Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage

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ABSTRACT

It is known that garlic bulbs preserved with traditional methods undergo considerable losses, ranging from 25 to 40%. A frequent cause of these losses is associated with the development of pathogenic fungi, such as those of the genus *Fusarium*. The effect of ozone on post-harvest garlic bulbs was evaluated.

Garlic cloves inoculated with *Fusarium proliferatum* F21 and F22 strains, were exposed to a continuous gaseous ozone flow (2.14 μg m^{-3}), during 4 days, 20 h a day. After ozone-treatment, the garlic samples were moved at 22 °C to mimic retail conditions (shelf life). The changes in several quality parameters such as fungal decay and aroma were evaluated on garlic samples, as whole bulbs, cloves with and without tunic, through a sensorial descriptive test, SPME analysis in GC/MS and microbiological approaches. The data collected showed that ozone treatment did not affect the aromatic profile of garlic. A significant detrimental effect of ozone treatment on garlic decay was observed. Our results encourage the use of gaseous ozone treatment for containing garlic fungal decay during its storage.

1. Introduction

Garlic, *Allium sativum* L., is an herbaceous, perennial, bulbous, and highly odorous plant belonging to the *Liliaceae* family, known and appreciated all over the world. Currently, the world's annual garlic production is around 28,51 million tons with a harvested area of 1,55 million hectares (FAO Statistical Division, 2018). To meet the needs of local and international markets, a large quantity of garlic production is stored. Commonly, bulbs are kept at room temperature (20–30 °C), if marketed within a short period, or at lower temperatures when the storage period is longer. Traditional garlic storage methods do not always guarantee high-quality products. Tripathi and Lawande (2006) reported that total losses during the bulbs storage under ambient conditions ranged from 25 to 40%, with as consequence the increase of the price and decrease of the local availability.

Losses are associated mainly with physiological disorders and pests. Among the most important pathogens are fungi, including *Aspergillus niger*, *A. ochraceus*, *Botrytis alli*, *Fusarium oxysporum*, *F. proliferatum*, *Penicillium purpurogenum*, *Rhizopus stolonifer* and *Stemphylium botryosum* (Valdez et al., 2009; Palmero et al., 2013).

The use of post-harvest technologies, such as irradiation, can contain microbial development, consequently reducing conservation problems. This technique, approved by 38 countries around the world, although it has proven more effective than traditional ones, is applied to less than 1% of total garlic production (Tripathi and Lawande, 2006). In recent years, several studies have supported the use of ozone as an efficient, inexpensive, and easy-to-use alternative to common storage methods (Cao et al., 2018; Kim et al., 1999, Mohd Aziz and Ding, 2018; Horvitz and Cantalejo, 2014; Vettraino et al., 2019a,b).

There are various types of ozone generators, both for the application of ozone in gaseous or aqueous phases, which allow different uses both in the field of disinfection and sanitation of drinking water or wastewater in various contexts (urban, hospital, industrial, etc.), and also of surfaces, environments or food products. The safety of products treated with ozone and the treatment efficacy have long been studied. In 1997, the use of ozone was recognized as safe (GRAS) and twenty years ago, ozone was approved for direct food contact (US FDA, Secondary Direct Food Additives Permitted in Food for Human Consumption). Already in 1948, Schomer and McColloch experimented treatments with ozone gaseous on apples. Later, ozone treatments, were applied to small fruits such as blueberries, strawberries and table grapes (Spalding, 1968).

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The efficiency of ozone treatments is related to several factors, including temperature, application time, pH, type and physiology of the treated product. Thus, specific ozonation methods should be developed for different hosts (Horvitz and Cantalejo, 2014).

Our work aimed to verify the effectiveness of gaseous ozone treatment for containing the decay of garlic by *F. proliferatum*, one of the most frequent causal agent of garlic clove rot, evaluating, at the same time, the preservation of its aromatic peculiarities, through a chemical and sensory approach.

2. Materials and methods

2.1. Plant material

In July 2019, a total of 2 kg of fresh garlic bulbs of the variety Morado de Cuenca, produced from the same company, were purchased from the local market and checked for the presence of disease symptoms. Three sub-samples of 10 (+/-2) bulbs were randomly chosen and used for all analyzes.

2.2. Microbiological analysis

2.2.1. Isolation and identification of F. proliferatum

A total of 10 diseased cloves were peeled and then surface disinfected (70% alcohol for 30 s, rinsed in sterile distilled water three times and air dried aseptic under conditions to remove surface water). Symptomatic tissues were treated as described by Vettraino et al. (2019a). Briefly, symptomatic cloves were cut in small pieces (5 × 5 mm). Five fragments, randomly selected were plated onto PDS (Potato dextrose agar (Oxoid, UK, 39 g/L−1) amended with streptomycin sulfate (0.06 g/L−1). Plates were incubated at 21 °C. Colonies resembling *F. Proliferatum* were single spore purified and identified on the base of microscopic observations (Eslie and Summerell, 2006). Two strains, F21 and F22, were further identified through molecular techniques. Rapid extraction of fungal DNA and PCR amplification were conducted as previously described, using primer sets ITS1/ITS4 and EF1/EF2 (Vettraino et al., 2005). The sequences obtained were analyzed by BLAST, for comparison against the GenBank non-redundant (NCBI-nr) database (http://blast.ncbi.nlm.nih.gov). Phylogenetic trees were constructed by the neighbor-joining method based on Kimura's two-parameter model with bootstrap values based on 1,000 replications (Saitou and Nei, 1987) using PHYLIP (Felsenstein, 1989). Trees were drawn using Tree View (Page, 2001). *Fusarium oxysporum* (Accession numbers: MK629372 for ITS and KM886216 for EF-1α), *F. proliferatum* (Accession numbers: MN594807 for ITS and KM873334 for EF-1α) and *F. equiseti* (Accession number: MG594514 for ITS and KM886212 for EF-1α) were used for the phylogenetic analysis.

2.2.2. Pathogenicity tests

Pathogenicity to garlic cloves was determined using F21 and F22 isolates. Apparently healthy cloves were sterilized as above described. Thirty cloves were injured using a 5 mm diameter cork bore to a depth of 4.5mm and the wound was inoculated with a PDA plug supporting mycelia taken from the margin of a 7-day-old culture. Cloves inoculated with sterile PDA served as controls. Inoculated and control cloves were placed in sealed plastic boxes and incubated at 25 ± 2 °C for 2 weeks (Dugan et al., 2007). *Fusarium proliferatum* was re-isolated to fulfil Koch's postulates.

2.3. Ozone treatment

Garlic samples were placed in glass jars (5L volume) and exposed to a continuous flow of 2.14 µg m^{-3} of gaseous ozone in the air, for 4 days, 20 h a day (T = 4.0 ± 1.0 °C and 90 ± 5% RH). An ozone generator (C32-AG, Industrie De Nora Spa, Milan, Italy) equipped with an oxygen concentrator (nominal production capacity of 32 g of ozone h^{-1}) was used to produce gaseous ozone. The ozone concentration in the cold room was constantly monitored by a UV-11 photometric ozone analyzer (BMT 146 Messtechnik Gmbh, DE).

Table 1. Experimental scheme ozone and air treatments.

	4 °C		20 °C
Garlic samples	Ozone	Air	Air
Bulbs	WOC	WAC	WAR
Cloves	BOC	BAC	BAR
Cloves without tunics	NOC	NAC	NAR

2.4. Sensory and volatile compounds evaluation

Garlic samples were analyzed as whole bulbs (W), intact garlic cloves (B) and garlic cloves without tunics (N). Each sample was stored both at room temperature (R) and 4 °C (C). Besides, the refrigerated samples (4 °C) were subjected to treatment with ozone (O) and air (A). The experimental plan is detailed in Table 1.

2.5. Effect of ozone on postharvest decay

Cloves were peeled and those apparently healthy were arranged in 6 groups: a) control cloves, wounded but not inoculated and kept at 22 °C (C1); b) cloves, wounded but not inoculated, treated with ozone and then kept at 22 °C (R2); c) cloves, wounded and inoculated with *F. proliferatum* F21, treated with ozone and then kept at 22 °C (FO1); d) cloves, wounded and inoculated with *F. proliferatum* F21 and then kept at 22 °C (F1). e) cloves, wounded and inoculated with *F. proliferatum* F22, treated with ozone and then kept at 22 °C (FO2); f) cloves, wounded and inoculated with *F. proliferatum* F22 and then kept at 22 °C (F2). Treatments were arranged in a completely randomized design. For each treatment a total of 15 cloves, free of damages and homogeneous size, were selected. Cloves were weighted using a technical balance (Adam Equipment Co. Ltd., Milton Keynes, UK). The percentage of weight loss (WL) at the end of the experiment was determined according to formula (1):

$$WL = (W0 - Wt/W0) *100 \qquad (1)$$

where W0 is the initial sample mass and Wt is the sample mass at time t. After 2 weeks, rot severity of garlic clove was estimated on the base of a visual scale according to Palmero et al. (2013), with some modifications, where: N1 = no symptoms; N2 = 1–69% rotted clove; N3 = more than 70% rotted clove.

Rot severity was calculated according to the following formula (2):

$$RS = (N1 \times 1) + (N2 \times 2) + (N3 \times 3)/ \text{ Number of total cloves} \qquad (2)$$

2.6. Analysis of volatile compounds by SPME-GC/MS

Extraction and concentration of head-space volatile compounds were carried out by solid-phase micro extraction (SPME) (De Santis and Frangipane, 2010). SPME holders and coating fibers used were obtained from Supelco (Bellefonte, PA, USA). For SPME sampling, one SPME device (50/30µm DVB/CAR/PDMS: divinylbenzene/carboxen/polydimethylsiloxane) was used. Prior to use SPME fiber was conditioned by heating in the injection port of a gas chromatograph at 250 °C for 30 min in order to remove traces of contaminants. Prior to analysis, a fiber blank was run to confirm the absence of contaminant peaks. To optimize SPME conditions such as the most suitable temperature and equilibration time to obtain a significant headspace fraction, were adjusted.

Two cloves of garlic were crushed, the obtained pulp was immediately placed in a 20 mL vial then capped with an aluminum cap. The SPME fiber was exposed to the headspace above the sample for 30 min at room temperature. After adsorption time, the SPME fiber was removed from the sample vial and immediately inserted into the injection port of the GC–MS system where thermal desorption was performed at 250 °C for 2 min.

GC-MS PerkinElmer Clarus 500 instrument equipped with flame ionization detector (FID) was used for chemical analysis.

Chromatographic separations were performed on a Restek Stabilwax fused-silica capillary column (length 60 m × 0.25 mm ID x 0.25 μm film thickness).

The oven temperature program was as follows: 50 °C, then a gradient of 6 °C/min to 220 °C (20 min). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. Split injection with a split ratio of 1:20 was used. The electron-impact ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200 °C; scan mode, 40.0–400.0 mass range. Mass spectral identification of the volatiles was carried out by comparing spectra with those in the NIST and Wiley mass spectral libraries. Furthermore, linear retention indices (LRIs) of each compound were calculated using a mixture of *n*-alkanes hydrocarbons (C8–C30, Ultrasci) injected directly into GC injector at the same temperature program reported above. The data were obtained by normalizing the peak area generated in FID (%) without using corrections factors (RRFs). All analyses were repeated twice.

2.7. Sensory analysis

2.7.1. Selection descriptive sensorial attributes

During three collective sessions (1h each), in air-conditioned (20 °C ± 2) departmental sensory lab (ISO-8589,1988), eight expert sensory assessors (2 males and 6 females, aged between 23 and 58 years), previously selected, trained and monitored, according to ISO standards (ISO-8586-1, 1993), received five different types of garlic, purchased in a local market, to developing a unanimous description of the flavour. An informed consent was obtained by all assessors, and relevant ethical standard were abided by for our sensory experiments.

The list of the related sensory flavor attributes was developed by the evaluators to build up an unanimous description of the flavour (ISO-13299, 2016), during three collective sessions of about 1 h each, using the consensus technique (De Santis and Frangipane, 2020; Rossini et al., 2016). Table 2 shows the definition of ten carefully chosen attributes of the garlic lexicon.

The selective sensory attributes of the profile sheet were assessed by all judges giving a score from 0 (null) to 9 (maximum).

Furthermore, to avoid problems related to subjectivity in the use of the intensity scale and to difficulty in finding the reference standards of garlic aroma was provided a definition of attributes of the profile sheet

Table 2. Lexicon for odor description of garlic samples studied in this research.

	Attributes	Definition
Aspect cloves	Colour	Intensity of coloring
	(1–5)	
Odour	Intensity	Intensity of flavour
	(1–9)	
	Pungency	The sensation of irritation of the epithelium inside the nasal cavities
	(1–9)	
	Persistence	Recovery time of the neutral smell condition
	(1–9)	
	Herbaceous	Aromatics associated with uncooked vegetables
	(1–9)	
	Balsamic	Odor reminiscent of conifer leaves
	(1–9)	
	Sulphurous	An aroma reminiscent of sulfur
	(1–9)	
	Hearty	An aroma that has a damp and earthy character similar to fresh mushrooms
	(1–9)	
	Vinegarish	A sour-smelling liquid containing acetic acid
	(1–9)	
	Cooked onion (1–9)	Smell associated with cooked but not burnt onion

(Table 2), and garlic samples were tested according to a procedure developed in this study and described below. This procedure was adopted with the main aim to verify any changes, on treated garlic samples, imposed by the storage treatments. All garlic samples were evaluated twice by the judges, one day apart.

2.7.2. Samples preparation and serving

Selected descriptors were used to draw up the profile sheet, provided to the judges for the evaluation of the garlic samples (Table 2).

An aliquot of 3/4 of cloves, randomly chosen from each thesis, separated into cloves, completely devoid of tunics (if any), were placed in frozen polyethylene bags. Each bag was placed in small plastic containers, closed with a lid, and then distributed to the judges for the evaluation of the aroma. Before each test, each judge proceeded to crush the cloves of garlic (about 5 g), closed in the bags, to activate the formation of aromas.

2.7.3. Test design and samples evaluation

Due to the difficulty of finding adequate standards for some specific descriptors of the garlic, the descriptive test was modified as follows.

The judges were given the garlic cloves simultaneously, in closed containers, as described above.

In each set, the untreated control fresh sample (CK) was the first sample. It was tested simultaneously by all judges, under the guidance of the panel leader, and taken as a standard reference for evaluating the sensory attributes of all garlic samples in each set. Assesses of the CK samples were excluded from processing. Subsequently, each judge individually proceeded to evaluate other garlic samples according to the experimental plane.

Whole garlic bulbs samples, as well as the cloves with and without tunics, were evaluated separately, on the same day, with an interval of 30 min between one session and the next.

The whole test was replicated the next day.

2.8. Statistical analysis

The Shapiro-Wilk test of normality was used to determine whether the data were normally distributed. ANOVA, with a post hoc HSD Tukey Test, were performed to determine significance between different treatment groups. P levels of <0.05 were considered significant. The data of disease incidence and severity of clove rot (%) were analyzed using Excel software (Microsoft, US). A Principal component analysis was conducted to investigate the correlation among the chemical and sensorial data. An ANOVA test was performed to discard the no-significant variables thus reducing to a 13-items matrix. The Principal Component Analysis (PCA) was used to determine the most useful descriptors for sensory and chemical differentiation of the samples, and it was carried out on sensory and GC/MS data. The statistical analysis was carried out using a statistical analysis software (XLSTAT premium, Addinsoft, Paris).

3. Results

3.1. Ozone effectiveness on F. proliferatum garlic infection

Collected diseased garlic bulbs showed visible symptoms consisting in dark purple spots distributed on the surface. From garlic tissues, a total of 10 white to light pink colonies were obtained and were single-spore purified. Isolates produced walled and straight macroconidia measuring around 29.1–87.3 μm × 2.5–3.5 μm. The apical cell was curved and formed with 3–5 septa. One-cell microconidia were oval to obovoid in shape measuring around 3.7–37.2 μm × 1.1–4.6 μm. Chlamydospores were absent.

Isolates F21 and F22, showed 100% identity with *F. proliferatum* (Accession numbers MN594807 for ITS and KM873334 for EFtef1-α genes). The phylogenetic clustering indicated that taxa F21 and F22 formed a distinct cluster together with *F. proliferatum* (Figure 1 A, B).
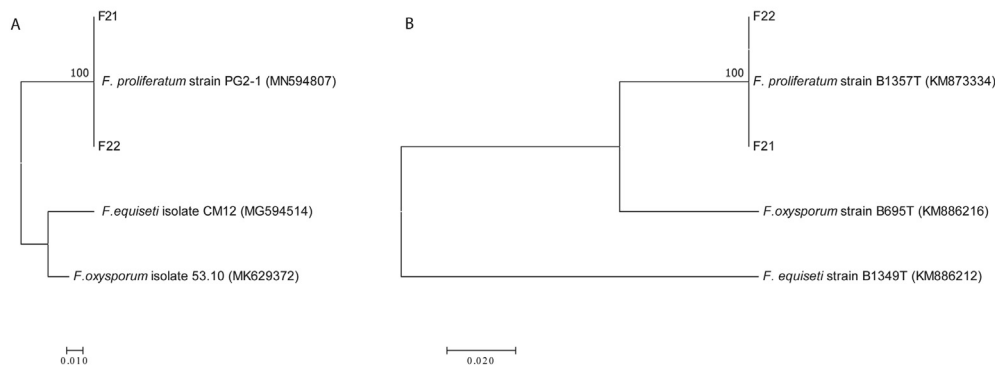
Figure 1. Neighbour-Joining (NJ) tree based on a concatenated alignment of ITS (A) and EF-1 α (B) genes of *F. proliferatum* F21 and F22 from garlic cloves and other *Fusarium* reference strains from NCBI database. The distances were determined by Kimura's two-parameter method. The numbers beside branches represent bootstrap values from 1000 replicates. Scale bar indicates 0.02 substitutions per site.

Pathogenicity tests showed that *F. profileratum* was very aggressive to garlic cloves, causing heavy brown decay areas around inoculation points. No significant differences in virulence were observed between treatments F21 and F22 (t-test; P > 0,05). Thus, data from the treatment with the two isolates were pooled. Similarly, values from C1 and R2 were combined (t-test; P > 0,05). On inoculated cloves, the necrotic spots were observed 4 days post-inoculation and enlarged over time resulting in a syrupy texture. No symptoms of rot were detected on control cloves. The exposure of cloves to 2.14 µg m^{-3} of O$_3$ for 4 days, 20 h a day, significantly decreased the decay incidence after 2 weeks of storage at 22 °C (Chi-square; P < 0,05). The disease severity index values from inoculated garlic cloves were significantly greater than in controls and ozone treated samples (ANOVA; P < 0,05).

3.2. GC/MS headspace analysis

All identified compounds by GC/MS analyses were listed in Table 3.

A total of seventeen components were identified and showed quantitative and qualitative differences between three garlic samples (cloves without tunics, cloves and bulbs) treated with ozone and untreated.

The untreated samples (NAR, BAR and WAR) had a similar chemical qualitative profile. Diallyl disulfide and disulfide, methyl 2-propenyl were the main compounds in all samples and remained so even after treatment with ozone. The SPME analysis highlighted the presence of some components in the treated samples, absent in the untreated ones.

The chemical composition of samples without tunics treated at 4 °C (NAC) and 20 °C (NAR) was very similar and the identified compounds were the same.

On the contrary, in NOC dimethyl 2-hydroxy-3-methoxysuccinate was missing while allyl mercaptan (0.12%) and methoxymethyl isothiacyanate (0.79%) were only found in this sample.

Unpeeled garlic cloves treated in air had the similar chemical composition but differed in the presence of acetic acid (2-propenylthio) (0.22%) and trimethyl-thiourea, (0.23%) when exposed at 20 °C (BAR). In BOC sample, butenal, (E) (0.16%), dimethyl sulfoxide (0.41%), acetic acid, hydroxy-, ethyl ester (0.68%) and benzo[h]cinnoline (1.47%) were found.

The chemical composition of WAC and WAR samples was superimposable with the exception of 1,2-dithiolane (0.57%) present only in WAR. On the other hand, some compounds, such as acetic acid 2-propenylthio (0.18%), acetic acid hydroxy-ethyl ester (0.26%), thiourea trimethyl, (0.08%) and benzo[h]cinnoline (4.24%), were only detected in WOC sample.

The obtained results show that the treatment with ozone alters the volatile chemical profile only in part, determining the appearance of some molecules absent in the untreated samples even if in rather small percentages.

Regarding the different stored temperatures (4 °C and 20 °C) used, the GC-MS analyses displayed no differences of the chemical profile according to the data obtained by sensory analyses.

3.3. Garlic samples sensory profile

Results of the descriptive tests conducted on the garlic samples clearly showed that the aroma is best characterized by organosulfur compound, *vinegarish*, and *cooked onion*, in addition to the more generic attributes of *aromatic intensity*, *persistence* and *pungency* (Table 4, P < 0.05). The selected attributes, used to describe the sensory characteristics of garlic, can well highlight the differences between samples analyzed. The ozone treatment slightly affected the aroma of garlic based on the type of product (bulbs, cloves with or without tunics) (Figure 2). In all bulbs, ozone-treated garlic (WOC) showed a slight reduction in some aromatic notes and the overall aroma (Table 4, P < 0.05, Figure 2). Particularly, all garlic bulbs showed a more pronounced *vinegarish* note, while the *cooked onion* aroma was less perceived. Moreover, a slight reduction of the overall *aromatic intensity* and *sulphurous* note was also perceived.

The differentiation in the descriptors found in all the ozone-treated bulbs were confirmed, although to a softly lesser extent, also in the treated cloves (BOC) (Figure 2). Conversely, the treated cloves without tunic (NOC) (Figure 2) reacted differently to the treatment, probably due to a more oxidizing effect.

Any of the descriptors determined differences between garlic samples stored at 4 °C and room temperature, regardless of the sample types analyzed (bulb, cloves with and without tunics).

Overall, the sensory analysis of the products clearly showed that the ozone treatment maintained a similar aroma in both treated and untreated garlic samples. Mainly the treatment brought a slight reduction of intensity, sulfur, and cooked onion notes, and a moderate increase in the vinegarish aroma.

3.4. Principal component analysis

The Principal Component Analysis (PCA) was used to determine the most useful descriptors for sensory and chemical differentiation of the samples, and it was carried out on sensory and GC/MS data.

Table 3. Chemical composition (%) of GARLIC (bulbs).

No.[1]	COMPONENT[2]	LRI[3]	LRI^lit[4]	NAC	NOC	NAR	BAC	BOC	BAR	WAC	WOC	WAR
1	thiirane, methyl	870	875	0.96	0.57	1.71	0.79	0.68	1.09	0.95	0.73	0.9
2	allyl mercaptan	889	891	-	0.12	-	-	-	-	-	-	-
3	2-butenal, (E)-	1030	1034	-	-	-	-	0.16	-	-	-	-
4	diallyl sulfide	1145	1143	0.98	1	0.92	1.06	0.99	0.96	1.04	1.07	1.06
5	thiophene, 3,4-dimethyl-	1250	1253	1.43	0.84	1.57	0.91	0.66	1.78	1.13	0.71	0.7
6	disulfide, methyl 2-propenyl	1277	1281	2.04	3.03	2.45	2.06	2.09	2.61	1.71	1.43	1.43
7	disulfide, methyl 1-propenyl	1288	1292	0.34	0.34	0.32	0.21	0.19	0.48	0.2	0.14	0.11
8	1,2-dithiolane	1350	*	0.2	0.32	0.37	0.37	-	0.29	-	-	0.57
9	diallyl disulfide	1470	1475	88.95	90.52	84.54	92	89.52	86.94	92.74	88.27	90.73
10	dimethyl 2-hydroxy-3-methoxysuccinate	1500	*	1.11	-	1.71	0.89	1.02	1.28	0.81	1.2	1.06
11	methoxymethyl isothiacyanate	1528	1533	-	0.79	-	-	-	-	-	-	-
12	dimethyl sulfoxide	1558	1560	-	-	-	-	0.41	-	-	-	-
13	acetic acid (2-propenylthio)-	1620	*	0.15	0.26	0.23	-	0.14	0.22	-	0.18	-
14	acetic acid, hydroxy-, ethyl ester	1650	*	-	-	-	-	0.68	-	-	0.26	-
15	thiourea, trimethyl-	1669	*	0.14	0.1	0.17	-	-	0.23	-	0.08	-
16	3-vinyl-1,2-dithiacyclohex-4-ene	1751	1750	3.71	2.11	6.01	1.71	-	4.12	1.42	1.7	3.32
17	benzo[h]cinnoline	1775	*	-	-	-	-	1.47	-	-	4.24	-
	Total identified (%)			100	100	100	100	100	100	100	100	100

[1] Indicates the compound identification number.
[2] The components are reported according their eluition order on polar column.
[3] Linear Retention indices measured on polar column.
[4] Linear Retention indices from literature.
* Not available.

Three main components reached eigenvalues greater than 1, and together explained more than 96% of the overall variance. As shown in Tables 5 and 6, the sensory *Persistence* (4.21%) and the chemicals *diallyl disulfide* (63.28%), *3-vinyl-1, 2-dimethylcyclohex-4-ene* (23.18%) contributed to the PC1, *Intensity* (15.08%), *Spiciness* (15.89%) and *Cooked onion* (35.65%), as sensory parameters, and *3-vinyl-1, 2-dimethylcyclohexes-4-ene* (23.14%), as a chemical component, contributed to the PC2, at last, the sensory *Vinegarish* (35.15%) and the chemical compound *3-vinyl-1, 2-dimethylcyclohex-4-ene* (23, 14%) mainly contributed to the third component.

The two bi-plots (Figures 3 and 4) showed the distribution of the products according to two parameters, the preparation of the sample (W, bulbs, B, cloves, and N, cloves without tunics) and the method of conservation (O, ozone, AC, air-4°C, and AR, air-room temperature).

As already emerged from the separate analysis of chemical and sensory data, all the samples treated with ozone acquired a slightly modified aromatic profile (mainly an increase in the vinegar sensory note and a slight reduction in the sulfurous notes), however more similar to the natural product, than that of the untreated clove kept in the air (BAR), or the one without tunics also refrigerated (NAC, NAR).

4. Discussion

Garlic is an important spice commodity consumed worldwide almost every day in every home. This spice, especially in some countries, due to their gastronomic traditions, is used daily in the kitchen, and it is, therefore, essential to guarantee the daily offer on the market. However, garlic harvesting is mostly annual, and therefore, an effective garlic storage system becomes inevitable to ensure regular supplies to consumers with high quality products, free from fungal decay (Valdez et al., 2009; Palmero et al., 2013).

Among the treatments proposed to contain the deterioration of the crop, during post-harvest storage, one of the most promising is certainly the use of ozone, which, even in combination with low-temperature storage or packaging, can positively influence the extension of the shelf life of fruit or vegetables (Karakosta et al., 2019; Panou et al., 2018).

The approval of the US FDA as a food additive (US FDA, 2001), has led to the expansion of the field of application of ozone, evaluating, among other things, the effectiveness in the control of fungal infections on various plant matrices, both by treatment as ozonated water and gaseous ozone (Akbas and Olmez, 2007; Aguayo et al., 2006; Venta et al., 2010; Karaca and Velioglu, 2020).

Table 4. Sensory evaluation of garlic samples (means and standard deviations).

Samples	Intensity	sd	Pungency	sd	Persistence	sd	Sulphurous	sd	Vinegarish	sd	Cooked onion	sd
WOC	5.67b	0.52	4.83a	0.75	4.67b	0.52	3.67b	0.52	4.67a	0.52	3.67b	0.52
WAC	6.67a	0.52	5.05a	0.82	5.17a	0.75	5.00a	0.63	2.67b	0.82	5.67a	0.82
WAR	6.50a	0.55	4.90a	0.55	5.50a	0.52	5.33a	0.52	3.00b	0.89	5.83a	0.98
BOC	4.17b	0.75	2.50a	1.22	4.50a	0.55	4.20a	0.63	3.80a	0.55	2.45b	0.75
BAC	5.17a	0.75	2.83a	0.75	4.33a	0.52	4.90a	0.75	2.50b	0.54	3.33a	0.66
BAR	5.33a	0.52	3.20a	0.52	3.67b	0.82	4.50a	0.55	2.50b	0.62	3.67a	0.59
NOC	4.37a	0.48	3.00a	0.84	3.50a	0.77	5.00a	0.63	2.05a	0.77	3.50a	0.55
NAC	4.38a	0.56	2.90a	0.73	3.83a	0.88	4.67b	0.88	1.83a	0.41	4.00a	0.63
NAR	4.35a	0.49	2.83a	0.74	3.00a	0.74	4.50b	0.55	1.67a	0.52	4.83a	0.75

Different lowercase letters indicate significant difference among treatments (P < 0.05).
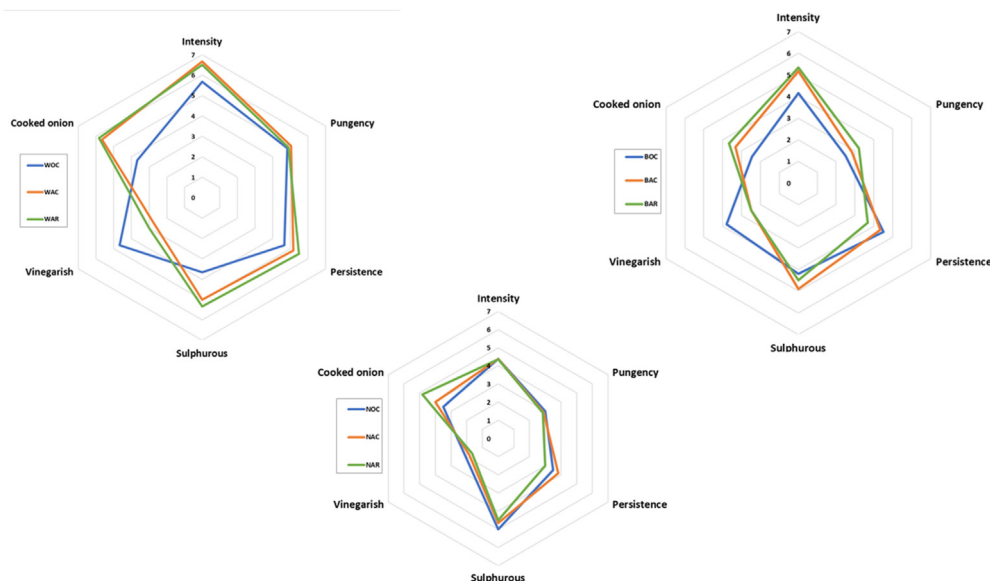
Figure 2. Sensory profile of garlic samples. Whole bulbs (WOC. WAC, WAR), intact garlic cloves (BOC, BAC, BAR), and cloves of garlic without coats (NOC, NAC, NAR), labeled according to Table 1.

The abundance of scientific publications on this subject provides clear evidence of the interest and consequently of the numerous applications that ozone has had in the food sector.

However, almost all the studies have mainly evaluated the effectiveness of the ozone treatment to enhance the shelf-life and safety of food products and, more rarely, the changes in the aromatic profile and the efficacy on fungi decay.

To our knowledge this is the first study that investigated the effect of gaseous ozone treatments on garlic in postharvest conditions, focusing on the decay caused by *F. proliferaum* and changes in the chemical and sensory profiles. Ozone treatment was effective in controlling decay development, confirming previous studies on other pathogen-host binomials (Vettraino et al., 2019 a,b; Kim et al., 1999). It is worthy to notice that pathogen was not killed, probably because protected within the tissue or due to the interaction between ozone and host (Elshahawy, Saied, N.M., Morsy, 2017). *Fusarium proliferatum* strains collected from garlics has been proved to produce a broad range of toxins, such as fumosins, fusaric acid, B1, B2, and B3, which may pose a risk for food safety (Galvez et al., 2017). Ozone is an oxidizer and reacts with the mycotoxin molecules changing their structures and forming less toxic products (Afsah-Hejri et al., 2020). In our laboratories, further studies are ongoing to investigate the influence and the mechanisms of ozone treatments on the degradation of mycotoxins in garlic and other products.

Gaseous ozone (2.14 µg m^{-3} for 20 h day for 4 days) slightly affected the sensory profile of garlic. Similarly, Song et al. (2000) stated that ozone treatment on onions caused a mild reduction of the pungent note, and the intensity, without significantly altering the overall aroma profile.

Venta et al. (2010) in recent study on tomato exposed to O$_3$ (25 and 45 mg for 2 hours/day, for 16 days) reported of no influence on the sensory characteristics of the products, even if indirectly assessed through chemical and physical parameters.

Akbas and Ozdemir (2008) observed slight significant changes (P < 0.05) in the flavor, appearance, and overall palatability of the ozone-treated red pepper flake samples between 5.0 and 9.0 ppm, while, confirming our results, no significant changes (P < 0.05) were found between the score of taste, flavor, appearance and overall palatability of the samples treated with ozone between 0.1 and 1.0 ppm, for 360 min (2008).

The descriptive analysis carried out on garlic samples treated with ozone showed that, the sensory attributes, significantly (P < 0.5) discriminating for the thesis, were mainly *intensity, sulphurous, vinegarish,* and *cooked onion*, the others, although at various levels, not appeared to be affected by the treatment.

As reported by Block et al. (1993), the chemical profile of garlic is complex and characterized by over 100 different compounds that

Table 5. Contribution of the variables (%) to PCA.

	PC1	PC2	PC3
Intensity	2.416	15.078	4.780
Pungency	2.132	15.886	14.134
Persistence	4.212	2.563	5.548
Sulphurous	0.270	2.023	6.075
Vinegarish	1.471	1.747	35.153
Cooked onion	0.027	35.647	0.026
thiirane, methyl	0.689	0.638	0.022
diallyl sulfide	0.019	0.003	0.013
thiophene, 3,4-dimethyl-	0.889	0.280	0.804
disulfide, methyl 2-propenyl	0.559	1.448	5.352
diallyl disulfide	63.280	1.197	18.574
dimethyl 2-hydroxy-3-methoxysuccinate	0.858	0.353	1.938
3-vinyl-1,2-dithiacyclohex-4-ene	23.180	23.139	7.579
Eigenvalue	8.538	2.969	1.824
Variability (%)	61.604	21.420	13.159
Cumulative %	61.604	83.024	96.184

Table 6. Factor scores of garlic samples.

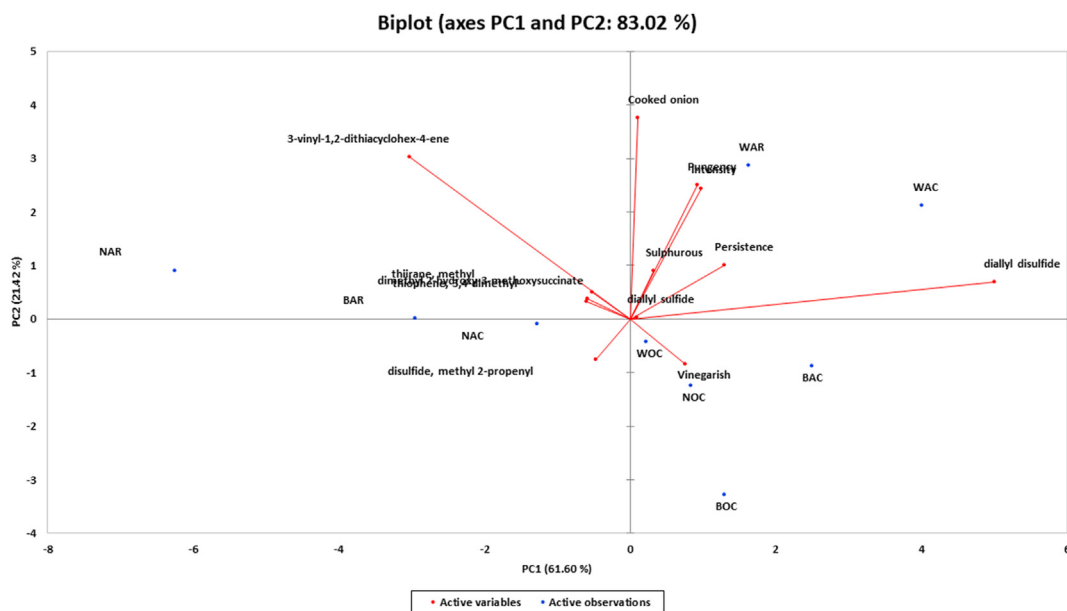|	PC1	PC2	PC3
WOC	0.215	-0.423	3.028
WAC	4.012	2.117	-0.11
WAR	1.623	2.865	0.47
BOC	1.298	-3.276	0.914
BAC	2.493	-0.871	-1.313
BAR	-2.951	0.007	0.158
NOC	0.84	-1.235	-1.726
NAC	-1.276	-0.092	-1.177
NAR	-6.255	0.909	-0.245

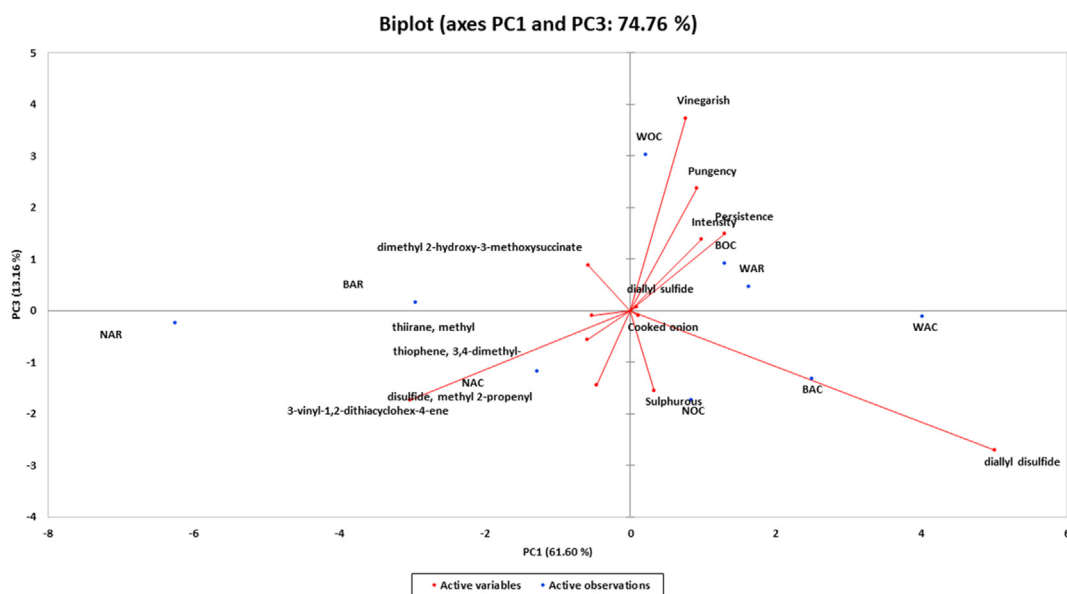**Figure 3.** Score loading bi-plot (PC1/PC2) for garlic samples.

Figure 4. Score loading bi-plot (PC1/PC3) for garlic samples.

contribute to its aroma and properties. Relevant and unique feature is its high content of organosulfur substances, which play a central role in defining its aromatic profile.

When the headspace is sampled by SPME, only compounds that are volatile, such as organosulfur components, can be trapped on the fiber. The ambient temperature was used in the adsorption (sampling) phase on the fiber to avoid artifacts. The SPME technique has already been used to discriminate the volatile profile in garlic, both after crushing and toasting with high-temperature treatments (Kim et al., 2001). Several sulfur-containing compounds, including diallyl disulfide as the main component, have been detected by SPME-GC/MS in elephant and normal garlic (Kim et al., 2018).

In this study, the SPME coupled GC/MS allowed us to appreciate small changes associated with ozone treatment on garlic. The SPME technique has also proven to be a particularly efficient, fast and sensitive tool to highlight the presence and small differences in the concentration

of organosulfur compounds typical of the aroma of garlic. It is worthy to note that Locatelli et al. (2014) also found slight variations in the SPME GC/MS analysis of organosulfur compounds, obtaining similar results on raw, microwave baked and steamed garlic. Based on these results, it is possible to hypothesize that the compounds that most characterize the aroma of garlic are fairly stable and not very sensitive to bulb treatments. The PCA confirmed these results.

5. Conclusion

Sensorial analysis supported by the obtained data by solid phase microextraction (SPME) combined with GC/MS analyses about chemical composition, clearly showed that the treatment with gaseous ozone, slightly modifies the aromatic profile of the garlic, without, however, producing an evident deviation, and therefore, without causing the loss of its flavoring capacity.

The value of this crop justifies the interest in a technique, as like the application gaseous ozone, able to reduce the post-harvest decay by *Fusarium proliferatum*, maintaining the best aromatic and functional characteristics of the bulbs.

Declarations

Author contribution statement

Diana De Santis, Anna Maria Vettraino: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Stefania Garzoli: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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