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Ready-to-use broccoli raab (*Brassica rapa* L. subsp. *sylvestris*) quality and volatilome as affected by packaging

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ABSTRACT

Broccoli raab (*Brassica rapa* L.) is a cruciferous vegetable primarily grown in the southern regions of Italy. Distinguished from the more common broccoli head (*B. oleracea*), its edible parts consist of sprouts, buds, younger stems, and leaves. Considering the high perishability of broccoli raab, it is necessary to apply post-harvest technologies to make it a ready-to-use product. In this paper, active modified atmosphere packaging (aMAP, closed at 3% O_2 and 97% N_2) was applied, in combination with two types of packaging materials (polypropylene/polyamide microperforated, PP/PA MF, and polypropylene, PP). Shrink-wrap packaging and air-storage were included as control measures. The purpose was to assess the effects of different packaging conditions on enhancing knowledge about volatile compound development and quality aspects of the produce. Both packaging materials resulted in a 10% accumulation of CO_2, combined with 1% O_2 for PP bags and 10% for PP/PA. Different gas mixtures led to varied sensory and volatile development in minimally processed broccoli raab during storage. PP exhibited the lowest odor score (1.2, well below the marketability limit) and the highest concentrations of off-odor-related volatile compounds (ethanol, acetaldehyde, dimethyldisulfide, dimethyltrisulfide, and methanethiol) while samples packed in PP/PA MF reached an intermediate odor score with superior visual quality evaluation up to 12 days of storage. Both packaging materials reduced weight loss compared to control samples, up to less than 2% and 6%, respectively. In conclusion, active MAP using PP/PA MF bags demonstrated the best suitability in maintaining broccoli raab quality, with a final gaseous composition of 10% O_2 and 12% CO_2 deemed optimal for storage.

1. Introduction

"Cima di rapa," also known as broccoli raab or broccoli rabe (*Brassica rapa* L. subsp. *sylvestris* L. Janch. var. *esculenta* Hort.), is a cruciferous vegetable commonly found in the southern regions of Italy, particularly cultivated in the Apulia region (approximately 4500 ha annually) (Conversa et al., 2016). Broccoli rabe represents a part of traditional recipes and culture. Despite its value, there are no patented cultivars available; instead, various landraces are prevalent, characterized by substantial phenotypic variability. These landraces are often named according to their harvesting time or based on the shape and size of the primary inflorescence (Mazzeo et al., 2019). The edible portion of broccoli rabe includes flower sprouts, buds, and small flowers blooming between the buds, along with younger leaves and stems. Cultivation of these landraces typically involves cycles lasting 50–90 days in open fields during the fall and winter seasons, with minimal agronomic practices and pesticide treatments applied (Giannino et al., 2020).

Consumers appreciate this product due to its aromatic taste, characterized by being slightly bitter and pungent, for the presence of glucosinolates (Renna et al., 2015). The nutritional value of plants from the Brassicaceae family depends on the presence of bioactive sulphoraphane isothiocyanate, derived from the enzymatic breakdown of glucoraphanin, (Wieczorek et al., 2022), alongside dietary fibre, and bioactive compounds such as phenolics, vitamin C and carotenoids (Cefola et al., 2010a; Cefola et al., 2010b; Conversa et al., 2016). Volatile compounds also play a crucial role in the aroma and flavor profile of plant tissue. *Brassica* species are rich in volatile components that contribute to the product's distinctive bitter and sharp aroma, which consumers highly value. Broccoli raab has a typical pungent flavor, like rocket leaves, that is mainly due to the presence of glucosinolates and isothiocyanates.

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However, volatiles significantly influence the occurrence of off-flavors, which is a function of their production and accumulation within tissues (Mastrandrea et al., 2017a,b). The broccoli volatilome comprises various chemical groups, including alcohols, sulphides, aldehydes, esters, and more. Neverthless, the volatile profile in fresh produce is dynamic, predominantly formed through enzymatic reactions and closely linked to environmental factors such as temperature, pH, and sampling conditions, storage duration, packaging, and processing methods (Wieczorek et al., 2022). Several factors can contribute to the production of sulfur volatile compounds (dimethyl disulfide and dimethyl trisulfide, hydrogen sulfide and methanethiol), which are responsible for off-odor (de Chiara et al., 2021).

The best quality of fresh vegetables is commonly expressed at harvest time and the objective of processing should be to store these fresh-like characteristics during their shelf life. In the case of local product, such as "cima di rapa", consumers often associate them with tradition, quality, and health, thereby showing a particular interest in consuming them and even being also available to increase the "willingness to pay" for high-quality food. Shrink-wrap packaging is currently used as a marketing method, serving as a form of passive modified atmosphere packaging which leads to a slight modification of the internal gaseous composition. However, due to the high perishability of minimally processed broccoli raab, it becomes necessary to implement postharvest technologies aimed at delaying yellowing of leaves, dehydration, flowering of the buds, and decay occurrence, as well as the production of off-odor-related volatiles compounds (de Chiara et al., 2021). The food industry is increasingly focused on optimizing postharvest technology to produce ready-to-use broccoli raab while preserving nutritional and organoleptic aspects.

However, there is a lack of comprehensive study in the literature on this subject concerning broccoli raab (*B. rapa* L. subsp. *sylvestris*). Among various processing strategies aimed at enhancing produce quality during its shelf life, modified atmosphere packaging (MAP) is one of the most effective and economical methods for extending the freshness of fresh-cut leafy vegetables (Liguori et al., 2023). Storage at low temperatures has also been shown to impact product quality significantly (Cefola et al., 2010a). Generally, the selection of packaging materials to design MAP should take into consideration several aspects such as permeability to gases, moisture, and light, as well as mechanical strength and cost-effectiveness. On the other hand, the choice of atmosphere composition to put inside the bags depends on diverse factors like the respiration rate of the product, as well as the tolerance of the product to low or high O_2/CO_2 concentration (Torrieri et al., 2010). The scientific literature on the product characteristics and quality parameters evolution under different gas compositions can help in justifying these choices. Even if few research articles regarding the proper packaging condition for broccoli rabe are available (Torrieri et al., 2010; Conte et al., 2011; Cefola et al., 2016; de Chiara et al., 2021; Liguori et al., 2023) no studies focused on changes in volatilome associated with packaging conditions were published.

Generally, it is reported that maintaining a low-oxygen concentration (3–5%) in the surrounding atmosphere is ideal for preserving the freshness and extending the marketability of broccoli raab during the storage period (Cefola et al., 2010a, 2015; de Chiara et al., 2021). However, it's crucial to avoid extreme CO_2 accumulation (above 10–15%), coupled with O_2 level below 2%, as well as fluctuations in storage temperature, to prevent the development of off-odors. The optimization of packaging for transporting and distributing minimally processed broccoli raab is influenced by several factors, with the characteristics of the plastic film used to pack the sample playing a significant role in gas accumulation (Conte et al., 2011; de Chiara et al., 2021). Consequently, the composition of the modified atmosphere, particularly low oxygen levels and increased carbon dioxide, can modify the physiological properties of the fresh product (Torrieri et al., 2010). A sub-optimal design of modified atmosphere packaging may reduce the shelf life of fresh produce, having a detrimental effect on physicochemical properties, sensory parameters (including volatiles development), and microbiological safety.

Based on the reported findings and to prevent excessive oxygen reduction within the packaging, two materials with different characteristics, one with microperforations and one without, were tested. Additionally, drawing upon previous studies (Cefola et al., 2010a, 2015; de Chiara et al., 2021) and to expedite the attainment of equilibrium, an initial low oxygen concentration was employed. This study aims to enhance the knowledge of how MAP impacts the development of volatile compounds, as well as the physicochemical and sensory qualities of minimally processed broccoli rabe. Since MAP efficacy is influenced by packaging type, two distinct plastic films (polypropylene, polypropylene/polyamide microperforated film) were utilized, along with active MAP application, to package ready-to-use "cime di rapa". To the best of our knowledge the existing literature on the evolution of volatile compounds profile in fresh-cut *Brassica rapa* L. subsp. *sylvestris* during low temperature storage is practically zero. Therefore, significant emphasis was placed on examining the development of volatile compounds and their correlation with the evolution of the gaseous mixture within the packages throughout storage time, alongside sensory quality.

2. Materials and methods

2.1. Sample preparation

"Cima di rapa" 'Novantina' flower sprouts with a corymb (approximately 3 cm wide) having closed flowers and 5–6 young leaves, were harvested at a commercial maturity stage from a commercial farm located in Foggia (Southern Italy) after 90d from transplanting. Broccoli raab were directly transported to the laboratory and immediately processed to obtain ready-to-use product by cutting them at a length of 10 cm. Then, samples in triplicate (about 100 g each one) were put in plastic bags (22 ×32 cm) of two different packaging materials described in Table 1 (Carton Pack S.p.A., Rutigliano, Italy), closed in active modified atmosphere (aMAP) at 3% O_2 and 97% N_2 (Boxer 50 GAS-Lavezzini, Fiorenzuola d'Arda, Italy). As control, fresh-cut broccoli raab were put in plastic trays closed using shrink-wrap packaging or microperforated bags.

To sum up the following packaging conditions were compared: i) PP/PA MF-aMAP; ii) PP-aMAP; iii) WRAP (shrink-wrap packaging using commercial plastic cling film); and iv) control (CTRL, air storage within macroperforated bags).

Each condition was replicated three times for each sampling time (4 packaging conditions x 3 replicate x 4 storage times). Minimally processed "cima di rapa" were stored up to 12 d at 5±1°C and the reported analysis were conducted after cut, and on the 2nd, 5th, 8th and 12th day.

2.2. Gaseous atmosphere composition, color analysis, weight loss

Oxygen and carbon dioxide inside PP/PA MF and PP packages headspace, were measured by using a hand-held gas analyzer (CheckPoint, PBI Dansensor, Mocon/Ametek, Minneapolis, USA). The color was measured on fresh-cut broccoli raab leaves surface using a spectrophotometer (Konica Minolta CM 2600d, Osaka, Japan) in the reflectance mode on the CIE L*a*b* color scale. From the primary L*, a*, and b* values the following indexes were calculated: Hue angle ($h° = \arctan \frac{b^*}{a^*}$); Chroma = $\sqrt{a^{*2} + b^{*2}}$; Global color variation ΔE $= \sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2}$. Measurements were taken over 3 random points on samples from each replicate and each sampling time. Three replicates for each sampling time were weighed and the weight loss was calculated as a percentage of the fresh weight at day 0.

2.3. Sensory determinations

Sensory parameters were scored by a group of 6 trained panelists on

Table 1
Technical characteristics of the plastic materials used for packaging.

Packaging material	Thickness (μm)	Oxygen Transmission Rate (mL m^{-2} d^{-1} atm^{-1})	Water Transmission Rate (g m^{-2} d^{-1})	Holes diameter (μm)	MF density (holes m^{-2})
Polypropylene/polyamide microperforated (PP/PA MF)	67.00	29.00	2.01	70.00	0.0001
Polypropylene (PP)	30.00	1100.00	5.00	–	–

each replicate for each sampling time. Visual quality was scored on a 9–1 scale, where 9=excellent, fresh appearance, 7=good, 5=fair (being 6 the limit of marketability), 3=fair (useable but not saleable), 1=unusable. Intermediate numbers are assigned where appropriate. Indicators for odor score were: 5 = no off-odor, 4 = slightly off-odor, 3 = moderate off-odor, 2 = strong off-odor, 1 = odor of mouldiness (de Chiara et al., 2021). Decay was scored using a 1–5 scale, where 1=no decay, 2=slight decay, but product salable, <2% affected; 3=moderate decay, product useable but not salable, <5% affected, 4=moderately severe, <15% affected and 5=severe, unusable. Discoloration scores (from 1 to 5) on the cut ends were 1=none, 2=slight (not reduce salability), 3=moderate (limit of marketability), 4=moderately severe, and 5=severe. The scale from 1 to 5 was also used for the yellowing of beads or leaves, where 1=green, 2=slight yellowing (1–2 beads per floret), 3=moderate yellowing (3–5 beads per floret), 4=moderately severe and 5=severe. Flowering was scored on a 5–1 scale: 5 = complete flowering, 4 = severe flowering, 3 =moderate flowering, 2 = slight flowering, 1 = no flowering (Cefola et al., 2010a).

2.4. Volatile compounds analysis

Volatile compounds, including the by-products of anaerobic metabolism ethanol and acethaldeyde, were evaluated across all the three replicates on each sampling day for each condition. Thirty grams of broccoli raab, with 0.6 g of $CaCl_2$ and 6 g of NaCl and 30 mL of distilled water, were homogenized for 6 min using a commercial disperser (T 18 ULTRA-TURRAX, IKA, Germany). Subsequently, 8 g of sample, added to 2 μL of internal standard solution (IS, 2-methyl-1-pentanol, 100 ppm), were placed into a 20 mL capped solid-phase microextraction vial and incubated at 40 °C for 20 min. Carboxen/polydimethylsiloxane (CAR/PDMS) 85 μm fiber was inserted into the vial for a headspace exposure time of 30 minutes and then introduced into the gas chromatograph injector port at 250 °C. A desorption time of 4 min was applied, using the split injection mode (1:20). The instrument used was a gas chromatograph Agilent model 6890 Series, paired with an Agilent 5975 C network mass selective detector. Separation of the volatiles were conducted using a HP-5 ms capillary column (60 m ×250 μm × 0.25 μm). Temperature program of the oven was the following: 40 °C for 4 min, rise to 140 °C at 3 °C min^{-1}, with a subsequent holding time of 10 min. Transfer line temperature was 280 °C. An electronic impact mode at 70 eV was used as mass detector conditions; source temperature was 230 °C; scanning rate was 2.88 scan/s, and mass scanning range was *m/z* 30–400. Helium was used as carrier at a flow of 1.0 mL min^{-1}. Compounds were identified by comparing the mass spectra with the data of a system library (NIST 02, p > 80) and the results (peak areas) were normalized postacquisition compared to the area of the internal standard and reported as "normalized peak area" (Mastrandrea et al., 2017a; 2017b).

2.5. Vitamin C, total phenols, antioxidant activity, and total chlorophyll

Five grams of fresh broccoli raab tissue were homogenized adding 10 mL of methanol: H_2O (5:95) containing citric acid (21 g L^{-1}) and EDTA (0.5 g L^{-1}). The homogenate was filtered using a two-layers cheesecloth and a C18 Bakerbond SPE column (Waters, Milford, MA, USA). Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents in the extract were evaluated by means of a HPLC analysis, using the method from (Zapata and Dufour, 1992), with some modifications. DHAA was derivatized into the fluorophore 3-(1,2- dihydroxyethyl) furol [3,4-b] quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples (20 μL) were injected in an Agilent 1200 Series HPLC equipped of a G1312A binary pump, a G1329A autosampler, a G1315 B photodiode array detector from Agilent Technologies (Waldbronn, Germany). The separation of the compounds of interest was achieved using a Zorbax Eclipse XDB-C18 column (150 mm× 4.6 mm; 5 μm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was composed by MeOH:H_2O (5:95) with 5 mM cetrimide and 50 mM potassium dihydrogen phosphate (pH 4.5). The flow rate was 1 mL min^{-1}. Results were reported as g of AA or DHAA per kg of fresh weight (g kg^{-1}), actual concentration of the compounds in the sample were obtained by comparing peak area with a standard solution. To evaluate both total phenol content and antioxidant activity, the same extract was used obtained homogenizing 5 g of plant tissue in a MeOH:water (80:20) solution. After centrifugation the supernatant was used for the assays. The antioxidant capacity assay was performed following the procedure described by (Brand-Williams et al., 1995), with slight modifications. After appropriate dilution the supernatant was mixed with 0.95 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution to start the reaction, the absorbance was read after approximately 30 min at 515 nm. Results was expressed in gram of Trolox per kg of fresh weight (g Trolox kg^{-1}). Using the same extract was measured the total phenol content according to Singleton and Rossi (1965). Properly diluted extract was mixed with distilled water, Folin-Ciocalteu reagent and 200 g L^{-1} sodium carbonate solution. After 2 hours of incubation in darkness, the absorbance was read at 765 nm. Total phenol content was assessed based on the gallic acid standard curve and expressed as grams per kg of fresh weight (g gallic acid kg^{-1}). Chlorophyll content was measured on five g of broccoli raab, randomly from flowers, stems and leaves to have a representative sample. Plant tissue was submerged in 20 mL methanol for 24 hours at 20 °C in darkness. Chlorophyll quantification was done spectrophotometrically at 653, 666 and 470 nm. Chlorophyll content was then expressed as g per kg of fresh weight (Wellburn, 1994). All the analysis were performed on three replicates for each sampling time.

2.6. Statistical analysis

To evaluate the effect of packaging conditions (PP/PA MF-aMAP; PP-aMAP; WRAP and control, including starting gaseous atmosphere and type of plastic film) and storage time on the measured parameters, all the data were subjected to a two-way analysis of variance (ANOVA), and means were separated by Tukey's test at $P<0.05$ (5% significance level) using Stat Graphics Centurion XVI.I (Stat Point Technologies, Inc., Warrenton, VA, USA) software. For each time of storage, a one-way ANOVA was performed to determine the effect of packaging treatment on the qualitative characteristic considered. Off-odor-related volatiles of broccoli raab on the 8th and 12th day, including the O_2 and CO_2 concentrations within the bags at these sampling dates and sensorially evaluated odor and visual quality were modeled by using a multivariate principal component analysis (PCA) to establish a relationship between the studied parameters. PCA describes the data by projecting it on a new set of axes in the multivariate space. Principal components are described as linear combinations of the original variables and each one accounts for the direction of maximum variability in the data (Bro and Smilde, 2014).

3. Results

3.1. Physicochemical and sensory parameters

The changes in the gas composition of packed broccoli raab samples over storage time are reported, except for CTRL samples stored in air (Fig. 1). It was possible to observe a rapid drop in the percentage of O_2 within PP packages, already after 2 d of storage. After that, the value remained very low (approximately 2%) but constant up to the end of storage time, with significant differences if compared to PP/PA MF bags, which showed an increase in O_2, reaching the equilibrium at values of about 10%. Conversely, the CO_2 levels increased in both types of packaging at similar rates. In the case of shrink-wrap packaging, achieving an air-like environment by the end of the storage period proved challenging due to imperfect sealing. Consequently, the trends in O_2 and CO_2 concentrations were variable, and significant alterations in their levels were not observed as depicted in Fig. 1.

It was observed that most of the measured parameters were not significantly influenced by either the packaging condition or the storage time (see Table S1). However, both factors had a significant impact on the content of AA and DHAA, weight losses, and several sensory attributes. Additionally, the total phenols content and antioxidant activity of the samples were only affected by the packaging condition (see Table S1). Regarding sensory parameters, the statistically significant differences were mainly due to the variations identified by the panelists during the last days of storage (Table 2). Notably, PP-stored samples, exhibited the lowest odor scores, suggesting the presence of a higher concentration of volatile compounds responsible for off-odor, as will be discussed below. In contrast, CTRL and commercially packed (WRAP) samples maintained higher values, like freshly processed products, while PP/PA MF-aMAP samples fell in between, with the development of off-odors closely associated with the oxygen and CO_2 concentrations within the packages.

Throughout the storage period, a notable discrepancy in weight loss was observed between the control (CTRL) sample and the others. After 12d CTRL samples exhibited significantly higher weight losses (5.83%), while a gradual, physiological weight loss was observed for the other samples that did not exceed 2% by the end of storage. At the end of the storage period, there were differences in the AA content among the samples (Fig. 2), with CTRL and PP/PA MF-aMAP samples maintaining the highest values.

Regarding the antioxidant activity and total phenolics content of broccoli raab, although the type of packaging had a significant effect, appreciable differences were not observed at the end of storage. Additionally, the final values were similar to the starting ones (data not shown), showing that despite the gas concentrations not being optimal for all samples, the impact on these aspects of broccoli raab was negligible.

Table 2
Odor score, visual quality, and decay score of the packaged broccoli raab as evaluated by panelists at the end of storage time (12th day). Data are means of three replicates for each sampling time. For each sensory attribute means with different letters are significantly different according to Tukey's test (P value ≤0.05).

Packaging condition	Odor score (5–1)	Visual quality score (9–1)	Decay score (5–1)
PP/PA MF-aMAP	3.0 ± 0.5b	6.0 ± 0.0a	2.5 ± 0.0b
PP-aMAP	1.2 ± 0.3c	5.5 ± 0.0b	2.0 ± 0.0c
CTRL	4.5 ± 0.0a	4.1 ± 0.3d	2.8 ± 0.3ab
WRAP	5 ± 0.0a	5.0 ± 0.0c	3.0 ± 0.0a

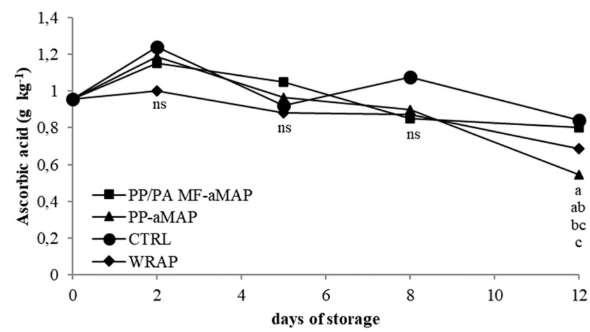

Fig. 2. Ascorbic acid content over storage time of minimally processed broccoli raab as affected by packaging condition. Data are means of three replicates for each sampling time. Means with different letters at the same time of storage are significantly different according to Tukey's test (P value ≤0.05).

3.2. Volatiles and sensory determination

Table 3 presents the outcomes of the two-way analysis of variance conducted on volatile organic compounds (VOCs) content of broccoli raab over the storage time. It was found that the impact of both factors (packaging condition and storage time) and their interaction was significant for most compounds. A total of 12 volatiles were identified, each of which contributes to a specific odor, as detailed in Table 4, with some being specifically associated with off-odor characteristics. The volatile compounds detected in broccoli raab tissue can be categorized into six chemical classes: (a) aldehydes: acetaldehyde, 2-pentenal, hexanal and 2-hexenal; (b) alcohols: ethanol, 2-penten-1-ol, 3-hexen-1-ol; (c) thiols: methanethiol; (d) volatile sulfur compounds: dimethyl disulphide, and dimethyl trisulphide; (e) esters: methyl thiocianate; (f) furan: 2-ethylfuran.

Fig. 3 reports the evolution over time of some of the identified volatiles. Despite the significance observed in the two-way ANOVA, it became apparent that by the end of the storage period, most of the values were not statistically different. However, concentrations of ethanol and acetaldehyde within PP-aMAP were notably higher than in

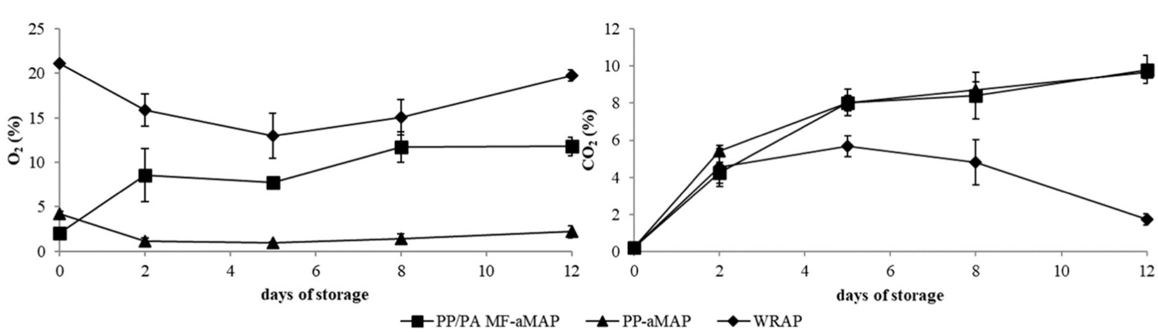

Fig. 1. Oxygen (O_2) and carbon dioxide (CO_2) concentration (%) within PP/PA MF-aMAP, PP-aMAP and WRAP samples over storage time at 5 ± 1 °C. Data are means of three replicates for each sampling time, error bars represent standard deviation.

Table 3
Effect of packaging condition (A), storage time (B) and their interaction (AxB) on volatiles compounds of broccoli raab during storage at 5 °C. Data are mean values of 12 samples (3 replicates × 4 storage time). For each volatile compound means with different letters are significantly different according to Tukey's test (P value ≤0.05).

Volatile compounds	Packaging condition				A	B	A x B
	PP/PA MF-aMAP	PP-aMAP	CTRL	WRAP			
Methanethiol	0.00±0.00b	0.06±0.12a	0.00±0.00b	0.00±0.00b	****	****	****
Acetaldehyde	0.40±0.41b	0.93±0.67a	0.39±0.53b	0.30±0.32b	****	****	****
Ethanol	0.00±0.00b	4.53±5.53a	0.07±0.15b	0.04±0.08b	****	****	****
Furan, 2-ethyl-	6.85±3.00	8.32±4.45	9.19±3.67	8.77±2.71	ns	****	ns
Disulfide, dimethyl	0.00±0.00b	0.30±0.75a	0.00±0.00b	0.00±0.00b	****	****	****
Hexanal	8.13±3.05b	14.54±7.03a	14.06±8.95a	12.13±5.47ab	**	****	ns
2-Pentenal, (E)-	1.98±0.55b	2.79±1.49ab	2.90±1.03ab	3.66±1.08a	**	*	ns
2-Hexenal, (E)-	166.92±75.12c	224.60±125.50bc	278.06±116.28ab	305.13±97.90a	***	***	*
Thiocyanic acid, methyl ester	2.12±1.06c	2.86±1.28bc	4.86±3.10ab	3.93±1.50a	****	****	**
2-Penten-1-ol, (Z)-	3.41±2.67	3.64±2.28	4.67±1.64	5.12±2.00	ns	*	ns
3-Hexen-1-ol, (Z)-	31.31±16.10b	37.21±20.54ab	44.26±23.06ab	48.56±25.61a	*	****	**
Dimethyl trisulfide	0.00±0.00b	0.21±0.51a	0.00±0.00b	0.00±0.00b	****	****	****

(****) P≤0.0001; (***) P≤0.001; (**) P≤0.01; (*) P≤0.05; ns, not significant

Table 4
Odour descriptor for each detected volatile compound.

Volatile compounds	Odour descriptor	Reference
Methanethiol	Rotten cabbage	(Cheng et al., 2020)
Acetaldehyde	Ethereal, pungent	(Mastrandrea et al., 2017b)
Ethanol	Alcoholic, ethereal	(Mastrandrea et al., 2017b)
2-Ethylfuran	Chemical	(Mastrandrea et al., 2017a)
Dimethyldisulfide	Sulfurous, onion, garlic	(Mastrandrea et al., 2017a; Cheng et al., 2020)
Hexanal	Fatty, green, grassy	(Mastrandrea et al., 2017b)
2-Pentenal	Oily, fatty, fruity	(Drabinska et al., 2022)
2-Hexenal	Almond-like, green, herbal	(Mastrandrea et al., 2017b)
Methyl thiocianate	Sulfury onion	(Marcinkowska & Jeleń, 2020)
2-Penten-1-ol	Fruity, green	(Mastrandrea et al., 2017b)
3-Hexen-1-ol	Fresh, green grass-like	(Mastrandrea et al., 2017b)
Dimethyltrisulfide	Onion, cabbage	(Cheng et al., 2020)

other samples starting from days 5 and 8 of storage, respectively. In addition, there was a significant increase in the production of dimethyldisulfide, dimethyltrisulfide, and methanethiol. Specifically, the normalized peak area for dimethyldisulfide was 1.1, for dimethyltrisulfide was 0.76, and for methanethiol was 0.19 in PP-aMAP samples at the end of the storage period, whereas these substances remained at 0 for all other samples throughout the entire storage duration (data not shown). This suggests that they are closely linked to the gas composition within PP-aMAP bags.

A principal component analysis was conducted on data collected from the last two sampling times (8th and 12th day), during which differences were more pronounced (Fig. 4). PC1 and PC2 accounted for the majority of the variability of the samples (91.36%). The analysis included the volatile compounds associated with off-odor (dimethyl disulfide, dimethyl trisulfide, methanethiol, ethanol, and acetaldehyde) and their relationship with gaseous concentrations and sensory parameters.

The bi-plot provided a visual representation of how various packaging conditions influenced the development of off-odor in broccoli raab over the storage period. Air-stored broccoli raab (CTRL) was clearly distinguished from samples stored in shrink wrap packages and in aMAP using the two different packaging materials (PP/PA MF or PP), indicating a correlation between O_2 concentration and the sensory evaluation of odor. Conversely, "cima di rapa" stored in PP-aMAP was grouped close to the off-odor related volatiles and fermentation by-products, exhibiting a positive correlation with carbon dioxide concentration. WRAP samples were most similar to CTRL samples due to comparable gas concentration within the packages, resulting in lower development of unfavorable volatiles compounds.

4. Discussion

4.1. Physicochemical and sensory parameters

The primary reason for the differences observed in gas accumulation within the broccoli raab bags is supposed to be the presence of microperforation in the PP/PA MF material, along with variations in gas permeability between the two packaging materials used in the trial. Selecting the most suitable packaging for storing minimally processed products is crucial for maintaining horticultural produce quality (Del Nobile et al., 2009).

The use of PP nearly led to a condition of anoxia. As will be discussed later, this is linked to the deterioration of the organoleptic quality of the packaged product. As noted by Lucera et al. (2011), the optimal MAP design for broccoli raab should aim for O_2 and CO_2 levels of approximately 10%, as achieved in our study using PP/PA MF-aMAP.

Regarding physicochemical and sensory aspects, as observed by Jacobsson et al. (2004a, 2004b) in storing broccoli florets (*B. oleracea*) using polypropylene, polyvinyl chloride and low density polyethylene (LDPE), different films resulted in varying gas concentration when combined with broccoli respiration. Anaerobic respiration, leading to off-odor development, should be avoided. Jacobsson et al. found that CO_2 concentration within PP packages exceeded 10%, potentially contributing to off-odor occurrence; low oxygen levels observed with LDPE film also resulted in off-odor production.

Weight loss is also associated with film permeability, with values exceeding 10% making leafy vegetables unacceptable due to shriveling and wilting (Nunes and Emond, 2007). Greater water loss corresponds to a loss of visual quality for CTRL samples (as reported in Table 2), mainly due to a loss of leaf turgor. Similar results were noted by Cefola et al. (2016), who observed approximately 18% weight loss in air-stored broccoli raab, significantly higher than that of samples packaged using polypropylene/polyethylene terephthalate (PP/PET) and microperforated polypropylene/polyamide (PP/PA MF), leading to greater quality loss. Lucera et al. (2011) confirmed this, showing losses of up to 40% for air-stored samples compared to 3% for packed "cima di rapa". The use of biodegradable films with high water vapor transmission rate caused a very high percentage of weight loss, highlighting the importance of selecting an appropriate plastic material (Conte et al., 2011). Similar effects of MAP application were observed by Jia et al. (2009) when storing broccoli florets, with weight losses of MAP-packed samples below 4% after 23 d of storage, indicating that maintaining a high relative humidity within packages is crucial for product weight retention.

Ascorbic and dehydroascorbic acid were significantly affected by

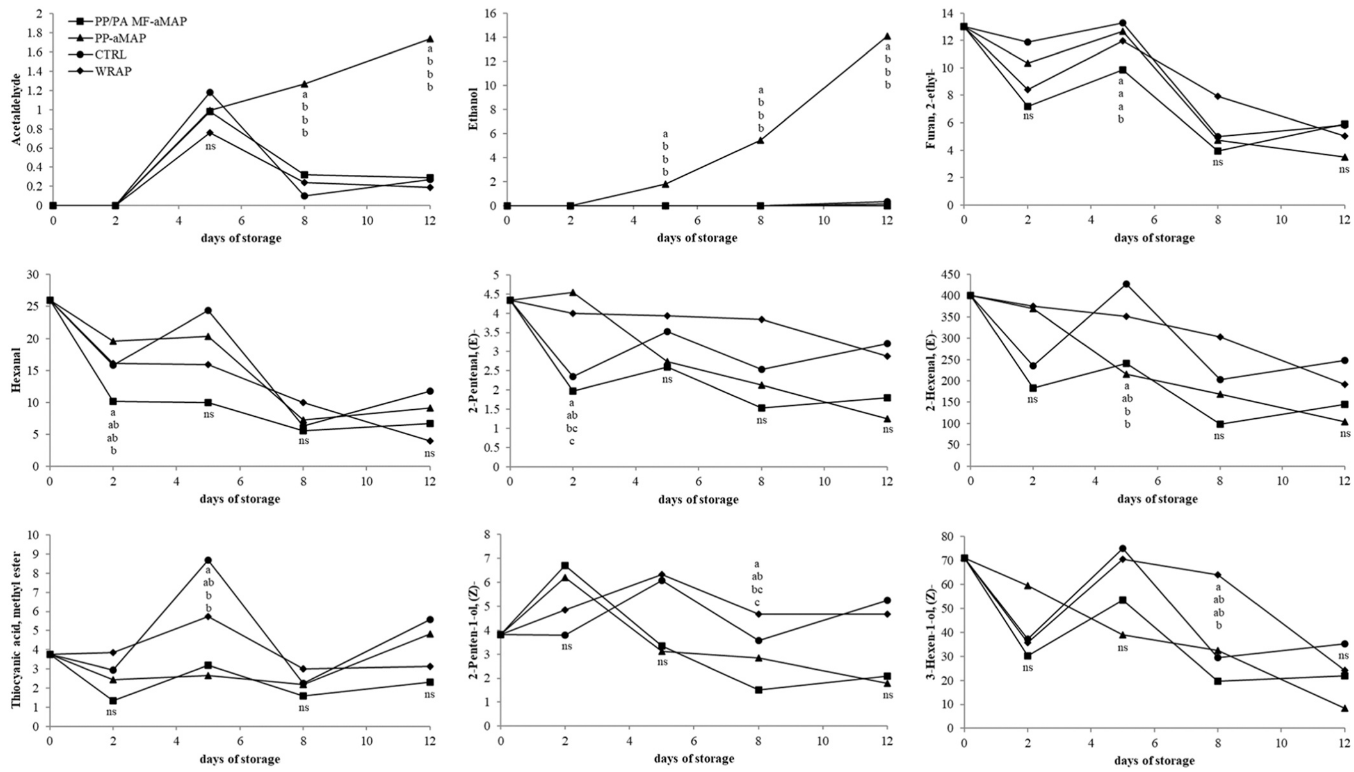

Fig. 3. Volatile organic compounds content expressed as IS-normalized peak area over storage time of minimally processed broccoli raab as affected by packaging condition. Data are means of three replicates for each sampling time. Means with different letters at the same time of storage are significantly different according to Tukey's test (P value ≤0.05), ns=not significant.

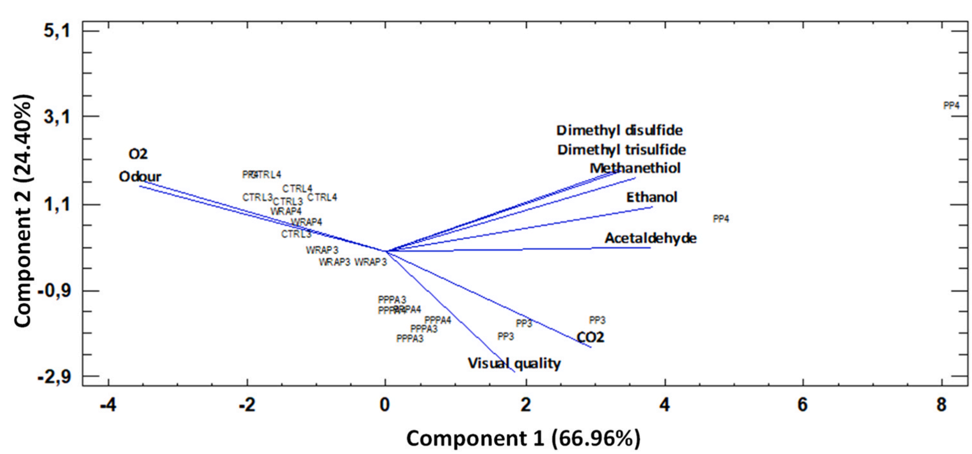

Fig. 4. PCA of the off-odor related volatiles of broccoli raab at 8d (CTRL3, PP3, PPPA3, WRAP3) and 12d (CTRL4, PP4, PPPA34, WRAP4) of storage, including the O_2 and CO_2 concentrations within the bags and sensorially evaluated odor and visual quality. The three replicates for each sample are reported with the same name.

packaging condition (Table S1, Fig. 2). A physiological reduction in AA was observed up to the end of storage, as also observed by Conversa et al. (2016). By the 12th day, differences between samples became significant, with CTRL and PP/PA MF-a MAP samples retaining the highest content of this substance. An increase in DHAA was observed by the end of the storage period, as previously reported by Cefola et al. (2010a) for "cima di rapa" stored in controlled atmosphere. These results can be partially ascribed to the greater weight loss of the CTRL sample, leading to a concentration of AA in plant tissue (Fernández-León et al., 2013). Cefola et al. (2010a) also observed that a controlled atmosphere did not affect total phenolic content and antioxidant activity throughout storage time, confirming that their behaviour was independent of the gases concentration of the surrounding atmosphere.

4.2. Volatiles and sensory determination

Besides the visible attributes used by consumers to assess the freshness of broccoli raab, such as visual quality, the odor within the packaging bags holds particular significance, and it is interesting to study its relationship with the content and changes over time of volatile compounds. Generally, the presence of microperforated packaging has been shown to influence the modification patterns of volatile compounds in fresh horticultural produce during storage (Boonthanakorn et al., 2020; Rux et al., 2017).

The characteristic *grassy* and *green* aroma of "cima di rapa" is given by the presence of active molecules from C18 fatty acids, along with sulfur components, primarily isothiocyanates and nitriles (Table 4). The detected volatiles generally align with those found in Brassicaceae-like leafy vegetables, where sulfur compounds are well represented. These sulfur-containing volatiles are linked to cell deterioration in lipid membranes and the loss of intracellular compartmentalization (Chen et al., 2019). The identified volatile organic compounds were all recognized as major contributors to the aroma and odor of *Brassica* species (Buttery et al., 1976).

The gaseous atmosphere has been proven to strongly impact the development of volatile compounds during storage, following previous findings (Makhlouf et al., 1989; Di Pentima et al., 1995). Results suggest that microperforation prevent the excessive increase of volatile sulfur compounds. Moreover, broccoli contains a great amount of sulfurous glucosinolate, similarly to other vegetables belonging to *Brassica* species, and it has been demonstrated that bacteria and yeasts can produce dimethyldisulphide and subsequently methanetiol or other sulfur compounds using this substrate as a source of energy (Caleb et al., 2016). While there is limited information on the microbial contamination of minimally processed broccoli raab, it can be assumed that PP-aMAP samples had a higher degree of contamination.

Nevertheless, all cited experiments refer to *Brassica oleracea*, and to our knowledge, there is no comparative information on the composition and evolution of volatilome in relation to the type of packaging in “cima di rapa”. However, given that the same volatile compounds have been identified, it is plausible to assume that findings for *B. oleracea* are applicable to *B. rapa*. For instance, storing broccoli florets within PP bags, led to an increase in dimethyl disulfide and dimethyl trisulfide compared to other different packaging types and air-stored samples (Jacobsson et al., 2004a; 2004b).

The absence of statistically significant differences between several volatiles compound after 12 d of storage (Fig. 3) was probably due to the cited instability and variability of plant tissue. Moreover, the consistent trend for volatile compounds linked to typical broccoli raab odors and flavors, suggests that the packaging condition does not have such an impact on this aspect of the product. However, a clear trend was observed for off-odor-related compounds.

Ethanol and acetaldehyde increased in the presence of low O_2 concentrations, representing by-products of anaerobic fermentation. Moreover, an increased production of ethanol can also be related to stress experienced by plant tissue, possibly due to suboptimal gas concentrations (Hansen et al., 1992; Forney and Jordan, 1998). Similar results were reported by Caleb et al. (2016) who observed higher concentration of by-product of fermentation, such as ethanol and ethyl acetate, in non-perforated-packed samples. Several authors have noted a similar trend when lower O_2 and higher CO_2 concentration occur within fresh or fresh-cut leafy green vegetables stored within plastic bags, leading to off-odor (Luca et al., 2016; Nielsen et al., 2008).

As described by Hansen et al. (2001), broccoli florets stored in low O_2 environments may maintain a good appearance with green leaves, but exhibit significantly higher off-odor score, with sour/fermented note, attributed to increased concentrations of acetaldehyde and ethanol, with ethanol concentration typically ten times higher than those of acetaldehyde, as observed for broccoli raab. Dimethyldisulfide, dimethyltrisulfide, and methanethiol are generally recognized as responsible for off-odors (Nielsen et al., 2008). The production of dimethyldisulfide in anaerobic conditions occurs through the degradation of proteins containing cysteine (Derbali et al., 1998). Similar results were obtained by Jacobsson et al. (2004a; 2004b) for broccoli florets packaged using PP film compared to LDPE and PVC, resulting in a higher concentration of sulfur compounds in the sample due to significant differences in the atmospheres within the packaging materials during storage.

Caleb et al. (2016), explored the impact of MAP on the postharvest quality of fresh-cut broccoli (*Brassica oleracea* L.). They observed a notable increase in sulfur compounds and alcohols within the bags over storage time, as a function of packaging condition applied. Similar results were observed by Yuan et al. (2021) in their study on *Brassica rapa* subsp. *chinensis* (pak choi). The presence of micro-perforated film significantly affected the concentration of volatile compounds in the product. Specifically, ethanol content, associated with anaerobic metabolism, was higher in non-microperforated packaging compared to microperforated packaging. The authors attributed this difference to the low O_2 concentrations and accumulation of CO_2, which altered the respiration process from aerobic to anaerobic. This shift led to the production of ethanol and off-odors. These observations align with previous studies (Rux et al., 2017; Tyagi et al., 2020).

Regarding PCA biplot results, different packaging condition affected the development of volatile compounds: a correlation was observed between oxygen concentration and sensory evaluated odor, though not significant. Polypropylene-aMAP samples showed a correlation with off-odor related volatiles and fermentation by-products, along with a positive correlation with CO_2 accumulation. Similar results were obtained by Wang et al. (2019). They observed that the application of a suitable controlled atmosphere, with an optimal balance of oxygen and carbon dioxide, during the storage of *B. oleracea,* increased the efficiency of energy production, also improving stress resistance, and significantly extending the shelf life of broccoli heads. However, excessive concentrations of CO_2 enhanced glycolysis and fermentation, altering the metabolism of sulfur compounds, thus resulting in the accumulation of unpleasant odors in the samples. The higher the CO_2 concentration, the more pronounced the development of unpleasant odors became. While shrink wrapped samples showed lower development of unfavorable volatiles compounds, they were accompanied by a deterioration in the external quality of the product. These findings should be taken into consideration in packaging design aimed at prolonging the shelf life of minimally processed broccoli raab.

5. Conclusion

The sensory quality and development of volatile compounds in minimally processed broccoli raab were influenced by both packaging conditions and storage duration, with these factors linked to the gas concentration inside the packaging. The utilization of polypropylene/polyamide microperforated material in combination with active MAP proved to be the most effective in preserving the visual quality of the product for up to 12 days of storage at low temperatures, without any adverse effects on chemical and nutritional attributes. Additionally, the presence of a plastic film helped regulate water loss from the "cime di rapa".

Considering all the findings, the application of active MAP with a composition of 3% O_2 and 97% N_2 within PP/PA MF bags was found to maintain the overall quality of fresh-cut broccoli raab, without causing the formation of harmful volatile compounds. This research can be used as a reference for further investigation into the volatile compounds of broccoli raab. It offers initial insights into the content of the product, and how MAP affects it. This technology is widely adopted and can be easily implemented at the company level. Although there may be a slight increase in costs associated with using this packaging for ready-to-use broccoli raab, consumers could be ready to accept it. Consumers hold a particular interest in this traditional product, and their willingness to pay may increase for a well-preserved product that retains its freshness and organoleptic properties unaltered throughout storage.

CRediT authorship contribution statement

Bernardo Pace: Supervision, Methodology, Data curation. **Michela Palumbo:** Methodology, Formal analysis, Data curation. **Maria Lucia Valeria de Chiara:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Cefola:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Maria Luisa Amodio:** Supervision, Data curation, Conceptualization. **Giancarlo Colelli:** Supervision, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Author Agreement Statement

The Corresponding Author declares that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

The Corresponding Author confirms that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

The Corresponding Author further confirms that the order of authors listed in the manuscript has been approved by all of us.

The Corresponding Author is the sole contact for the Editorial process. She is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2024.112961.

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