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Changes in the bioactive content and aroma profile of aged garlic with processing conditions

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ABSTRACT

The use of aged garlic (AG) for food supplement manufacturing benefits from the evaluation of how the processing conditions may affect its bioactive content and sensory profile. In this study, aging of untreated or crushed garlic bulbs under controlled temperature and humidity conditions (ActiveNature™ Technology) was evaluated for 0–40 days. To that aim, a multicomponent characterization including the LC-MS analysis of bioactive *S*-allyl-L-cysteine (SAC) and *S*-1-propenyl-L-cysteine (S1PC), the volatile profiling by HS-SPME-GC-MS, and the identification of key odourants by HS-SPME GC-O was addressed for the first time. Results were compared with those of fresh garlic and of commercial black garlic samples. Aging of uncrushed bulbs for 21 days afforded a trade-off to meet the highest content of SAC and S1PC (1.55 and 0.11 mg g^{-1}, respectively) in the shortest time, and a better aroma associated with a lower content of pungent organosulfur compounds (diallyl sulfide, allyl methyl disulfide, diallyl disulfide, allyl methyl trisulfide, etc) and higher levels of volatiles with fresh, green and sweet notes (*e.g.* 1.2 and 0.07 μg g^{-1} of benzaldehyde and furfural, respectively). These findings are a contribution to satisfy industry and consumer demand for processing methods and analytical approaches providing AG with premium quality.

1. Introduction

Garlic (*Allium sativum* L.) is widely used, not only as a condiment for culinary applications, but also as the basis for a number of garlic-based products including garlic supplements, due to its wide variety of biological properties (Shang et al., 2019). However, the consumption of garlic supplements is sometimes conditioned by their strong aroma and taste, and its tendency to cause stomach upset in sensitive individuals (Rais et al., 2023). To overcome these limitations, alternative garlic-based products such as aged garlic (AG) are increasingly being employed for food supplement manufacturing. As processing conditions are known to affect AG composition in different manner, their careful optimization has to be addressed if AG supplements with improved organoleptic properties and better consumer acceptance are intended.

Although fermentation of raw garlic with microorganisms (*Saccharomyces cerevisiae*, *Lactobacillus plantarum*, etc.) (Kim et al., 2011; Nam et al., 2022; Rais et al., 2023; Ríos-Ríos et al., 2019) or by soaking in hydroalcoholic mixtures for an extended period of time (*e.g.* 15–20 % ethanol for at least 18 months) (Chen et al., 2020) have been described for garlic aging, heating under controlled temperature (60–90 °C) and humidity conditions (70–90 %) for 1–3 months is by far the most common aging procedure implemented in industry (Kodera et al., 2002; Lee et al., 2016; Xu et al., 2015; Yang et al., 2019). As a result of non-enzymatic browning reactions (*e.g.* Maillard reaction), caramelization, and chemical oxidation taking place during aging, AG with a brown or even black colour (depending on processing conditions), sticky jelly-like texture, sweet taste, reduced pungency and a more pleasant aroma is obtained. Moreover, as compared to fresh garlic, AG provides enhanced antioxidant activity, cardiovascular benefits (*e.g.* reduction of high blood pressure and cholesterol levels), etc. (Ahmed & Wang, 2021), bioactivities supporting its preferred use for manufacturing of food supplements.

One of the most significant changes during garlic aging is the increase in bioactive molecules, including *S*-allyl-L-cysteine (SAC) and its stereoisomer *S*-1-propenyl-cysteine (S1PC) (Ahmed & Wang, 2021; I. Jiménez-Amezcua et al., 2023). As SAC content is generally considered

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for the standardization of AG supplements and preparations, a number of studies have focussed on the selection of the optimal processing conditions providing SAC-enriched AG (Chen et al., 2020). Moreover, the immunomodulatory and antihypertensive effects ascribed to S1PC (Matsutomo et al., 2017; Suzuki et al., 2016) have recently contributed to propose S1PC as an additional standardization marker (Kodera et al., 2017), although its determination is not yet widely considered.

Several green sample preparation methods, such as those based on headspace solid phase microextraction (HS-SPME), coupled to gas chromatography-mass spectrometry (GC-MS) or GC-olfactometry (GC-O), have been reported for the rapid isolation of a number of food volatiles (Piergiovanni et al., 2024). These methodologies have been previously used to evaluate the changes in the volatile composition of AG, either with the pre-treatment of raw garlic bulbs (*e.g.* crushing) (Tocmo et al., 2017; Varga-Visi et al., 2019), or with the aging conditions (Manoonphol et al., 2023; Molina-Calle et al., 2017; Ríos-Ríos et al., 2019). SPME approaches have also been reported for the evaluation of garlic odour-active compounds, either lost by volatilization or formed as a result of processing conditions (Abe et al., 2020; Hwang & Kim, 2022; Sasmaz et al., 2024; Yang et al., 2019). However, no published work has considered either the HS-SPME-GC-MS volatile profile of AGB subjected to crushing once the thermal processing has been initiated, or the combination of volatile data and bioactive content when the processing of AGB intended for food supplement manufacturing is to be optimized.

In this study, aging of garlic (either untreated or crushed during their processing) under mild temperature conditions (ActiveNature™ Technology) was optimized in terms of processing time to simultaneously provide AGB with higher bioactive content and improved sensory profile, by means of a multianalytical approach. To that aim, SAC and S1PC contents were determined by liquid chromatography-mass spectrometry (LC-MS), whereas HS-SPME-GC-MS and HS-SPME GC-O methods were optimized for volatile profiling and identification of aged garlic key odourants, respectively. Fresh garlic and commercial black garlic samples were also analysed for comparative purposes.

2. Materials and methods

2.1. Standards and samples

Analytical standards (purity >99 %) of *S*-1-propenyl-L-cysteine (S1PC) and 1,2-dichlorobenzene were obtained from Sigma-Aldrich (St. Louis, MO, USA), and of *S*-allyl-L-cysteine (SAC) from Tokyo Chemical Industry (Zwiindrecht, Belgium).

Garlic samples under study included: sun dried fresh garlic (FG) cultivated in Las Pedroñeras (Castilla-La Mancha, Spain), and aged garlic samples obtained from FG using an innovative 100 % natural aging process (ActiveNature™ Technology) under controlled temperature (<65 °C) and humidity (60–90 %) conditions. In addition to aged garlic samples from untreated garlic bulbs (AGB), garlic bulbs subjected to crushing after 11 aging days (aged garlic crushed bulbs, AGCB) were also considered for aging. In both cases, sampling was done after 0, 14, 21, 28 and 40 days of thermal processing. For the comparison of these results with those of samples usually employed at industry for manufacturing of AG supplements, commercial black garlic samples (CBG1 and CBG2; aging process non specified) were purchased online. Before analysis, all samples were frozen, freeze-dried, ground to fine particles using an IKA A10 basic mill (IKA-Werke, Germany), sieved through a 500 μm mesh and stored in dry and dark conditions at room temperature.

2.2. Bioactive content

2.2.1. Sample preparation

Garlic samples (0.1 g) were subjected to extraction with 1 mL of Milli-Q water (Millipore, Bedford, MA, USA) using an ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) operating at 35 °C for 25 min, 80 Hz of frequency and 30 % of power. The extracts were centrifuged (MiniSpin®, Eppendorf, Hamburg, Germany) at 4401 *g* for 10 min, diluted 10 times and filtered (0.2 μm × 13 mm, UptiDisc PTFE filters, Montluçon, France) prior to their LC-MS analyses. All experiments were carried out in triplicate.

2.2.2. LC-MS analysis

LC-MS analysis of garlic bioactives and other typical garlic polar compounds was carried out following the method previously optimized and validated, including recovery experiments, by Jiménez-Amezcua et al. (2023). An Agilent 1100 series system equipped with an autosampler, a quaternary pump, a temperature-controlled column compartment and a diode array detector coupled to a 6195 mass spectrometry detector provided with an electrospray ionization (ESI) source (Agilent Technologies, Santa Clara, CA, US) was used. Chromatographic separation was done using an InfinityLab Poroshell 120 Bonus-RP column (150 mm × 4.6 mm, 2.7 μm; Agilent Technologies) operating at 0.4 mL min^{-1} and 30 °C. A binary gradient consisting of water (A) and acetonitrile (B), both with 0.1 % formic acid, was performed as follows: 0–7 min, 7 % B; 21 min, 60 % B; 22–32 min, 95 % B; 33–48 min, 7 % B. The injection volume was 5 μL.

ESI parameters were set as indicated below: capillary voltage, 3 kV; drying gas temperature (N_2, 99.5 % purity), 300 °C; drying gas flow, 12 L min^{-1}; nebulization pressure, 276 kPa and fragmentor voltage, 40 V. Mass spectra were simultaneously acquired in SCAN (90–2000 *m/z* range), and in SIM mode for the quasimolecular ion corresponding to the $[M+H]^+$ adduct of both SAC and S1PC (*m/z* 162).

Compound identification was based on chromatographic retention and MS data and was confirmed, when possible, by co-injection of the corresponding commercial standards and by comparison with literature data (Jiménez-Amezcua et al., 2023, 2024; Woo et al., 2022). Quantitative analysis of bioactives was performed in triplicate using an external standard calibration curve of SAC (0.5–50 mg L^{-1}) and S1PC (0.1–50 mg L^{-1}). Data acquisition and processing were carried out using HP Chemstation Rev. B.04.02 software (Agilent Technologies). Results were expressed as mg g^{-1} dry sample.

2.3. Volatile profiling and key odourant analysis

2.3.1. HS-SPME-GC-MS analysis

Garlic volatiles were sampled by HS-SPME using two different fibres (both from Supelco, Bellefonte, Palo Alto, USA): (i) a 75 μm carboxen/polydimethylsiloxane (C/PDMS) fiber and (ii) a 50/30 μm divinylbencene/carboxen on polydimethylsiloxane (DVB/CAR/PDMS) fibre. First, the sample amount (20–200 mg) to be optimized, together with 0.5 mL of salty (NaCl, Sigma-Aldrich, St. Louis, MO, US) water and 0.1 mL of internal standard (2 μg mL^{-1}, 1,2-dichlorobenzene, Sigma-Aldrich), were placed into a 20 mL vial fitted with a screw cap and a predrilled PTFE-lined septum (Thermo Scientific™, MA, USA) and vortexed for 2 min. Other operating conditions such as extraction temperature (T_{ext}: 40–55 °C) and extraction time (t_{ext}: 5–30 min) were further optimized for a fixed equilibrium time (t_{eq}: 20 min). Constant stirring (220 rpm) was set during the whole procedure.

Volatiles isolated by HS-SPME ($n = 3$) were analysed on an Agilent 7890-5977A GC-MS system equipped with an CTC120 autosampler (both from Agilent Technologies, Stock-port, UK). The SPME fibre was desorbed into the injection port, after evaluation of different temperatures (200–250 °C) and splitless times (0.3, 0.75, 1.5 and 3 min), using a SPME liner (0.75 mm, Supelco). Chromatographic separations were performed on a Zebron ZB-5MS 5 % phenyl methyl polysiloxane capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Phenomenex, CA, USA), using helium as carrier gas (0.9 mL min^{-1}). The oven temperature was 40 °C, rising by 4 °C min^{-1} to 250 °C, and held for 8 min.

Mass spectra were acquired by electron ionization (EI) at 70 eV, scanning the 40–350 *m/z* range. Quadrupole, source and interface temperatures were set at 150, 230 and 280 °C, respectively. For data

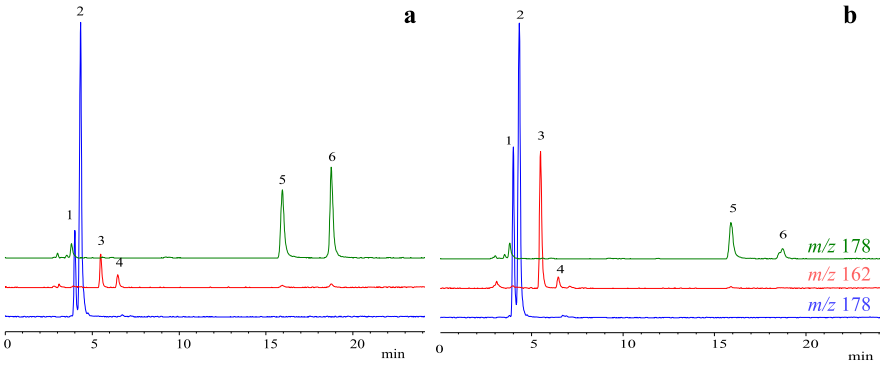

Fig. 1. LC-MS profile of garlic (**a**) before aging (FG) and (**b**) after 21 days of processing (AGB21). Labeled peaks are as follows: (1) Isoalliin, (2) Alliin, (3) *S*-allyl-L-cysteine (SAC), (4) *S*-1-propenyl-L-cysteine (S1PC), (5) γ-glutamyl-*S*-allyl-L-cysteine (γ-GSAC) and (6) γ-glutamyl-*S*-1-propenyl-L-cysteine (γ-GS1PC).

acquisition and analysis, MSD ChemStation software (Agilent Technologies) was used.

Volatile compounds were tentatively identified by comparison of their experimental mass spectra with data from mass spectral libraries (Wiley 6.0, NIST 2020). Identifications were further confirmed by using linear retention indices (*LRI*) calculated from retention data of suitable *n*-alkanes (from C_6 to C_{30}) analysed under identical conditions. For all compounds, concentrations recovered by HS-SPME (expressed as μg g^{-1} dry sample) were calculated by comparing the peak area against that of the internal standard, using a response factor of 1.

2.3.2. HS-SPME GC-O analysis

Analysis of garlic key odourants (20 mg sample) was carried out following the procedure previously optimized in section 2.3.1., using a manual SPME holder from Supelco rather than an automated device. For HS-SPME GC-O analyses, an Agilent HP5890 Series II ODO 2 (SGE) GC–O system equipped with the same column used for SPME-GC-MS analysis was used. The outlet of this column was split between a flame ionization detector (FID) and a sniffing port. After HS-SPME isolation, volatiles were desorbed at 250 °C for 3 min in splitless mode onto five small loops of the column in a coil, which were cooled by using the solid carbon dioxide placed in a 250 mL beaker. Once desorption was complete, the solid carbon dioxide was removed. The oven temperature was held at 40 °C during desorption and for a further 1 min and then the temperature was raised at 4 °C min^{-1} to 250 °C, and held for 5 min. Helium at 2.0 mL min^{-1} was used as carrier gas and the detector temperature was set at 280 °C. *n*-Alkanes (C_6–C_{30}) were analysed under these operating conditions to obtain *LRI* for comparison with the GC-MS data.

Three assessors were involved in the detection and verbal description of the odour active components of the extracts. The sniffing time was approximately 20 min. Each odour was scored on a nine-point line scale (1–9) where 1–3 = weak, 4–6 = medium and 7–9 = strong.

2.4. Statistical analysis

Statistica 7.0 software (StatSoft, Inc., Tulsa, OK, USA) was used for the statistical analysis of data. Significance ($p < 0.05$) of differences was determined by the analysis of variance (ANOVA, Tukey test).

3. Results and discussion

3.1. Evolution of the bioactive content with garlic aging

Taking into account the intended selection of the processing conditions giving rise to aged garlic with the highest content of SAC and S1PC to be used for manufacturing of supplements, the quantitative determination by LC-MS of these two bioactives and the qualitative profiling of related compounds, was first addressed in AG samples subjected to different processing conditions. For comparison purposes, these analyses were also carried out on the FG and CBG samples previously described in section 2.1.

As shown in Fig. 1, the LC-MS profiles of both FG and AGB samples were characterized by a number of compounds typical of garlic, including isoalliin, alliin, SAC, S1PC, γ-glutamyl-*S*-allyl-L-cysteine (γ-GSAC) and γ-glutamyl-*S*-1-propenyl-L-cysteine (γ-GS1PC), whose concentrations were dependent on the sample considered. SAC and S1PC were present in all the samples under study (Table 1), except for commercial black garlic samples (CBG1 and CBG2), where only traces of these bioactives were detected. In both FG and garlic bulbs subjected to aging for 14–40 days, S1PC concentration was lower than that of SAC. In a study on raw garlic by Kodera et al. (2017), it was stated that γ-GS1PC, precursor of S1PC, is found at higher concentration than γ-GSAC, precursor of SAC. However, as a consequence of heat treatment, γ-GS1PC is affected to a greater extent by S-oxidation and deglutamylation to give rise to isoalliin or cycloalliin, as compared to its competitive hydrolysis to S1PC (Kodera et al., 2017; Lee et al., 2016). This explanation would

Table 1
Concentrations of SAC and S1PC (mg g^{-1} dry sample) in fresh garlic (FG), and their evolution in aged garlic bulbs (AGB) and aged garlic crushed bulbs (AGCB) with aging time (up to 40 days).

Aging time (days)	[SAC] (mg g^{-1} dry sample)		[S1PC] (mg g^{-1} dry sample)	
0 (FG)	0.18 (0.01)*[a]		0.099 (0.006)[a]	
	AGB	AGCB	AGB	AGCB
14	1.24 (0.02)[b,1]	1.46 (0.02)[b,2]	0.121 (0.003)[b,c,1]	0.089 (0.001)[b,2]
21	1.55 (0.06)[c,1]	1.12 (0.01)[c,2]	0.111 (0.006)[a,b,1]	0.037 (0.001)[c,2]
28	1.5 (0.1)[c,1]	1.01 (0.04)[d,2]	0.13 (0.01)[c,1]	0.023 (0.001)[d,2]
40	1.48 (0.05)[c,1]	0.74 (0.03)[e,2]	0.047(0.002)[d,1]	0.002 (0.001)[e,2]

* Average ($n = 3$) and standard deviation in brackets.
[a-e] Different letters within the same column indicate significant ($p < 0.05$) differences for SAC and S1PC content associated with aging time.
[1,2] Different numbers within the same row indicate significant differences ($p < 0.05$) for SAC and S1PC content associated with the crushing treatment of aged garlic bulbs.

agree with the experimental results here reported.

As compared to FG (0.18 mg g^{-1} dry sample), the aging process increased by nine the SAC concentration. These results are in good agreement with previous studies where aging was reported to increase the SAC content of fresh garlic in a ratio of 3–10 (Bae et al., 2014, p. 19.61–124.67 μg g^{-1}; Xu et al., 2015, pp. 98–198 μg g^{-1}; Jiménez-Amezcua et al., 2023, pp. 320-2320 μg g^{-1}), depending on the garlic variety and harvesting conditions, the aging process considered, etc. S1PC levels of AG samples were close to that of the FG sample here analysed (0.099 mg g^{-1} dry sample), and similar to data previously reported by other authors such as Woo et al. (2022) for a number of aged garlic products (in the range 120–530 μg g^{-1} dry sample).

High SAC contents have been reported to be promoted by mild (below 60 °C) thermal processing of garlic bulbs, as higher temperatures give rise to the reduction of γ-glutamyl-*S*-allyl-cysteine (γ-GTP) activity affecting the conversion of γ-GSAC into SAC (Bae et al., 2014; Manoonphol et al., 2023). In agreement with this, SAC and S1PC contents of the AGB samples here processed increased with aging time (Table 1), reaching the maximum values of 1.55 and 0.13 mg g^{-1} dry sample after 21–28 aging days, respectively. From day 21 onwards, SAC values remained stable, whereas S1PC content significantly decreased after 40 aging days (0.047 mg g^{-1} dry sample), according to the lower thermal stability of this compound previously reported (Woo et al., 2022).

Regarding the processing of crushed garlic bulbs, aging for 14 days was shown to provide a significantly ($p < 0.05$) higher content of SAC over that of the corresponding non-treated sample (AGB14). However, a marked decrease from then onwards was observed, ending in a 2-fold lower level of this bioactive at the end of the process. Moreover, SAC contents of AGCB samples processed for intermediate times (21–28 days) were always significantly lower than those of untreated garlic bulbs. Similarly, levels of S1PC in AGCB were found to noticeably decrease (45 fold) during processing and were always significantly lower than those of AGB samples, irrespective of the aging period considered.

3.2. Characterization of the aromatic composition of garlic samples

As previously mentioned, SPME has shown to be advantageous for the green, fast and sensitive isolation of volatiles in a number of food matrices with different purposes (Merkle et al., 2015; Xu et al., 2016). Therefore, the optimization of a HS-SPME procedure for garlic volatile isolation and preconcentration was considered prior to the volatile fingerprinting and the identification of key odourants by SPME-GC-MS and SPME GC-O, respectively, in the aged garlic samples under study.

3.2.1. Optimization of the HS-SPME-GC-MS procedure

As selectivity of SPME greatly depends on the fiber coating considered, two fibers (C/PDMS and DVB/CAR/PDMS) with different characteristics were evaluated under identical experimental conditions (200 mg AGB14, $T_{ext} = 40$ °C, $t_{eq} = 20$ min, $t_{ext} = 15$ min) for sampling of garlic volatiles. Despite C/PDMS fiber is usually recommended for isolation of high volatility compounds such as organosulfur compounds (*e.g.* allyl methyl sulfide, diallyl disulfide, 3-vinyl-1,2-dithiacyclohex-5-ene) present in garlic samples, no noticeable differences in the recovery of most of these compounds were observed over the results obtained by DVB/CAR/PDMS fiber. Moreover, this last fiber provided a richer GC-MS profile in the low-medium volatility range and, therefore, it was selected for further analyses (Fig. S1).

The development of a miniaturized method for garlic volatile profiling was then addressed, not only to fulfil the requirements of green chemistry, but also to minimize the unpleasant effect associated with the handling of high amounts of garlic for analysis. To that aim, sample amounts in the range 20–200 mg were evaluated. As garlic samples are rich in volatiles, 100 mg were shown to be enough to achieve the required sensitive characterization of garlic minor volatiles. In addition, this sample amount also prevented the unwanted detection as saturated peaks of some major garlic volatiles observed when higher amounts were considered for HS-SPME sampling.

Considering 20 min as equilibrium time, the extraction temperature and time were further evaluated in the ranges previously described in section 2.3.1. (40–55 °C and 5–30 min, respectively). Temperature has usually been described as one of the operating factors having a more significant effect on volatile recovery by SPME (Soria et al., 2017). In this work, although GC-MS profiles gathered at different temperatures were shown to be qualitatively similar in the whole elution range (Fig. S2), quantitative differences particularly in the recovery of several medium volatility compounds were observed with the extraction temperature considered. However, and taking into account the intended preservation of the original volatile composition of garlic samples under study (avoiding the potential generation of thermal degradation artifacts) and the intended development of a sensitive but also sustainable (in terms of energy consumption) approach, 40 °C were selected as optimal SPME extraction temperature. On the other hand, although longer extraction times (30 min) were shown to positively affect the recovery of several volatiles (*e.g.* 5-methyl-1,2,3,4-tetrathiane), this beneficial effect on a reduced number of peaks was not found to justify an extended analysis time and, therefore, 20 min were considered as a trade-off value for the intended sensitive and fast characterization of the garlic samples here considered.

Once optimal HS-SPME operating conditions were selected (100 mg sample, $T_{ext} = 40$ °C, $t_{eq} = t_{ext} = 20$ min), the amount of volatiles thermally desorbed from the SPME fiber and further introduced in the GC column was evaluated by means of the optimization of the injector temperature and splitless time. Again, the combination of a higher injector temperature and a short splitless time (250 °C for 0.75 min) provided a better detection of some major organosulfur volatiles as non-saturated peaks, and a better focusing of high volatility garlic compounds.

3.2.2. Identification and quantitation of aged garlic volatiles

The optimized HS-SPME-GC-MS procedure was further applied to the characterization of the volatile composition of the different garlic samples under study. A total of 61 compounds with different functionality (organosulfur, alcohols, esters, aldehydes, ketones, furan derivatives and pyrazines) were detected in the FG, AGB, AGCB and CBG samples here analysed. Table S1 lists a compilation of these compounds together with their experimental *LRI*. Moreover, Fig. 2 shows, for comparative purposes, the volatile profiles gathered by HS-SPME-GC-MS for two of these samples: FG and aged garlic bulbs processed for 40 days (AGB40).

OSC were the predominant class of volatiles, with 32 compounds identified in the different garlic samples evaluated. Among them, 12 OSC were selected for their quantitative monitoring because of their significant role in garlic aroma, as described in previous literature (Kilic-Buyukkurt et al., 2023; Sasmaz et al., 2024), and/or their higher variability with the aging time or the crushing treatment of bulbs during processing (Table 2 and Table S2). Most of OSC here detected were derivatives of alliin, an odourless sulfoxide generated from γ-GSAC by a series of reactions, including oxidation (Rais et al., 2023). It has been described that when fresh garlic is cut, crushed, etc., alliinase enzyme is released and activated, converting alliin and the remaining *S*-alk(en)-yl-L-cysteine-*S*-oxides into sulfenic acid intermediates (Abe et al., 2019, pp. 1585–1593; Molina-Calle et al., 2017). In the cellular environment, these intermediates are quickly condensed into thiosulfinates such as allicin, which is the unstable precursor of a number of volatile OSC such as diallyl sulfide, diallyl disulfide, allyl methyl disulfide, diallyl trisulfide, among others (Abe et al., 2019, pp. 1585–1593; Kim et al., 2011). These compounds, experimentally detected at different concentrations in the garlic samples here analysed, have been reported to be responsible for the pungent aroma of FG, their concentration decreasing with aging (Colín-González et al., 2012; Kang et al., 2022; Sasmaz et al., 2024), as evidenced in the present study. Among major

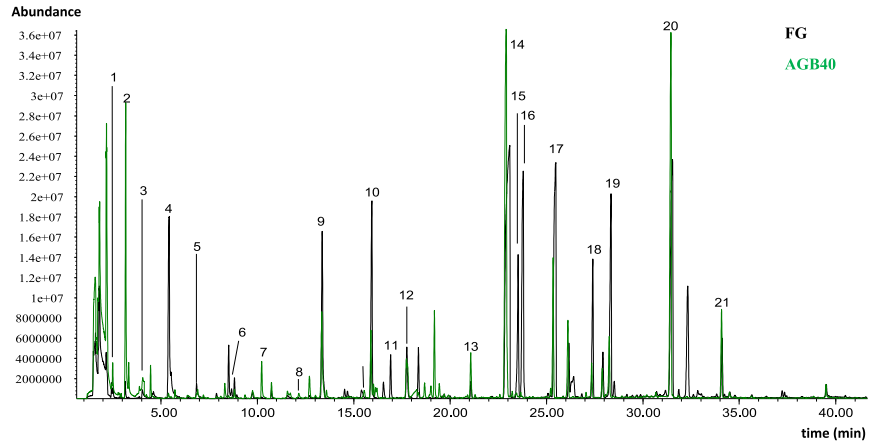

Fig. 2. HS-SPME-GC-MS chromatographic profiles of FG and AGB40 samples. 1. 2-Propenal, 2. 2-Propen-1-ol, 3. Allyl mercaptan, 4. 2-Butenal, 5. Allyl methyl sulfide, 6. Dimethyl disulfide, 7. 5-Hexenal, 8. Furfural, 9. Diallyl sulfide, 10. Allyl methyl sulfide, 11. Benzaldehyde, 12. 3-H-1,2-Dithiole, 13. Internal Standard, 14. Diallyl disulfide, 15. (*Z*)-1-propenyl propyl disulfide, 16. (*E*)-1-propenyl-2-propen-1-yl disulfide, 17. Allyl methyl trisulfide, 18. 4-Methyl-1,2,3-trithiolane. 19. 3-Vinyl-1,2-dithiacyclohex-5-ene, 20. Diallyl trisulfide, 21. 5-Methyl-1,2,3,4-tetrathiane.

OSC, concentrations of diallyl sulfide and diallyl disulfide were the most variable in the garlic samples evaluated, with FG and AGB samples showing significantly higher contents of both compounds (in the ranges 1–5.4 and 10–38 μg g^{-1} dry sample, respectively) than those of commercial black garlic samples (0.02–0.24 and 0.2–3 μg g^{-1} dry sample, respectively) (Table 2). In agreement with this, recent literature has reported values up to 0.012 μg diallyl sulfide g^{-1} dry sample and in the range 0.005–0.248 μg diallyl disulfide g^{-1} dry sample for black garlic samples aged under different processing conditions (65–85 °C, 70–85 % relative humidity, 24 and 50 days) (Sasmaz et al., 2024). The milder conditions here employed for garlic aging involving, among others, lower processing temperatures would justify these differences.

In order to evaluate the potential beneficial effect of crushing on the reduction of aged garlic pungent volatiles, garlic bulbs were subjected to this treatment on the 11th day of processing and further aged to complete the processing time. These results (AGCB samples) were compared with those of untreated aged garlic bulbs (AGB samples) in Table S2. In general, and similarly to AGB samples, garlic bulbs subjected to crushing were found to decrease their OSC concentration with aging time (*e.g.* from 3.7 to 1 μg diallyl sulfide g^{-1} dry sample for up to 40 days). As

Table 2
Concentrations (μg g^{-1} dry sample) for selected volatiles determined by HS-SPME-GC-MS in fresh garlic (FG), aged garlic samples processed from bulbs (AGB) for different times (14, 21, 28 and 40 days) and in commercial black garlic samples (CBG).

LRI[1]	Compounds[2]	FG	AGB14	AGB21	AGB28	AGB40	CBG1	CBG2
	Thiols							
598	Allyl mercaptan	0.87^{a} (0.04)	0.88^{a} (0.05)	0.54^{b} (0.03)	0.50^{b} (0.02)	0.23^{c} (0.02)	–	0.08^{d} (0.04)
	Monosulfides							
556	Dimethyl sulfide	0.05^{a} (0.01)	0.06^{a} (0.002)	0.056^{a} (0.003)	0.04^{b} (0.02)	0.04^{b} (0.01)	–	0.013^{c} (0.007)
698	Allyl methyl sulfide	1.2^{a} (0.6)	0.28^{b} (0.06)	0.24^{b} (0.06)	0.2^{b} (0.1)	0.10^{b} (0.04)	0.09^{b} (0.03)	0.142^{c} (0.001)
862	Diallyl sulfide	5.4^{a} (0.4)	3.5^{b} (0.3)	$2.5^{b,c}$ (0.8)	$2.0^{c,d}$ (0.8)	1.0^{d} (0.4)	0.02^{e} (0.02)	0.24^{f} (0.07)
	Disulfides							
746	Dimethyl disulfide	0.47^{a} (0.01)	0.06^{b} (0.01)	$0.054^{b,c}$ (0.004)	0.06^{b} (0.02)	0.03^{c} (0.01)	0.4^{a} (0.1)	0.22^{d} (0.02)
922	Allyl methyl disulfide	15.1^{a} (0.5)	3.1^{b} (0.1)	2.7^{b} (0.1)	2.5^{b} (0.5)	1.3^{c} (0.1)	0.5^{d} (0.2)	1.4^{c} (0.5)
1092	Diallyl disulfide	38^{a} (7)	24^{b} (5)	19^{b} (3)	18^{b} (1)	10^{c} (1)	0.2^{d} (0.1)	3^{e} (2)
	Trisulfides							
978	Dimethyl trisulfide	3.31^{a} (0.03)	0.35^{b} (0.03)	0.25^{c} (0.01)	0.22^{c} (0.01)	0.1^{d} (0.01)	0.2^{c} (0.1)	0.4^{b} (0.1)
1153	Allyl methyl trisulfide	18^{a} (4)	9^{b} (2)	6^{c} (2)	4.7^{c} (0.4)	1.7^{d} (0.2)	0.12^{e} (0.08)	2.4^{f} (0.02)
1318	Diallyl trisulfide	22^{b} (3)	30^{a} (2)	21^{b} (1)	18^{b} (0.5)	7.2^{c} (0.3)	–	2^{d} (0.5)
	Cyclic sulfides							
1171	4-Methyl-1,2,3-trithiolane	2.4^{a} (0.4)	$6^{b,1}$ (1)	$3.6^{b,1}$ (0.3)	$3.3^{b,1}$ (0.2)	$1.4^{c,1}$ (0.1)	0.01^{d} (0.008)	0.98^{e} (0.01)
1229	3-Vinyl-1,2-dithiacyclohex-4-ene	3.7^{a} (0.5)	1.6^{b} (0.6)	$1^{b,c}$ (0.3)	$0.8^{b,c}$ (0.2)	0.3^{c} (0.1)	–	0.07^{d} (0.04)
	Alcohols							
566	2-propen-1-ol	0.78^{a} (0.01)	2.9^{b} (0.1)	7.3^{c} (0.2)	9.5^{d} (0.7)	8^{c} (1)	0.16^{e} (0.01)	1.2^{f} (0.03)
776	2,3-butanediol	–	0.09^{a} (0.05)	0.1^{a} (0.01)	0.12^{a} (0.05)	0.15^{a} (0.03)	0.09^{a} (0.03)	0.010^{b} (0.001)
	Aldehydes							
541	2-propenal	0.4^{a} (0.1)	0.13^{b} (0.02)	0.2^{c} (0.01)	0.18^{c} (0.01)	0.12^{b} (0.01)	0.01^{d} (0.001)	0.020^{e} (0.001)
965	Benzaldehyde	0.39^{a} (0.04)	0.5^{a} (0.2)	1.2^{b} (0.3)	1.4^{b} (0.2)	0.6^{a} (0.08)	0.05^{c} (0.02)	0.08^{c} (0.03)
	Pyrazines and furan derivatives							
823	Methyl pyrazine	–	0.015^{a} (0.003)	0.06^{b} (0.01)	0.10^{c} (0.01)	0.12^{d} (0.003)	0.007^{e} (0.001)	0.27^{f} (0.05)
833	Furfural	0.005^{a} (0.001)	0.01^{a} (0.003)	0.07^{b} (0.01)	0.15^{c} (0.01)	0.14^{c} (0.01)	1.5^{d} (0.3)	2.2^{e} (0.6)
854	2-Furanmethanol	–	–	0.004^{a} (0.001)	0.02^{b} (0.0003)	0.03^{c} (0.003)	0.015^{d} (0.002)	0.015^{d} (0.005)

[1] Experimental linear retention index (*LRI*) on DB-5 column, calculated from retention data of *n*-alkanes (C_6-C_{30}) analysed under identical experimental conditions.
[2] Compounds identified by comparing experimental and literature *LRI* values and experimental MS spectra with data from mass spectral libraries (Wiley, NIST).
* Average ($n = 3$) and standard deviation in brackets.
[a-f] Different letters within the same row indicate significant ($p < 0.05$) differences for FG, AGB and CBG samples.
-: Non detected.

regard the comparison of AGCB and AGB samples, whereas crushed garlic bulbs were found to provide a higher release of a number of unwanted OSC such as allyl methyl disulfide, diallyl disulfide, dimethyl trisulfide, allyl methyl trisulfide, etc. for most aging times considered, similar contents of others were detected in AGB and AGCB samples either for specific aging times (*e.g.* allyl methyl sulfide in AGB14 and AGCB14) or irrespective of the processing time (*e.g.* diallyl sulfide).

In addition to unpleasant OSC, a number of volatiles from different chemical families and showing positive aromatic attributes (sweet, fruity, etc. notes) were found to increase their concentration during garlic aging (Fig. S3). Thus, aldehydes and furan derivatives such as benzaldehyde and furfural, present in low concentrations in FG, were detected at higher levels in garlic samples thermally processed (up to 1.4 and 0.15 μg g^{-1} dry sample of benzaldehyde and furfural in AGB28, Table 2), in agreement with previous references (Bae et al., 2014; Molina-Calle et al., 2017). As compared to CBG samples, benzaldehyde was significantly higher in the AGB21/AGB28 samples, whereas furfural was significantly lower. Furfural, an aroma compound formed via Maillard reaction and present in a number of foods (Gong et al., 2021), together with other products of this non-enzymatic browning reaction such as 2-furanmethanol, has been previously described in aged garlic as a result of the thermal degradation of sugars (Nam et al., 2022; Ríos-Ríos et al., 2019). Furfural content has also been reported to vary widely (0.062–0.519 μg g^{-1}) with the garlic aging conditions employed (Molina-Calle et al., 2017; Sasmaz et al., 2024). In agreement with this, the mild conditions employed in this study for garlic aging (Active-Nature™ Technology) would justify the lower levels of these Maillard reaction derivatives detected in AGB samples, as compared to the two commercial black garlic samples analysed (*e.g.* AGB28 and AGB21 showed 15 and 31 times lower content of furfural than CBG2, respectively). As for the comparison of crushed and untreated garlic bulbs (Table S2), crushing gave rise to a significantly higher content of both furfural and 2-furanmethanol irrespective of the aging time considered (0.15 *vs* 0.22 μg furfural g^{-1} dry sample and 0.02 *vs* 0.26 μg 2-furanmethanol g^{-1} dry sample in AGB28 and AGCB28, respectively).

Regarding the alcohols showing a greater variability, 2-propen-1-ol (allyl alcohol) content of FG (0.78 μg g^{-1} dry sample) was found to significantly increase during aging, particularly for garlic processed for 21–40 days. As compared to crushed bulbs, untreated garlic provided values of this compound significantly higher irrespective of the aging period considered (*e.g.* 9.5 *vs* 4 μg g^{-1} dry sample for AGB28 and AGCB28, respectively). These results match well with those published by Martínez-Casas et al. (2017), who reported that 2-propen-1-ol was not detected in purple garlic but it was abundant in black garlic, and with those of Kim et al. (2011), who described a 2-fold increase of this compound when raw crushed garlic was subjected to a high temperature treatment (72 °C for 14 days) to obtain black crushed garlic. CBG1 and CBG2 samples showed significantly lower contents of 2-propen-1-ol (0.16–1.2 μg g^{-1} dry sample) than the AGB and AGCB samples processed in this study, irrespective of the aging time considered.

3.2.3. *Analysis of aged garlic key odourants*

As previously mentioned, optimal processing conditions of aged garlic intended for supplement manufacturing should be chosen as a trade-off to meet the highest content of bioactives (SAC and S1PC, Table 1), the lowest level of unpleasant volatiles (*e.g.* OSC, Table 2 and Table S2) and a processing time as short as possible. Taking into account these considerations, the appropriate balance between bioactivity, organoleptic properties and processing efficiency was found for garlic samples subjected to aging for 21 days (AGB21 and AGCB21 samples) and, therefore, these samples were chosen for further sensory analyses.

The GC-O technique was applied to aged garlic samples for evaluation of their key odourants, and, for comparison purposes, to FG and CBG2 samples. Identification of the most active odour compounds was done in combination with HS-SPME-GC-MS data (Table S1). As shown in Table 3, GC-O analyses yielded a total of 14 odour-active compounds

Table 3
Aroma descriptors and GC-O odour scores for volatiles identified in selected garlic samples.

Experimental *LRI*[a]		Aroma descriptors	Identity	GC-O Score[b,c]			
GC-O	GC-MS			FG	AGB21	AGCB21	CBG2
<600	556	Sulfurous Onion Green	Dimethyl sulfide	6	9	6	3
<600	566	Pungent Mustard	2-Propen-1-ol	–	4	4	1
601	598	Sulfurous Garlic	Allyl mercaptan	5	–	–	–
612	616	Garlic cooked	Methyl-thiirane	–	3	4	–
703	698	Garlic	Allyl methyl sulfide	1	3	4	2
799	800	Fresh Green Fatty	Hexanal	4	1	6	–
829	833	Almond-like Sweet	Furfural	–	–	2	4
847	853	Fresh Green	2-Hexenal	3	2	6	–
853	854	Musty Sweet	2-Furanmethanol	3	3	5	–
865	862	Garlic Metallic Meaty	Diallyl sulfide	5	1	–	2
874	874	Herbaceous, sweet	5-Methyl-3(2H)-furanone	3	–	3	7
920	922	Sulfurous Garlic	Allyl methyl disulfide	5	4	4	–
972	978	Sulfurous Meaty	Dimethyl trisulfide	5	4	5	–
1089	1092	Strong garlic	Diallyl disulfide	5	5	5	4

[a] Experimental linear retention index (*LRI*) on DB-5 column for volatiles detected by HS-SPME GC-O analysis and for volatiles separated and identified by HS-SPME-GC-MS analysis.

[b] GC-O scores for fresh garlic (FG), aged garlic bulbs and aged garlic crushed bulbs processed for 21 days (AGB21 and AGCB21, respectively) and for a commercial black garlic sample (CBG2).

[c] Odour score: weak (1–3), medium (4–6) and strong (7–9).

which were mainly detected in the FG and aged garlic samples analysed. OSC (dimethyl sulfide, diallyl sulfide, diallyl disulfide, etc.), which are responsible for the sulfurous odour of FG, have been described to undergo decomposition or transformation into other substances during the heat processing of black garlic. As a result of the reduction of these compounds, experimentally evidenced in this paper (section 3.2.2.), the pungent smell of black garlic is significantly diminished compared to its white counterpart (Kilic-Buyukkurt et al., 2023). In good match with this and with previous literature (Sasmaz et al., 2024; Yang et al., 2019), the odour scores for OSC such as diallyl sulfide, and particularly for allyl mercaptan (compound responsible for the persistent odour of human breath after garlic consumption), in AGB21 and AGCB21 samples were found to be noticeably lower than those of FG.

Regarding the comparison of aged garlic processed from crushed and untreated garlic bulbs, crushing did not noticeably affect the contribution of OSC other than dimethyl sulfide to the overall odour profile of aged garlic. Except for allyl mercaptan and diallyl sulfide, GC-O scores for OSC were always higher for both AGB21 and AGCB21 over those of CBG2. Dimethyl sulfide has been previously identified by sensory-directed flavour analysis (odour active values (OAV), aroma extraction dilution analysis (AEDA), etc) as one of the key aroma-active compounds of black garlic (Yang et al., 2019). In agreement with this, dimethyl sulfide was the sulfur volatile whose odour score was the highest for the FG, AGB21 and AGCB21 samples analysed in the present work.

On the other hand, fresh, green and sweet were the characteristic notes of aged garlic which were mainly associated to compounds such as hexanal, 2-hexenal, furfural and 2-furanmethanol (Table 3). In good match with the experimental results of the present study, it has been reported the influence of furan derivatives (furfural, 2-furanmethanol, etc.) and aldehydes (*e.g.* hexanal) on appealing flavour notes of aged garlic (Abe et al., 2019, pp. 1585–1593; Ríos-Ríos et al., 2019). Furthermore, the scores for these odour active compounds have been reported to be affected by the different processing of garlic bulbs (Najman et al., 2022; Ríos-Ríos et al., 2019; Sasmaz et al., 2024). Thus, in this study, whereas CBG2 showed the highest GC-O score for 5-methyl-3(2H)-furanone, its score was much lower for the FG and AG samples here analysed. The null contribution of furfuryl alcohol in CBG2 sample could be due to the known decomposition of this compound under high temperature and long processing times (Sasmaz et al., 2024). Finally, the high flavour dilution factor reported for 2-propen-1-ol by Yang et al. (2019) would also support the high GC-O scores determined and its key role in the characteristic flavour of the aged garlic samples processed in this study.

4. Conclusions

A multianalytical approach based on LC-MS, HS-SPME-GC-MS and HS-SPME GC-O analyses has been used to evaluate the differences in the bioactive content and aroma profile of aged garlic samples associated with their different processing methods (crushed *vs* untreated garlic bulbs; aging time: 0–40 days). Regarding the intended use of aged garlic samples processed here for manufacturing of supplements, garlic bulbs subjected to aging for 21 days with no crushing during their processing were chosen as a trade-off to meet the highest content of SAC and S1PC, an improved overall aroma provided by the balance between OSC with pungent notes and desirable odours, and the shortest possibly processing time. Moreover, the preliminary results obtained by HS-SPME GC-O, which deserve further investigation, have allowed the identification of the key aromatic components of black garlic. In conclusion, the data gathered in this study is a valuable contribution to the field of food science and technology as it addresses the optimization of aged garlic processing for the first time, considering bioactivity, aroma and sensory profile, all of which are crucial for consumer's acceptance of aged garlic supplements.

CRediT authorship contribution statement

Ignacio Jiménez-Amezcua: Formal analysis, Investigation, Methodology, Writing – original draft. **María Luz Sanz:** Conceptualization, Resources, Investigation, Methodology, Supervision, Writing – review & editing. **Marina Díez-Municio:** Funding acquisition, Resources, Investigation, Methodology, Writing – review & editing. **Jane K. Parker:** Resources, Investigation, Methodology, Writing – review & editing. **María José Oruna-Concha:** Resources, Investigation, Methodology, Writing – review & editing. **Ana Cristina Soria:** Funding acquisition, Project administration, Conceptualization, Resources, Investigation, Methodology, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.118003.

Data availability

Research data are already included in the paper

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