogen growth. In nature, ascorbic acids are very sensitive, and therefore are used to determine the freshness of produce [52,53]. Along with other antioxidants, they are known for their activity against free radicals. L-ascorbic acid (AA) is the principal biologically active form, while L-dehydroascorbic acid [54], an oxidation product, also exhibits biological activity. In this work, vitamin C content is defined as the sum of the two forms [32]. The vitamin C content decreased with times in all treatments, but it was significantly lower with CO_2 treatments (Figure 2D). This is in agreement with work conducted by Lee and Kader [55], suggesting the adverse effects of CO_2 in storage on the vitamin C content of leafy commodities.

3.3. Volatile Organic Compositions

The dynamic headspace of the volatile compounds from the samples were analysed by SPME. It was found that as many as 58 volatile metabolites were detected from freshly cut spearmints (Supplementary Table S1). Of those, the major compound group was terpene hydrocarbons, along with the sesquiterpenes and alcohols. The major volatile compounds representing the specific aroma of spearmints include D-carvone (C51, 31.6%), (-)-.beta.-bourbonene (C37, 13.5%), D-limonene (C12, 17%), caryophyllene (C42, 9.0%) and .beta.-myrcene (C11, 4.0%). These were comparable to the composition analysed of the essential oil of spearmint in different studies (carvone (40.0–70.0%), limonene (10.0–15.2%) and myrcene (8.93%) [20,56]), depending on the growing stages and cultivation areas. Factors such as ecotype, phenophases and the environment, including temperature, relative humidity, irradiance and photoperiod, are also crucial for the variation of the aromatic profiles [20,56]. In our study, volatile organic compounds changed during the storage of spearmint, and a heatmap analysis clustered the samples according to the aromatic profiles to two major groups (Figure 3A). Compounds such as spiro (2.4) heptane, 1,5-dimethyl-6-methylene-/Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)- (C52), (E)-.beta.-Famesene+Humulene (C46), beta-cubebene (C39), 1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]- (C49), 1H-cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- (C38), 4-isop ropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene (C53), alpha.-cubebene (C34), (+)-epi-bicyclosesquiphellandrene (C47), 1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methy lethyl)-, [S-(E,E)]-.beta.-copaene (C41), and caryophyllene (C42) were clearly separated from the rest of the treatments. To have a better picture of the relationship between CO_2 treatments during storage, a principal component analysis (PCA) was utilised. The variables were depicted across PC1 (48.94%) and PC2 (17.58%). The results illustrated three distinguishable clusters, in which the samples at the initial day clustered in one group. Over time, the aroma of the sample stored in air conditions shifted to a different cluster (arrow indicated in Figure 3B), while the treatments of CO_2 treatments, especially those of higher concentrations, were categorised into different groups. A biplot analysis (Figure 3C) elucidated the relationship of these samples with ethanol (C2), cyclohexanone, 2-methyl-5-(1-methylethenyl)- (C43), pulegone (C45), alpha-terpineol (C48), cyclohexene, 3-methyl-6-(1-methylethenyl)-, (3R-trans)- (C50), 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, and cis (C55).

During storage, minimally processed vegetables, including cut spearmints, are prone to quality degradation due to both microbiological spoilage and physiological activities. The primary benefit of lowering O_2 and elevating CO_2 in the CA is the reduction in the respiration/metabolic rate of the commodity, thereby retarding the senescence process [57]. This was, however, not effective in reducing microbiological growth [58]. In fact, the high CO_2 concentrations during the production of fermentative metabolites, viz., ethanol

and acetaldehyde, represent the off-odour of the produce [59]. The results of this study, however, showed that the content of acetaldehyde was much lower in the air enriched with 20% CO_2, compared with those of the lower concentration and the CTRL during the initial 5 day storage (Figure 2E). In contrast, the ethanol concentration seemed to peak at day 5, and decreased thereafter. The rest of the treatments were able to maintain the ethanol level throughout (Figure 2F). These findings are in line with other works that reported an increase in the levels of ethanol during storage in high CO_2 (10–23%) and low O_2, which was possibly due to the microbiological activity caused by mesophilic microorganisms [60,61].

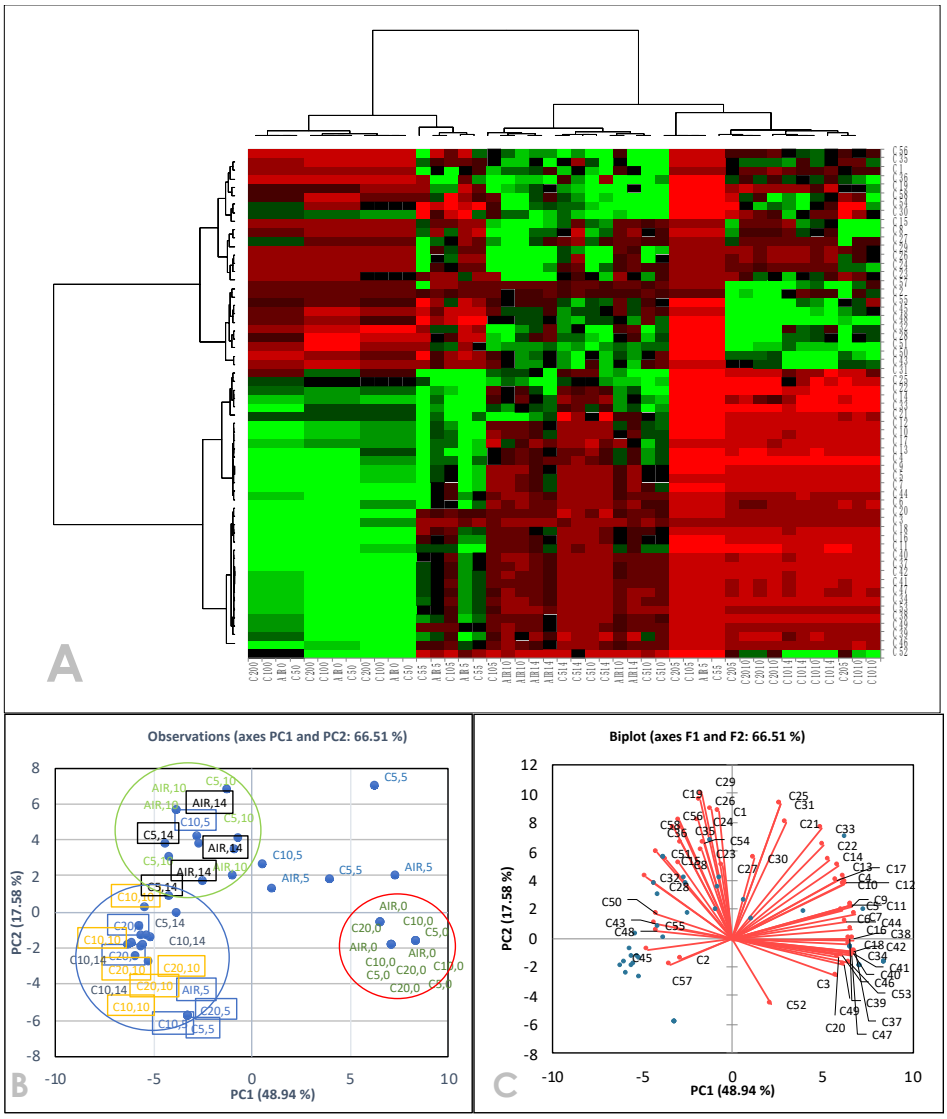

Figure 3. Heatmap of the volatile profile of spearmint during low temperature storage in air (AIR, *n* = storage day) and in low CO_2 concentrations (5, 10 and 20%, *n* = storage day) (**A**). Score plot clustering of the treatments (**B**) as well as their correspondent volatile compounds (**C**) (Cn; where *n* is the compound number assigned in supplementary Table S1).

3.4. Effect of High CO_2 in CA on the Sensory and Flavour Attributes

As shown in Figure 4, the CO_2 treatments affected the sensory attributes, as determined by 10 panellists. These panellists were asked to compare the sensorial attributes of spearmint stems with the control reference sample in each day of analysis. All samples were randomly labelled with three-digit numbers so that the judges were unable to tell which samples belonged to which treatments. The reference sample was named as REF. At

day 5, the judges could tell the difference between the control sample with 5% and 20% CO_2 treatments in terms of aromatic odour, off-flavour, degree of browning, and development of off-odour. The lowest appearance score was found in the 20% CO_2 treatment at day 10, thus its quality assessment was thereafter terminated. The 5% CO_2 treatment seemed to improve aroma and visual quality, while browning and off-odour were noticeable in the 20% CO_2 treatments. At day 10, the aroma and visual quality decreased significantly, and this treatment was removed from the experiments. At day 14, browning was greater in the CO_2 treatments and the visual quality decreased. Ranjitha et al. [54] described that high CO_2 and humidity accumulated in the package are key factors that have an adverse effect on the sensorial quality of minimally processed herbs. A further explanation was that excessive CO_2 may lead to the acceleration of the plant metabolism, mainly through the respiration and the development of off-odour.

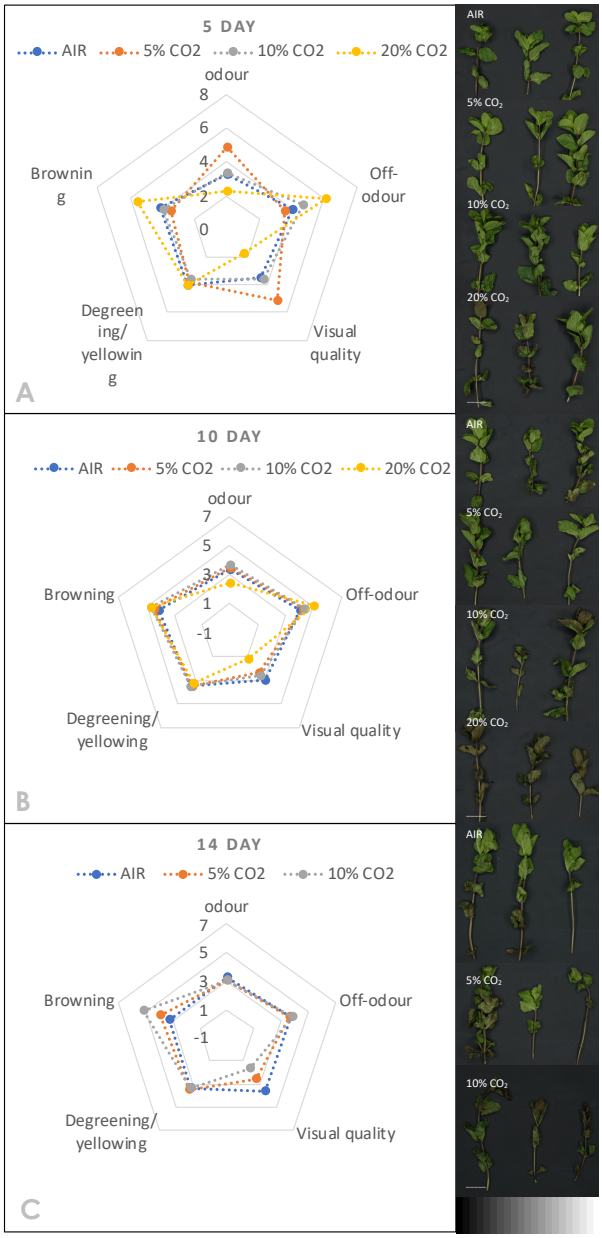

Figure 4. Sensory attributes scores (viz., odour intensity, off-odour, visual quality, yellowing or degreening and browning) evaluated by 10 panellists at the sampling days 5 (**A**), 10 (**B**) and 14 (**C**), on a 7-point scale, where 7 was described as having a much higher overall appearance than the reference (CTRL). Scale bar = 1 cm.

4. Conclusions

Unlike any other freshly cut produce, elevated CO_2 did not improve physical qualities during the storage of freshly cut spearmint stored at a low temperature over a 14 day storage period. CO_2 treatments enhanced weight loss, overall colour alteration and cell toxicity. Additionally, the elevated CO_2 concentration had an adverse effect on the contents of bioactive ingredients as well as functional properties, while the aromatic profiles were similar to the untreated sample. Consequently, as it is a highly perishable crop, cut spearmint possesses a short shelf life and applying a high CO_2 treatment was not suitable during postharvest low temperature storage in the controlled atmosphere condition. The antibacterial characteristics of the gas composition, as well as the usage of limited oxygen during storage, will be further investigated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8020126/s1. Table S1: Volatile compounds detected in fresh spearmint (and in spearmint stored in air) in the presence of 5% CO_2, 10% CO_2 and 20% CO_2 in 5, 10 and 14 days of storage. Mean values of three replicates ± standard error (n = 3).

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