JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Effect of Processing and Storage Time on the Contents of Organosulfur Compounds in Pickled Blanched Garlic

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Supporting Information

ABSTRACT: The influence of processing, with and without fermentation, on the contents of organosulfur compounds, namely, γ-glutamyl peptides, *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), and *S*-allyl-L-cysteine (SAC), in pickled blanched garlic was evaluated. For each processing type, the effect of the preservation method and storage time was also analyzed. Blanching in hot water (90 °C for 5 min) hardly affected the individual organosulfur compound content. The fermentation and packing steps negatively affected the levels of all compounds except for SAC. The content of this compound increased during storage at room temperature whereas γ-glutamyl peptides and ACSOs were degraded to various extents. The pasteurization treatment itself had no significant effect on the concentrations of organosulfur compounds. Use of the corresponding fermentation brine in the case of the fermented product in conjunction with refrigerated storage was found to be the best method to preserve the levels of organosulfur compounds in pickled garlic stored for up to one year.

KEYWORDS: *garlic, Allium sativum L., organosulfur compounds, processing, pickles, storage, preservation*

INTRODUCTION

Garlic (*Allium sativum* L.) is used in all parts of the world as a spice and as a food. The health properties of garlic depend on its bioactive compounds, especially its organosulfur compounds.[1] These include three γ-glutamyl peptides, namely, γ-L-glutamyl-*S*-allyl-L-cysteine (GSAC), γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC), and γ-L-glutamyl-*S*-methyl-L-cysteine (GSMC); their corresponding *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), which are (+)-*S*-allyl-L-cysteine sulfoxide (alliin), (+)-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (isoalliin), and (+)-*S*-methyl-L-cysteine sulfoxide (methiin), respectively; and (1*S*,3*R*,5*S*)-3-carboxy-5-methyl-1,4-thiazane 1-oxide (cycloalliin). It has been reported that the contents of these compounds in garlic bulbs change during the growth and storage periods[2−4] and they are affected by the cultivar and location of growth.[5,6] In addition, intermediate compounds in the biosynthesis of the ACSOs from γ-glutamyl-*S*-alk(en)yl-L-cysteine have demonstrated interesting health properties, such as those reported in the case of *S*-allyl-L-cysteine (SAC).[7] The chemical structures of all these organosulfur compounds are shown in Table 1.

Among the different garlic-based products on the market, pickled blanched garlic appears to be fairly well-accepted by consumers, who could be influenced, at least partially, by the above-mentioned beneficial effects of garlic on health. Different procedures for the preparation of pickled blanched garlic, with or without a fermentation step, have been previously proposed and the chemical, organoleptic, and nutritional characteristics of the respective products have been evaluated by our research group.[8−10] The main stage these procedures have in common is blanching with hot water to inactivate the enzyme alliinase, which is involved in the production of the pungent flavor and green discoloration ("greening").[11−13] It remains to be studied whether the pickled blanched garlic is as beneficial to health as raw garlic; or, in other words, whether the bioactive compounds in garlic are significantly affected by processing steps and storage time. The literature about this subject is rather scarce, and the pickled products subjected to study were not blanched in any case. Kim et al.[11] analyzed the level of allicin, the compound formed from alliin through the action of the enzyme alliinase, during aging of whole garlic cloves in brine containing 2% acetic acid. Lawson and Wang[14] studied the changes in several organosulfur compounds including SAC during aging of chopped garlic in 5% acetic acid.

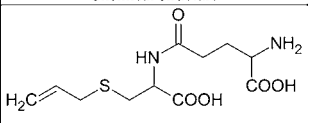
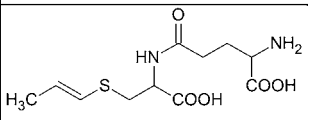
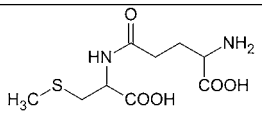
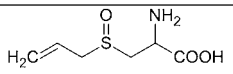
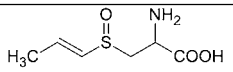
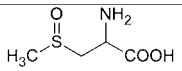
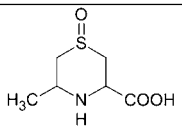
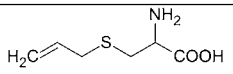
The objectives of the present work were to study the contents of organosulfur compounds (γ-glutamyl peptides, ACSOs, and SAC) in pickled blanched garlic processed with and without fermentation and to evaluate the influence of different packing methods and storage time on the above-mentioned compounds.

MATERIALS AND METHODS

Chemicals. GSMC, GSAC, GSPC, and isoalliin were isolated from Chinese chive seeds or onion bulbs as previously described.[5] Cycloalliin was obtained from the cyclization of isoallin as described by Carson et al.[15] Alliin and SAC were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methiin was synthesized as described by Shen and Parkin[16] using *S*-methyl-L-cysteine (Sigma) as the starting material. The purity of compounds except γ-glutamyl peptides was greater than 95% as determined by analytical HPLC. For the γ-glutamyl peptides, the purity (around 75%) was calculated on the basis of the corresponding extinction coefficients obtained by Lawson et al.[17] These values were taken into account for quantification purposes. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals and solvents were of

Received: October 21, 2011
Revised: March 13, 2012
Accepted: March 15, 2012
Published: March 15, 2012

Table 1. Chemical Structures of Organosulfur Compounds in Garlic

Compound	Chemical structure
γ-L-glutamyl-*S*-allyl-L-cysteine (GSAC)	
γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC)	
γ-L-glutamyl-*S*-methyl-L-cysteine (GSMC)	
(+)-*S*-allyl-L-cysteine sulfoxide (Alliin)	
(+)-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (Isoalliin)	
(+)-*S*-methyl-L-cysteine sulfoxide (Methiin)	
(1*S*,3*R*,5*S*)-3-carboxy-5-methyl-1,4-thiazane 1-oxide (Cycloalliin)	
S-allyl-L-cysteine (SAC)	

analytical or chromatographic grade and were obtained from various suppliers.

Preparation of Pickled Garlic. Two different processes for preparing pickled garlic were studied: (1) packing directly with acidified brine after blanching (unfermented pickled garlic), and (2) packing after the blanching and fermentation steps (fermented pickled garlic) (Figure 1). Each preparation was carried out with a separate batch of garlic cloves. The garlic used was of the "purple" type, Gardos cultivar, and was supplied peeled by the firm La Abuela Carmen from Montalbán (Córdoba, Spain). Before processing, the defective and small cloves were discarded. In the first preparation type, the garlic cloves were blanched in water at 90 °C for 5 min and immediately packed. A portion of blanched garlic cloves was packed into "B250" glass bottles (150 g of garlic cloves plus 110 mL of cover liquid capacity) using a brine acidified with lactic acid (packing solution 1, PS1) as cover liquid. Concentrations (in %) of lactic acid and NaCl in this brine were calculated to give equilibrium values of 1.5% acidity (as lactic acid) and 3.0% NaCl. One half of the packed product was pasteurized for 5.5 min in a water bath at 90 °C (cover brine was added at 70 °C, approximately), and the remainder was left as the nonpasteurized control (packing PNF and CNF, respectively). Both packed products were stored at room temperature. In the second preparation type, garlic cloves were blanched in the same way as for the first preparation and then subjected to lactic acid fermentation. For this, blanched garlic cloves were placed in two fermentation vessels (6 kg of garlic cloves plus 3.3 L of cover liquid capacity each), covered with brine (9% NaCl, w/v) and inoculated (2 h after brining) with a starter culture of *Lactobacillus pentosus*. The initial population in brine after inoculation was 1.8×10^7 CFU/mL. Fermentation was carried out in a room maintained at 30 °C for 10 days. At the end of this period, the population of lactobacilli reached 3.6×10^8 CFU/mL, Enterobacteriacea and yeasts were not detected (<20 CFU/mL), and the pH and titratable acidity of the brine were 3.9 and 1.2% (expressed as lactic acid), respectively. Then, the garlic cloves were divided into three portions. Two portions were packed and preserved in the same way as for the first preparation type (packing PF and CF), while the third portion was packed with its own fermenting brine (packing solution 2, PS2). This brine was centrifuged (16000*g*, 30 min) before use, to remove particles in suspension. The packed product was kept refrigerated during storage (packing RF).

Brine Analyses. The pH and titratable acidity of brines were measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Titratable acidity was determined by titrating to pH 8.3 with 0.2 M NaOH and expressed as lactic acid. The microbiological analysis comprised an aerobic plate count (APC), Enterobacteriaceae (VRBD), lactobacilli (MRS ± 0.02% w/v sodium azide), enterococci (Slanetz and Bartley), molds and yeasts (OGYE), and sulfite-reducing clostridia (DRCM). Media were from Oxoid Ltd., Basingstoke, England (PCA, MRS, Slanetz and Bartley, and OGYE), and Merck, Darmstadt, Germany (VRBD and DRCM).

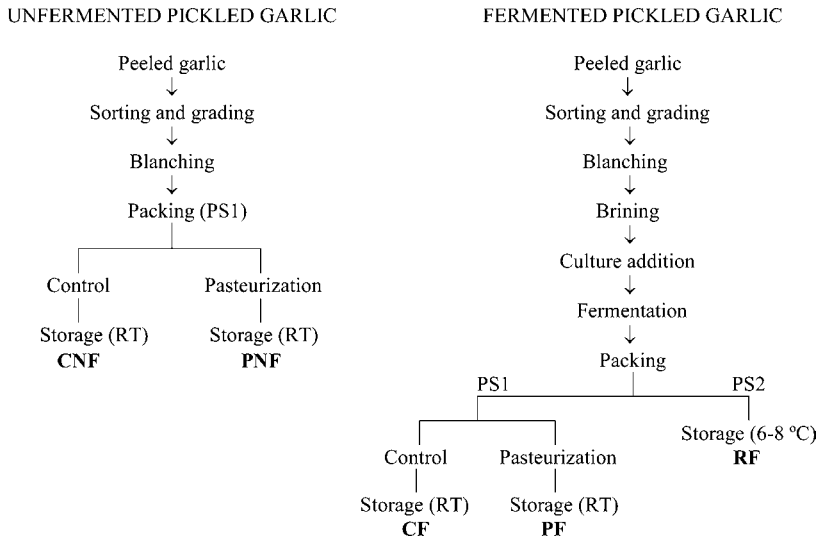


Figure 1. Processing steps for the two preparations of pickled garlic studied in the present work. PS1 = packing solution 1 = acidified fresh brine. PS2 = packing solution 2 = fermenting garlic brine. RT = room temperature. CNF = control, nonfermented pickled garlic. PNF = pasteurized, nonfermented pickled garlic. CF = control, fermented pickled garlic. PF = pasteurized, fermented pickled garlic. RF = refrigerated, fermented pickled garlic.

Moisture Content. The moisture content was determined using an MB35 moisture balance (Ohaus, Pine Brook, NJ, USA). Garlic cloves from each bulb were peeled and homogenized using a blender. The homogenate (1 g) was placed on the balance and dried at 80 °C. The determination was performed in duplicate.

Analysis of Organosulfur Compounds. Prior to extraction of organosulfur compounds, the samples (garlic cloves) were frozen in liquid nitrogen and freeze-dried. The resulting lyophilisate was ground into powder with a mortar and pestle to pass through a 500 μm sieve and stored in sealed plastic bottles at −30 °C until analysis.

Alliin, isoalliin, methiin, and cycloalliin were analyzed by HPLC as previously described.[5] In a 25 mL flask, garlic powder (0.5 g) was added to 15 mL of a 90% methanol solution containing 0.01 M HCl, and the mixture was shaken for 30 min at 900 rpm using a multipoint magnetic stirrer (SBS, Barcelona, Spain). An additional methanolic solution was added to the mixture to make exactly 25 mL. The resulting mixture was filtered through Whatman No. 40 filter paper, and an aliquot of the filtrate was centrifuged at 11600*g* for 5 min using a Hettich microcentrifuge (Andreas Hettich GmbH & Co., Tuttlingen, Germany). The supernatant was analyzed under the following conditions: column, Shodex Asahipak NH2P-50 2D (5 μm, 150 × 2 mm, Showa Denko, Tokyo, Japan); column temperature, ambient; flow rate, 0.2 mL/min; mobile phase, acetonitrile/water (84/16 v/v) containing 0.1% (v/v) concentrated (85%) orthophosphoric acid; detection, 210 nm; injection volume, 1 μL. Typical chromatograms from a standard mixture and a raw garlic extract by this HPLC method are shown in Figure 2. It can be noted that the synthetic standards (i.e., alliin and methiin) eluted as double peaks corresponding to the two diastereomers. By comparing with the garlic extract, it is clear that the (+)-isomer eluted earlier than the (−)-isomer, as only the (+)-isomer is found in natural products.[18] In order to confirm the peak identities, an extract from pickled garlic was analyzed by HPLC interfaced with an electrospray ionization mass spectrometer (ESI-MS). The chromatographic conditions were the same as those mentioned above, with the exception that formic acid instead of orthophosphoric acid was used in the mobile phase. The LC flow was directed to the ESI-MS without using a flow splitter. Typical settings of the main tuning parameters were as follows: capillary voltage, 3 kV; cone voltage, 15 V; source temperature, 100 °C; and desolvation temperature, 350 °C. Ions were formed using ESI in positive mode. The expected ions, as shown in Table 1 in the Supporting Information, were obtained in all cases confirming the peak identities.

GSMC, GSAC, GSPC, and SAC were analyzed by HPLC following the method described by Arnault et al.[19] A sample (0.5 g of garlic powder) was extracted with 25 mL of methanol/water (80/20, v/v) + 0.05% formic acid for 30 min at room temperature. The chromatographic conditions were as follows: column, Luna 3 μm C18(2) (150 × 4.6 mm inner diameter, Phenomenex, Torrance, CA); column temperature, 38 °C; flow rate, 0.4 mL/min; mobile phase, 20 mM sodium dihydrogen phosphate + 10 mM heptanesulfonic acid, pH 2.1 (adjusted with 85% orthophosphoric acid) (eluent A) and acetonitrile/eluent A (50/50, v/v) (eluent B); gradient, 0 to 5 min from 0% to 30% B (linear gradient), 5 to 25 min from 30% B to 54% B (linear gradient), 25 to 26 min from 54% B to 100% B (linear gradient), 26 to 28 min with 100% B, 28 min to 30 min from 100% B to 0%B (linear gradient); detection, 208 nm; injection volume, 10 μL. Typical chromatograms from a standard mixture and a raw garlic extract by this HPLC method are shown in Figure 3. Again, in order to confirm the peak identities, one run on HPLC/ESI-MS was performed. Instead of changing the mobile phase composition to be compatible with ESI-MS, separate collections of each peak (repeated injections from an extract of raw garlic) were performed under the above-mentioned chromatographic conditions. The isolated peaks were dried under reduced pressure and dissolved in 0.5 mL of water. Then, each peak was subjected to derivatization with FMOC reagent followed by HPLC/ESI-MS, as described by Gartenmann and Kochhar[20] with modifications. The advantage of derivatization is a mass shift of the molecular ion by 222 mass units per FMOC derivative and an improved ionization of peptides/amino acids. The FMOC-derivatized compounds, prepared according to Montaño et al.,[21] were analyzed under the following conditions: column, Kinetex C18 (2.6 μm, 150 × 4.6 mm inner diameter, Phenomenex); column temperature, 40 °C; flow rate, 1 mL/min; mobile phase, 0.1% formic acid in water (eluent A) and 0.1% formic acid in acetonitrile (eluent B); gradient, 0 to 20 min from 35% to 50% B (linear gradient), 20 to 23 min from 50% B to 80% B (linear gradient), 23 to 28 min with 80% B, 28 to 30 min from 80% B to 35%B (linear gradient). The ESI-MS conditions were the same as above-mentioned. For each FMOC-derivatized compound, the expected ion $[M + H]^+$ as well as the sodium adduct ion $[M + Na]^+$ was obtained, confirming the peak identities (Table 1 in the Supporting Information).

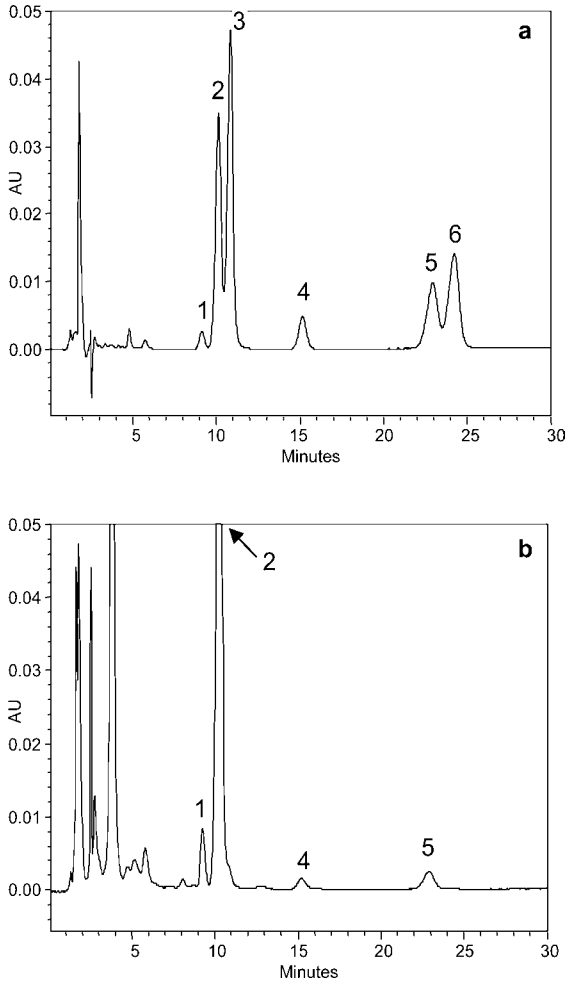


Figure 2. Typical separation of *S*-alk(en)yl-L-cysteine sulfoxides in (a) a standard mixture and (b) an extract from raw garlic by the HPLC method described in the text. Conditions: column, Shodex Asahipak NH2P-50 2D (150 mm × 2 mm); flow rate, 0.2 mL/min; detection, 210 nm; column temperature, ambient; mobile phase, acetonitrile/water (84/16 v/v) containing 0.1% (v/v) concentrated (85%) orthophosphoric acid. Peak identities are as follows: 1, isoalliin; 2, (+)-alliin; 3, (−)-alliin; 4, cycloalliin; 5, (+)-methiin; 6, (−)-methiin.

All determinations (in triplicate) were performed by using a Waters 2695 separation module (Waters Assoc., Milford, MA, USA) connected to a Waters 996 photodiode array detector and controlled using Empower software (Waters). The HPLC/ESI-MS system consisted of a Waters 2695 separation module connected to a Waters ZMD mass detector and controlled by MassLynx software (Micromass, Wythenshawe, U.K.).

Statistical Analyses. The data were subjected to analysis of variance using the STATISTICA software, version 7.0 (Statsoft, Inc., Tulsa, OK). The Scheffé test was used for means comparisons. Significant differences were determined at $p < 0.05$.

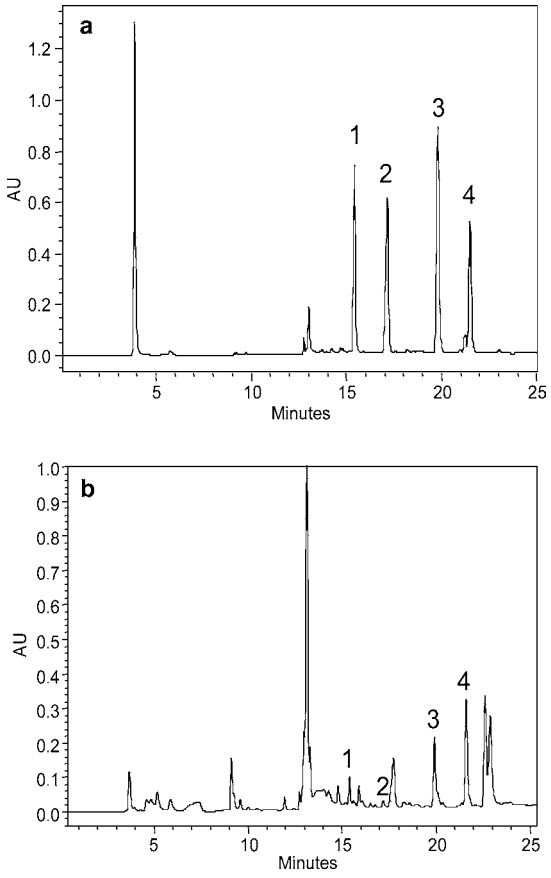


Figure 3. Typical separation of γ-glutamyl peptides and SAC in (a) a standard mixture and (b) an extract from raw garlic by the HPLC method described in the text. Conditions: column, Luna 3 μm C18(2) (150 mm × 4.6 mm); rate, 0.4 mL/min; detection, 208 nm; column temperature, 38 °C; mobile phase, gradient elution as described in the text. Peak identities are as follows: 1, GSMC; 2, SAC; 3, GSAC; 4, GSPC.

RESULTS AND DISCUSSION

In a previous publication[5] we determined the contents of organosulfur compounds in garlic from different cultivars grown in different locations. In conclusion, we found that the purple-type cultivars studied (Gardos, Morasol, Moraluz, and Morado de Santa Monica) showed on average higher contents of GSMC, GSAC, alliin, and methiin but lower contents of isoalliin than cultivars of the white type (Garcua, Gardacho, Thermidrôme, Vigor Supreme, Messidor, Therador, and Ajolvi) or Chinese type (Garpek and Chino Blanco). Therefore, from a health standpoint, the purple-type cultivars would be more appropriate for processing purposes than the other garlic types. Thus, the Gardos cultivar was chosen for the present work. The experimental design in the present work was similar to that previously used[10] to study the effect of processing, with and without fermentation, on the nutritional composition of pickled garlic. The physicochemical and microbiological characteristics during storage for the packed products are given in Table 2 in the Supporting Information. The physicochemical characteristics of the packed products after equilibration remained practically unchanged during storage time (up to 1 year) under the different packing conditions. The pH of the brine in packing RF was always higher than the pH of the other preparations (CNF, PNF, CF, and PF) (4.0 versus 3.5−3.7). The garlic cloves of packing CF or PF had higher water content than those of packing CNF, PNF, or RF (71.5−76.9% versus 65.0−69.8%). As expected, lactobacilli were detected in the fermented packed product except for packing PF, although only in packing RF these microorganisms remained after prolonged storage (around 10^6 CFU/mL after 1 year). Enterobacteria, clostridia, and yeasts were not detected in any of the products, which agrees with previous results.[10]

The changes in individual organosulfur compounds (expressed on a dry basis) during the processing of unfermented and fermented pickled garlic are shown in Figure 4. As

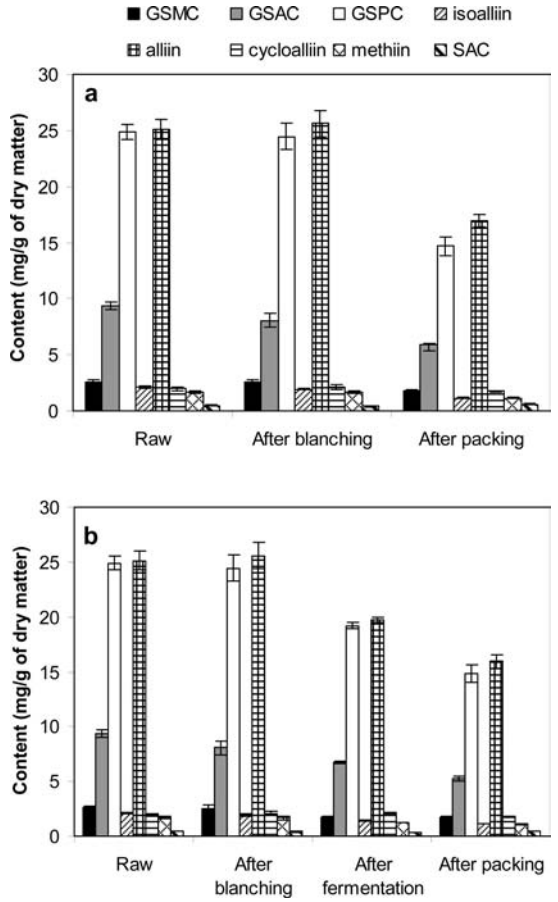


Figure 4. Changes in organosulfur compounds during the processing of pickled garlic processed (a) without and (b) with the fermentation step. Data represent mean values (n = 18 in raw garlic, garlic after blanching or garlic after fermentation; n = 60 or 90 in packed, unfermented or fermented garlic, respectively, corresponding to two or three treatments, each analyzed five times during storage, with analyses of two bottles in triplicate). Error bars indicate 95% confidence intervals.

expected, GSPC and alliin were the major organosulfur compounds in raw garlic, with contents of ≈25 mg/g of dry matter (dm) each, which is lower than the average value (≈ 35 mg/g of dm) found in our previous study.[5] The content of GSAC (9.4 mg/g of dm) was also lower than that of our previous study (22.9 mg/g of dm). The alliin/methiin/isoalliin ratio was 87:6:7, showing a relatively high proportion of isoalliin in comparison with previous studies.[5,6,22] Although other authors[23,24] have also observed relatively high levels of isoalliin (7−11%), this fact might indicate that the garlic cloves had been stored at a low temperature for some time prior to being analyzed for isoalliin, as this compound has been

reported to be formed from GSPC during storage at low temperatures.[25] The SAC content in raw garlic (0.5 mg/g of dm), which was not determined in our previous study, was much higher than the SAC content mentioned by Kodera et al.[7] for raw garlic (30 μg/g of fresh weight), but lower than the level found by Park et al.[26] of 1.7 mg/g of dm. The content of total identified sulfur compounds (TISC) in raw garlic was 68 mg/g of dm.

Blanching in hot water (90 °C for 5 min) did not significantly ($p < 0.05$) affect the individual organosulfur compound contents, with the exception of GSAC and SAC contents, which slightly decreased (Figure 4). However, after the fermentation step, in the case of the product subjected to fermentation, the garlic had lower contents of organosulfur compounds compared with garlic after blanching, with the only exception of the cycloalliin content, which did not change significantly. Leaching could be the main mechanism of most losses. Hydrolysis reactions of the γ-glutamyl peptides would be theoretically possible but quite unlikely considering that the reaction rates of hydrolysis of γ-glutamyl peptides are slow at room temperature (as can be demonstrated during storage time, see below). Microbial degradation of organosulfur compounds by the starter culture (*L. pentosus*) has not been investigated, but by comparing the levels of organosulfur compounds found in the fermented product after the fermentation step with those in the packed unfermented product (the garlic cloves being subjected to the same number of "washes" in both cases) it is deduced that losses due to microbial action, if any, are negligible. In fact, levels (as shown in Figure 4) were slightly higher in the fermented product as result of the higher weight/volume ratio of fermenter (1.8) in comparison with glass bottle (1.4).

Finally, after packing, additional losses in organosulfur compounds except for SAC occurred. In the case of the packed, unfermented product (averaging the two packing treatments, CNF and PNF), the losses in organosulfur compounds except for SAC compared to raw garlic ranged from 11% (cycloalliin) to 45% (isoalliin); on the contrary, the SAC content increased ≈20% in this product. As explained below, this can be attributed to an SAC formation from GSAC hydrolysis during storage time. In the packed, fermented product, when packing RF was excluded, the losses in individual compounds except for SAC were greater than those found in the unfermented product, ranging from 18% (cycloalliin) to 52% (isoalliin) whereas the SAC content did not change significantly ($p < 0.05$). If packing RF is included in the packed, fermented product (Figure 4), the losses were similar to those of the packed, unfermented product. Processing, including packing step, resulted in 42 and 44 mg TISC/g of dm for the fermented and unfermented products, respectively. These values are higher than those reported by Lawson and Wang[14] for chopped garlic aged in 5% acetic acid after two years of storage (24 mg TISC/g of dm).

The packing RF was superior to the other packings with regard to the content of individual organosulfur compounds except for SAC (Figure 5). This fact can be explained partially by the use of the corresponding fermenting brine instead of fresh cover brine for packing, but mainly by the increased stability of the organosulfur compounds during storage due to refrigeration, as illustrated in Table 2. The three γ-glutamyl peptides were significantly degraded during storage at room temperature in the packed, unfermented product, mainly GSPC (49% loss after 1 year storage, both in CNF and PNF). It is

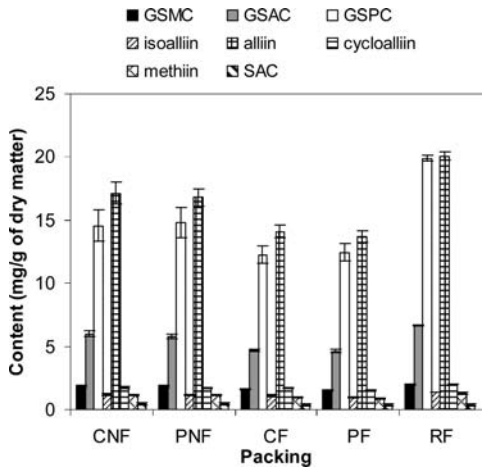


Figure 5. Comparison of levels of organosulfur compounds in the different preparations of packed, pickled garlic studied. Data represent the mean values of five samplings with analyses of two bottles in triplicate ($n = 30$). Error bars indicate 95% confidence intervals.

reasonable to argue that GSMC, GSAC, and GSPC were slowly degraded by chemical hydrolysis during storage to yield the corresponding *S*-alk(en)yl-L-cysteine. This is consistent with the increase in SAC content during storage. In fact, between 2 and 52 weeks storage, the molar conversion of GSAC to SAC was found to be around 1:1 for CNF, PNF, and PF. However, such a conversion was not apparent for CF and RF, as the degradation of GSAC throughout storage was not significant. The contents of *S*-methyl-L-cysteine and *S*-(*trans*-1-propenyl)-L-cysteine, derived from GSMC and GSPC, respectively, were not evaluated in the present work. The involvement of the enzyme γ-glutamyl-transpeptidase in the above-mentioned hydrolysis could be ruled out since the blanching treatment would inactivate this enzyme. In fact, the main purpose of blanching was the inactivation of the enzyme alliinase,[27] whose thermal stability seems to be similar to that of γ-glutamyl-transpeptidase.[28] Of the ACSOs, alliin and isoalliin were the most degraded compounds due to prolonged storage (23−28% loss after 1 year of storage) whereas the changes in cycloalliin and methiin were negligible or nonsignificant. In the packed, fermented product (excluding the packing RF), the losses in organosulfur compounds during storage were in general lower in comparison with the unfermented product, with the only exception of methiin, whose content significantly decreased during prolonged storage (22−25% loss after 1 year).

The effect of pasteurization on the contents of organosulfur compounds can be evaluated by comparison of the values in packing CNF versus packing PNF or in packing CF versus packing PF. It is clear from Figure 5 and Table 2 that the pasteurization treatment did not significantly affect the contents of individual organosulfur compounds.

In summary, from the above results it can be stated that processing affects the contents of organosulfur compounds in pickled garlic, but it is reasonable to assume that a great deal of the health benefits of garlic are maintained following pickling. However, it must be pointed out that although the content of γ-glutamyl peptides, ACSOs, and SAC remains relatively high in pickled blanched garlic, the numerous bioactive compounds produced from alliin and other ACSOs by the action of the enzyme alliiinase cannot be formed in pickled blanched garlic, as alliinase is deactivated. In fact, the objective of blanching treatment is the total deactivation of this enzyme in order to

Table 2. Effect of Storage Time on Organosulfur Compounds in Different Preparations of Packed Pickled Garlic

	organosulfur compound (mg/g of dry matter)[a]							
time (weeks)	GSMC	GSAC	GSPC	isoalliin	alliin	cycloalliin	methiin	SAC
				CNF				
2	2.04 ± 0.26 b	5.97 ± 0.15 b	17.03 ± 0.48 c	1.34 ± 0.09 b	18.86 ± 1.54 c	2.01 ± 0.19 b	1.18 ± 0.17 a	0.45 ± 0.09 a
5	2.02 ± 0.26 b	5.97 ± 0.39 b	17.17 ± 1.02 c	1.38 ± 0.07 b	18.99 ± 1.23 c	1.69 ± 0.05 a	1.21 ± 0.08 a	0.45 ± 0.06 a
8	2.07 ± 0.22 b	6.11 ± 0.21 b	16.98 ± 0.13 c	1.27 ± 0.11 b	18.70 ± 1.23 c	1.67 ± 0.13 a	1.29 ± 0.08 a	0.53 ± 0.02 a
29	1.60 ± 0.05 a	6.83 ± 0.35 c	12.70 ± 0.35 b	0.93 ± 0.40 a	16.12 ± 0.41 b	1.70 ± 0.16 a	1.12 ± 0.07 a	–[b]
52	1.50 ± 0.18 a	5.20 ± 0.15 a	8.93 ± 0.25 a	1.03 ± 0.06 a	13.56 ± 0.28 a	1.65 ± 0.10 a	1.09 ± 0.06 a	0.95 ± 0.04 b
				PNF				
2	2.03 ± 0.07 b	6.39 ± 0.47 b	18.4 ± 0.65 d	1.20 ± 0.05 b	18.0 ± 1.41 c	1.73 ± 0.10 ab	1.16 ± 0.12 a	0.40 ± 0.02 a
5	1.90 ± 0.13 b	5.62 ± 0.31 a	15.8 ± 0.07 c	1.32 ± 0.05 b	18.0 ± 0.65 c	1.63 ± 0.08 a	1.10 ± 0.05 a	0.46 ± 0.01 b
8	2.01 ± 0.08 b	5.73 ± 0.24 ab	17.5 ± 0.64 d	1.25 ± 0.09 b	18.0 ± 0.22 c	1.89 ± 0.16 b	1.14 ± 0.08 a	0.52 ± 0.02 b
29	1.70 ± 0.06 a	5.77 ± 0.15 ab	12.9 ± 0.31 b	1.00 ± 0.07 a	16.4 ± 0.32 b	1.75 ± 0.06 ab	1.16 ± 0.06 a	–
52	1.57 ± 0.11 a	5.47 ± 0.51 a	9.4 ± 0.56 a	0.92 ± 0.10 a	13.9 ± 0.20 a	1.66 ± 0.16 a	1.06 ± 0.09 a	0.99 ± 0.07 c
				CF				
2	1.79 ± 0.12 b	4.72 ± 0.13 ab	13.9 ± 0.28 d	1.48 ± 0.03 c	15.3 ± 0.73 b	1.88 ± 0.09 b	1.04 ± 0.04 b	0.32 ± 0.02 a
5	1.64 ± 0.11 ab	4.95 ± 0.16 b	13.0 ± 0.61 c	1.14 ± 0.08 b	15.1 ± 0.94 b	1.64 ± 0.13 a	0.95 ± 0.07 b	0.40 ± 0.04 b
8	1.56 ± 0.04 a	4.74 ± 0.18 ab	13.9 ± 0.20 d	1.08 ± 0.02 b	14.0 ± 0.30 b	1.63 ± 0.03 a	0.94 ± 0.04 b	0.39 ± 0.01 b
29	1.55 ± 0.08 a	4.65 ± 0.05 ab	11.3 ± 0.18 b	0.89 ± 0.08 a	14.2 ± 0.66 b	1.61 ± 0.09 a	0.97 ± 0.06 b	–
52	1.46 ± 0.11 a	4.35 ± 0.44 a	9.3 ± 0.62 a	0.85 ± 0.06 a	11.8 ± 0.58 a	1.58 ± 0.08 a	0.81 ± 0.05 a	0.74 ± 0.07 c
				PF				
2	1.52 ± 0.06 ab	4.83 ± 0.15 b	14.4 ± 0.20 c	0.99 ± 0.04 b	14.5 ± 0.46 c	1.53 ± 0.10 a	0.93 ± 0.04 b	0.32 ± 0.02 a
5	1.57 ± 0.02 b	4.95 ± 0.16 b	13.6 ± 0.22 c	1.05 ± 0.02 b	15.0 ± 0.28 c	1.53 ± 0.05 a	0.97 ± 0.02 b	0.40 ± 0.02 b
8	1.59 ± 0.06 b	4.43 ± 0.18 a	13.5 ± 0.62 c	1.03 ± 0.04 b	13.7 ± 0.51 b	1.50 ± 0.03 a	0.93 ± 0.08 b	0.38 ± 0.01 b
29	1.53 ± 0.04 ab	4.80 ± 0.13 b	11.6 ± 0.37 b	0.85 ± 0.03 a	13.7 ± 0.27 b	1.52 ± 0.04 a	0.94 ± 0.02 b	–
52	1.46 ± 0.06 a	4.16 ± 0.20 a	9.6 ± 0.56 a	0.85 ± 0.05 a	11.8 ± 0.26 a	1.51 ± 0.06 a	0.70 ± 0.10 a	0.68 ± 0.01 c
				RF				
2	2.02 ± 0.08 a	6.62 ± 0.13 a	19.5 ± 0.27 a	1.38 ± 0.07 a	20.1 ± 0.71 a	2.05 ± 0.06 a	1.28 ± 0.05 a	0.45 ± 0.02 a
5	2.05 ± 0.04 a	6.68 ± 0.01 a	20.6 ± 0.60 b	1.45 ± 0.02 a	20.1 ± 1.19 a	1.98 ± 0.06 a	1.30 ± 0.11 a	0.44 ± 0.02 a
8	1.94 ± 0.11 a	6.76 ± 0.14 a	20.2 ± 0.80 ab	1.40 ± 0.15 a	20.6 ± 0.68 a	2.05 ± 0.09 a	1.35 ± 0.09 a	0.45 ± 0.01 a
29	2.04 ± 0.13 a	6.66 ± 0.26 a	19.6 ± 0.25 a	1.34 ± 0.02 a	19.9 ± 0.31 a	1.99 ± 0.07 a	1.29 ± 0.07 a	–
52	1.96 ± 0.08 a	6.67 ± 0.19 a	19.5 ± 0.28 a	1.38 ± 0.04 a	19.8 ± 0.71 a	1.97 ± 0.06 a	1.23 ± 0.27 a	0.55 ± 0.04 b

[a]Each value is the mean ± standard deviation of 6 determinations (two bottles with analyses in triplicate). For each treatment, means within a column sharing a common letter were not significantly different at the $p < 0.05$ level. CNF, unfermented garlic packed with acidified brine, left at room temperature; PNF, unfermented garlic packed with acidified brine and then pasteurized, left at room temperature; CF, fermented garlic packed with acidified brine, left at room temperature; PF, fermented garlic packed with acidified brine and then pasteurized, left at room temperature; RF, fermented garlic packed with its corresponding fermentation brine, left refrigerated (6–8 °C). [b]– = not analyzed.

eliminate the characteristic pungent flavor of raw garlic and to prevent the appearance of "greening" during storage of product. Both in unfermented and in fermented pickled garlic the concentrations of all compounds except for SAC decreased significantly, mainly as a result of their solubilization into the cover brine (packing step in the unfermented product; fermentation and packing steps in the fermented product). On the other hand, the SAC content increased as a result of GSAC hydrolysis during the storage step, but the increase in comparison with raw garlic was only significant in the unfermented product (≈20% increase). The highest retention of organosulfur compounds except for SAC was obtained in the fermented pickled garlic by packing with the corresponding fermentation brine plus refrigerated storage. This preservation treatment proved to be effective in preventing the degradation of organosulfur compounds during storage (up to 1 year), although refrigeration inhibited the SAC formation. The pasteurization treatment itself had no effect on the concentrations of the organosulfur compounds in pickled garlic. All in all, the information presented in this work could be interesting for pickled garlic producers in order to improve their marketing strategies and for consumers in order to assist them in their purchasing decisions.

■ ASSOCIATED CONTENT

Supporting Information

Table of observed masses of ACSOs and FMOC derivatives of γ-glutamyl peptides and SAC from garlic extracts by HPLC/ESI-MS analyses and table of microbial and physicochemical characteristics for each packing brine, and moisture content of the corresponding garlic cloves during storage. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was supported by the Junta de Andalucia (Spain) through the Excellence Project AGR02495. The Junta de Andalucia is also grateful for the research contract of V.M.B.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACSOs, *S*-alk(en)yl-L-cysteine sulfoxides; APC, aerobic plate count; CF, control fermented pickled garlic; CFU, colony former unit; CNF, control nonfermented pickled garlic; DRCM, differential reinforced clostridial medium; ESI-MS, electrospray ionization mass spectrometer; FMOC, 9-fluorenylmethyl chloroformate; GSAC, γ-L-glutamyl-*S*-allyl-L-cysteine; GSMC, γ-L-glutamyl-*S*-methyl-L-cysteine; GSPC, γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine; HPLC, high-performance liquid chromatography; MRS, de Man, Rogosa and Sharpe agar; OGYE, oxytetracycline glucose yeast extract agar; PCA, plate count agar; PS1, packing solution 1; PS2, packing solution 2; RF, refrigerated fermented pickled garlic; SAC, *S*-allyl-L-cysteine; PF, pasteurized fermented pickled garlic; PNF, pasteurized nonfermented pickled garlic; TISC, total identified sulfur compounds; VRBD, violet red bile dextrose agar

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