JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Effect of Genetic Characteristics and Environmental Factors on Organosulfur Compounds in Garlic (*Allium sativum* L.) Grown in Andalusia, Spain

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ABSTRACT: The content of organosulfur compounds was determined in selected garlic cultivars grown at four locations in Andalusia, Spain. The organosulfur compounds studied were three γ-glutamyl peptides, namely, γ-L-glutamyl-*S*-(2-propenyl)-L-cysteine (GSAC), γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC), and γ-L-glutamyl-*S*-methyl-L-cysteine (GSMC), and four cysteine sulfoxides (alliin, isoalliin, methiin, and cycloalliin). There was a significant effect of the location, cultivar, and garlic ecotype on individual organosulfur compound contents. Purple-type cultivars showed on average the highest contents of GSMC, GSAC, alliin, and methiin but the lowest isoalliin content. The impact of genotype was relatively high for GSAC, whereas this factor hardly contributed to the total variability in alliin and isoalliin content. Planting date had a significant effect on the content of alliin and isoalliin. Discriminant analysis evidenced the ability of organosulfur compounds to distinguish among garlic bulbs from different locations or ecotypes with 81 or 86% accuracy, respectively.

KEYWORDS: Garlic, *Allium sativum* L., organosulfur compounds, genotype, environment, location, cultivar, planting date, chemometrics

INTRODUCTION

Garlic (*Allium sativum* L.) is grown worldwide and its (potential) medical properties have been known for thousand of years. The health properties of garlic depend upon its bioactive compounds, especially its organosulfur compounds.[1] These include three γ-glutamyl peptides, namely, γ-L-glutamyl-*S*-(2-propenyl)-L-cysteine (GSAC), γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine (GSPC), and γ-L-glutamyl-*S*-methyl-L-cysteine (GSMC); their corresponding sulfoxide derivatives, that is, (+)-*S*-(2-propenyl)-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 content of these compounds in garlic bulbs changes during the growth and storage periods.[2,3] Other bioactive organosulfur compounds in garlic are formed from the above-mentioned γ-glutamyl peptides and *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs). When garlic cloves are cut or crushed, the enzyme alliinase (EC 4.4.1.4) cleaves sulfoxides, except cycloalliin, to give pyruvate, ammonia, and thiosulfinates, with the latter being closest to the taste and aroma of the freshly cut garlic.[4] Differences in the ACSO profiles of *Allium* tissues may be due to genetic or environmental factors. Huchette et al.[5] demonstrated that cultivar and sulfur fertilization had significant influence on the alliin accumulation in garlic. Hong et al.[6] demonstrated that the alliin content in a selected garlic cultivar varied in different cultivating areas of Korea. In addition, cultural practices, such as planting date, appear to affect the garlic yield,[7] but their influence on the content of bioactive compounds has not yet been investigated.

Spain is the biggest garlic producer in Europe. Spanish production reached about 158 000 tons in 2009,[8] and a significant percentage (34%) of this production was from Andalusia, southern Spain. However, studies for the characterization of garlic cultivars grown in Spain in terms of bioactive compounds have not yet been carried out. This information would be valuable for garlic processors (e.g., pickled garlic producers) to improve the quality of processed garlic and to assist the increasing demand for natural food with a high added health value for consumption.

The main objective of the present study was to perform a comparative study on the content of organosulfur compounds (γ-glutamyl peptides and ACSOs) in garlic, by analyzing the influence of genetic (type and cultivar) and environmental (location and planting date) factors. A strong genotype influence may allow breeders to develop stable varieties with higher or lower levels of bioactive components depending upon nutritional and/or technological needs. Another aim of the study was to assess the usefulness of chemometric techniques [principal component analysis (PCA) and discriminant analysis (DA)] based on the organosulfur compound content for product differentiation.

MATERIALS AND METHODS

Chemicals. Methiin was synthesized as described by Shen and Parkin[9] using *S*-methyl-L-cysteine (Sigma) as the starting material.

Received: November 22, 2010
Accepted: December 21, 2010
Revised: December 20, 2010
Published: January 19, 2011

Isoalliin was isolated from onion following the method by Carson et al.[10] Cycloalliin was obtained from cyclization of isoallin as described in ref 10. GSMC, GSAC, and GSPC were isolated from Chinese chive seeds (Thompson and Morgan, Suffolk, U.K.) according to the method described by Lawson et al.,[11] with slight modifications, using a semi-preparative Luna 5 μm C18(2) (250 × 10 mm inner diameter, Phenomenex, Torrance, CA) high-performance liquid chromatography (HPLC) column. The chromatographic conditions were as follows: eluent A, 0.1% trifluoroacetic acid (TFA) in water, and eluent B, 0.1% TFA in acetonitrile; linear gradient, 0−40% B in 70 min; flow rate, 4 mL/min; and absorbance detection, 235 nm. Alliin was purchased from Sigma-Aldrich (St. Louis, MO). Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers.

Plant Material. The study was carried out with 13 selected garlic cultivars from four locations in Andalusia, namely, Cordoba (37° 51′ N, 4° 51′ W, 102 masl), Santaella (37° 33′ N, 4° 50′ W, 263 masl), Granada (37° 10′ N, 3° 37′ W, 639 masl), and Mengíbar (37° 58′ N, 3° 48′ W, 287 masl). Four cultivars (Gardos, Morado de Santa Mónica, Moraluz, and Morasol) were of the "purple" type; seven cultivars (Garcua, Gardacho, Thermidrôme, Vigor Supreme, Messidor, Therador, and Ajolvi) were of the "white" type; and two cultivars (Garpek and Chino Blanco) were of the "Chinese" type. Planting dates ranged from December 4 to 19, 2007. In addition, to study the influence of the planting date on bioactive compounds, garlic cultivars of the "purple" type from Cordoba were also planted on October 19, 2007 (Gardos cultivar) and November 14, 2007 (Gardos, Morado de Santa Mónica, Moraluz, and Morasol cultivars). All cultivars were planted in four-row plots arranged in a randomized complete block design with four replications, in agronomic experimental fields in the Andalusian agricultural trial network (RAEA) in each location. Each plot was 3 m long, with a spacing of 50 cm between rows and 7 cm between plants within a row (285 000 plants/ha), except in Santaella, where the distance between plants was 8 cm (278 000 plants/ha). The agronomic practices (N fertilization, pre-emergence herbicide, etc.) were similar at each location. All plants were harvested per plot at maturity on June 2008. A total of 10 plants were sampled randomly from the two external rows of each plot, and three of them were selected. Therefore, 12 bulbs (3 bulbs × 4 plots) per cultivar were taken from each location. After a drying period in an open space for 6−8 days, bulbs were sent to our laboratories for analysis of their organosulfur compounds.

There was some viral disease in the Thermidrôme cultivar from Cordoba throughout the growth period, which significantly affected the bulb yield. Therefore, we decided not to include this particular sample in the study.

The temperature between planting and harvesting was similar in Santaella (T_{min}, 8.9 °C; T_{max}, 21.6 °C; and T_{mean}, 14.8 °C) and Córdoba (8.1, 21.5, and 14.3 °C, respectively), which was higher than those of Mengíbar (5.5, 21.2, and 13.0 °C, respectively) and Granada (4.9, 19.7, and 12.0 °C, respectively). The total precipitation from planting to harvest was highest in Córdoba (573.2 mm) and lowest in Granada (222.4 mm), whereas it was 378.2 mm in Mengíbar and 491.6 mm in Santaella.

Sample Preparation. For each cultivar, 5−10 bulbs were separately crushed into garlic powder to study intracultivar variability (or variability between bulbs). In addition, to study the variation between cloves, two cloves were separated from a number of bulbs of different cultivars and independently crushed into garlic powder. The garlic powder was prepared as follows: garlic cloves were frozen in liquid nitrogen, immediately peeled, and kept at −30 °C in a freezer until lyophilization. After this step, the resulting lyophilizate 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.

Analysis of Organosulfur Compounds. The organosulfur compounds (GSMC, GSAC, GSPC, methiin, alliin, isoalliin, and cycloalliin) were analyzed by HPLC according to the method described by Ichikawa et al.,[3] with slight modifications. In a 25 mL flask, garlic powder (0.5 g) was added to 15 mL of a 90% methanol solution containing 0.01 N 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 and Co., Tuttlingen, Germany). The supernatant was analyzed by HPLC using two different chromatographic conditions: (1) GSAC and GSPC were analyzed using a Jasco model PU-2089 pump coupled with a Jasco model AS-2057 autosampler, a Jasco model UV-2075 ultraviolet−visible (UV−vis) detector, and a computer with Jasco-Borwin chromatography software (Jasco Corp., Tokyo, Japan). The HPLC conditions were the following: column, Luna 5 μm C18(2) (150 × 4.6 mm inner diameter, Phenomenex, Torrance, CA); column temperature, ambient (22−23 °C); flow rate, 0.8 mL/min; mobile phase, 50 mM phosphate buffer (pH 2.6)/methanol (80:20, v/v); wavelength, 205 nm; and injection volume, 10 μL. (2) GSMC, alliin, isoalliin, methiin, and cycloalliin were analyzed using a Waters 2690 separation module (Waters Association, Milford, MA) connected to a Waters 996 photodiode array detector and controlled with Millenium 32 software (Waters). Chromatographic conditions were as follows: 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) of concentrated (85%) phosphoric acid; wavelength, 210 nm; and injection volume, 1 μL. All determinations were performed at least in duplicate.

Statistical Analysis. All of the data were compiled and calculated using a combination of Microsoft Excel 2002 (Microsoft Corporation, Redmond, WA) and Statistica software, version 7.0 (Statsoft, Inc., Tulsa, OK). Analysis of variance (ANOVA) was performed to assess the effect of the environment (location and planting date), cultivar, and garlic type on the levels of organosulfur compounds. The means were compared using the Student−Newman−Keuls test. Significant differences were determined at the $p < 0.05$ level. Variance component analysis was conducted to quantify the contributions of location, genotype, and their interaction to the total variance in the levels of organosulfur compounds. Location and cultivar were considered as random factors. The ANOVA method was used to estimate the variance components and to test their significance. Simple linear correlation was used to determine the relationship among different compounds for all samples analyzed. Chemometric techniques, such as PCA and DA, were applied to the data set to assess their potential in classifying the garlic samples according to garlic type, cultivar, or growing location. The DA model was built following the forward stepwise option. The values of *F* to enter or remove were fixed at 1 and 0, respectively. The minimum tolerance was fixed at 0.01. Prior probabilities were established in proportion to the number of samples in each group.

RESULTS AND DISCUSSION

Before the influence of genetic and environmental (location and planting date) factors on the organosulfur compounds of garlic was analyzed, the variation between cloves and bulbs was investigated (Table 1). Analytical errors (average coefficient of variation for triplicate analyses of a single sample) for the compounds analyzed ranged from 2.8% (GSPC) to 7.7% (isoalliin). The variation between cloves of the same bulb was higher than analytical error for each compound ranging from 8.7% (alliin) to 25.7% (isoalliin). In turn, the variation between cloves was less than the variation between bulbs, with the latter ranging from 15.9% (GSPC) to 36.4% (isoalliin). Significant variations in the

Table 1. Comparison of the Variation in the Contents of Organosulfur Compounds between Garlic Cloves and between Garlic Bulbs

	average coefficient of variation (%)						
	GSMC	GSAC	GSPC	isoalliin	alliin	cycloalliin	methiin
analytical error	6.3	3.6	2.8	7.7	5.6	6.8	5.9
variation between two cloves from the same bulb (n = 32)	18.3	14.6	11.1	25.7	8.7	12.4	8.5
variation between five bulbs from the same cultivar and same location (n = 46)	30.8	27.0	15.9	36.4	21.1	28.8	27.6

Table 2. Average Value, Standard Deviation (SD), and Range for the Levels of Organosulfur Compounds (mg/g of dm) for All Garlic Samples (Bulbs) Analyzed[a]

	average	SD	range
GSMC	2.57	1.89	0.10−12.35
GSAC	22.90	10.27	2.27−65.07
GSPC	34.56	10.78	14.28−90.35
isoalliin	0.27	0.23	0.01−1.21
alliin	34.65	15.30	2.71−73.47
cycloalliin	2.56	1.21	0.13−7.53
methiin	3.44	1.66	0.38−9.49
total ACSOs[b]	38.36	16.76	3.11−80.75

[a] The number of samples analyzed was 279. [b] Total cysteine sulfoxides, except cycloalliin.

Table 3. Correlation Coefficients between Individual Organosulfur Compounds for All Garlic Samples from Different Locations[a]

variable	GSMC	GSAC	GSPC	isoalliin	alliin	cycloalliin	methiin
GSMC		0.40[b]	−0.14[c]	−0.18[d]	0.37[b]	0.43[b]	0.61[b]
GSAC			0.28[b]	−0.19[d]	0.23[b]	0.10	0.46[b]
GSPC				0.30[b]	0.35[b]	0.11	0.16[d]
isoalliin					0.51[b]	0.45[b]	0.12[c]
alliin						0.77[b]	0.79[b]
cycloalliin							0.66[b]

[a] Number of cases included in the analysis = 279. [c] p value < 0.05. [d] p value < 0.01. [b] p value < 0.001.

content of organosulfur compounds between garlic cloves and between garlic bulbs have been previously reported. Lawson et al.[12] found variations in the contents of allicin and other thiosulfinates, 4.4−9.7% (between cloves) and 7.3−16.6% (between bulbs). Hughes et al.[13] found that levels of alliin and two γ-glutamyl peptides were significantly higher in outer than inner cloves. Variations within and between bulbs may occur as a result of the microenvironment in which the bulb is grown.[13]

Total Variation in the Levels of Organosulfur Compounds. The average values and ranges for the levels of organosulfur compounds for all garlic samples from the different cultivars and locations are summarized in Table 2. A large variation can be observed for all compounds. Alliin, GSPC, and GSAC were the major organosulfur compounds, with average contents of 34.65, 34.56, and 22.90 mg/g of dry matter (dm), whereas the level of isoalliin (0.27 mg/g of dm) was the lowest. Varying contents for the γ-glutamyl peptides and ACSOs are in agreement with the results of other authors.[3,14,15] The total content of ACSOs (except cycloalliin, which is not cleaved by the enzyme alliinase when garlic cloves are cut or crushed) was, on average, 38.36 mg/g of dm, corresponding to 15.9 mg/g of fresh weight (fw), assuming a moisture content in garlic of 58.58%,[16] which is within the range (10.55−30.22 mg/g of fw) reported by González et al.[17] for different garlic cultivars from Mendoza, Argentina. The wide range of pungency precursor compounds (alliin, methiin, and isoalliin) indicates a large variation in the sensory quality related to the flavor of garlic samples analyzed in the present study. On average, alliin comprises about 85% of the ACSOs, with methiin making up about 8%, whereas cycloalliin and isoalliin are responsible for approximately 6 and 0.7%, respectively. Excluding cycloalliin, the alliin/methiin/isoalliin ratio was 90:9:1, which differs from that found by other authors, such as 84:5:11,[18] 76:14:10,[19] and 83:16:1.[20] A relatively high proportion of isoalliin (18−39%) was reported by González et al.[17] in different garlic cultivars, which were stored at 0 °C prior to analysis. Because isoalliin has been demonstrated to be formed from GSPC when garlic is stored at low temperatures,[13] the relatively high level of isoalliin reported in the literature, contrary to our low level (1%), could be a consequence of low-temperature storage prior to analysis. Our cycloalliin proportion (6%) was similar to values (3−5%) reported by Ichikawa et al.[3] It has been demonstrated[21] that cycloalliin is formed from isoalliin during garlic storage at room temperature (23 °C).

Correlations between the contents of all bioactive compounds in all samples analyzed are shown in Table 3. The strongest correlations were observed between the contents of alliin and methiin (r = 0.79, p < 0.001) and between alliin and cycloalliin (r = 0.77, p < 0.001). The correlation between isoalliin and alliin was relatively high (r = 0.51, p < 0.001), which is in agreement with that by González et al.[17] However, a significant correlation between alliin and methiin was not found by these authors. Methiin was relatively highly correlated with its precursor GSMC (r = 0.61, p < 0.001), but correlations between alliin and its precursor GSAC or between isoalliin and its precursor GSPC were weaker (r = 0.23 and 0.30, respectively).

Location and Cultivar Effects. The garlic samples harvested on the different locations showed significant average differences in the organosulfur compounds (Table 4). In general, garlic samples from Córdoba and Santaella (the distance between these locations is only about 50 km) showed the highest content of organosulfur compounds. The amount of GSAC, alliin, cycloalliin, and total ACSOs, except cycloalliin (pungency), were not significantly different between these two locations. It is worth noting that the difference between the average pungency levels for garlic from Cordoba/Santaella and that from Mengíbar or Granada was higher than 40%.

The above results appear to be related to the weather conditions in the locations. Cysteine sulfoxides showed very highly significant (p < 0.001) correlations with the average temperature between planting and harvesting (r values ranged from 0.44 for cycloalliin to 0.70 for alliin) and with total precipitation between planting and harvesting (r = 0.33−0.68), whereas the γ-glutamyl

Table 4. Mean Values[a] of the Contents of Organosulfur Compounds (mg/g of dm) in Garlic Grown at Four Different Locations

location	GSMC	GSAC	GSPC	isoalliin	alliin	cycloalliin	methiin	total ACSOs (pungency)[b]
Cordoba	1.81 a	22.72 b	42.34 c	0.51 d	45.60 c	3.02 b	3.41 b	49.52 c
Granada	2.52 b	26.49 c	30.04 a	0.09 a	20.84 a	2.02 a	2.63 a	23.55 a
Santaella	3.45 c	23.34 b	31.78 ab	0.32 c	45.04 c	3.22 b	4.90 c	50.25 c
Mengíbar	2.42 b	18.95 a	34.58 b	0.14 b	26.31 b	1.94 a	2.75 a	29.20 b

[a] Mean values with different letters within a column are significantly different according to the Student−Newman−Keuls test ($p < 0.05$). [b] Total cysteine sulfoxides, except cycloalliin.

Table 5. Mean Values[a] of the Contents of Organosulfur Compounds (mg/g of dm) in Garlic According to Cultivar and Type Averaged over Four Locations

main effect	GSMC	GSAC	GSPC	isoalliin	alliin	cycloalliin	methiin	total ACSOs (pungency)[b]
cultivar								
Morasol	4.74 d	30.47 ef	32.86 a−d	0.19 ab	37.12 b−d	2.62 b−e	4.04 cd	41.35 b−d
Gardos	2.84 bc	22.03 b−d	40.88 cd	0.23 a−c	40.75 cd	3.10 de	3.88 b−d	44.89 cd
Moraluz	3.61 c	29.11 d−f	32.89 a−c	0.23 a−c	42.57 d	3.44 e	4.40 d	47.21 d
Morado de Santa Monica	2.80 bc	23.78 b−e	29.60 ab	0.21 ab	35.85 a−d	2.80 c−e	3.81 b−d	39.86 a−d
Messidor	1.46 ab	25.85 c−f	42.87 d	0.22 a−c	27.65 ab	1.91 a−c	3.22 a−d	31.09 ab
Garcua	1.15 a	20.98 bc	38.77 b−d	0.37 c−e	32.86 a−d	2.46 b−e	3.23 a−c	36.45 a−d
Vigor Supreme	1.05 a	21.63 b−d	36.25 a−d	0.32 b−d	30.44 a−c	2.12 a−d	2.48 a	33.23 ab
Thermidrôme	2.74 bc	31.62 f	36.15 a−d	0.20 ab	30.81 a−c	1.38 a	4.47 d	35.49 a−c
Therador	2.06 ab	16.75 b	32.28 a−c	0.09 a	26.54 ab	1.83 a−c	2.93 a−c	29.56 ab
Gardacho	0.93 a	20.70 bc	36.05 ad−	0.31 b−d	25.82 a	1.67 ab	2.23 a	28.35 a
Ajolvi	2.43 a−c	22.85 b−d	36.26 a−d	0.19 ab	25.92 a	1.22 a	2.00 a	28.10 a
Chino Blanco	2.08 ab	9.09 a	29.05 ab	0.38 de	30.94 ab	2.47 b−d	2.07 a	33.40 ab
Garpek	1.74 ab	6.70 a	27.92 a	0.47 e	34.48 a−d	3.03 de	2.67 ab	37.62 a−d
type								
purple	3.51 b	26.47 c	33.94 b	0.22 a	39.13 c	2.99 b	4.04 c	43.39 b
white	1.49 a	23.39 b	37.66 c	0.27 b	29.12 a	1.89 a	3.00 b	32.39 a
Chinese	1.92 a	7.93 a	28.50 a	0.43 c	32.66 b	2.74 b	2.36 a	35.45 a

[a] Mean values with different letters within a column for each effect are significantly different according to the Student−Newman−Keuls test ($p < 0.05$). [b] Total cysteine sulfoxides, except cycloalliin.

peptides showed a weaker correlation (GSPC: $r = 0.19$, $p < 0.01$ and $r = 0.33$, $p < 0.001$, respectively) or no correlation at all (GSAC or GSMC: $r < 0.1$, $p > 0.1$). Previous knowledge of the variation in garlic organosulfur compounds among growing locations is scarce. Huchette et al.[5] determined the alliin content in three garlic cultivars grown in France and Spain under similar growing protocols and found a significant higher alliin content in Spain.

The content of organosulfur compounds in 13 garlic cultivars averaged over four locations is shown in Table 5. There were statistically significant differences among the cultivars in all compounds analyzed. Purple-type cultivars (Morasol, Gardos, Moraluz, and Morado de Santa Monica) showed on average the highest content of GSMC, GSAC, alliin, methiin, and pungency but the lowest content of isoalliin. The isoalliin level in garlic appears to be related to the formation of "greening" discoloration,[22−24] which is an unwanted effect for the garlic processing industry (e.g., the manufacture of garlic puree). Therefore, it appears that the purple-type cultivars would be more appropriate for processing purposes than the other garlic types. Although only two Chinese-type cultivars were included in the present study, ANOVA revealed that, on average, the Chinese-type cultivars had higher alliin, cycloalliin, and isoalliin levels but lower GSAC, GSPC, and methiin levels than the white-type cultivars. In particular, the GSAC levels in the Chinese-type cultivars were about one-third of those in the white- or purple-type cultivars.

The above pungency results are in agreement with Pardo et al.,[25] who found the most pungent (evaluated by a panel test) to be the purple-type cultivar, Morasol, in comparison to other selected cultivars of the white and Chinese types. In our study, the organosulfur compound content in the Morasol cultivar was not significantly different from the rest of the purple-type cultivars analyzed, with the exception of the GSMC content, which was higher in the Morasol cultivar. However, the pungency of this cultivar tended to vary widely according to the location (Figure 1). Of the purple-type cultivars, the widest variation in pungency because of the grown location was observed in Gardos, whereas the most stable cultivar among the grown locations was Morado de Santa Monica.

Variance component analysis showed that the contribution of genotype to the total variation was relatively high for GSMC (34%) and GSAC (45%) but low for alliin or pungency (<10%) and not significant for GSPC and isoalliin (Figure 2). Alliin and isoalliin levels and, consequently, pungency were mostly determined by environmental (location) factors (≈60%), whereas for GSMC and GSAC, the impact of the location was low or negligible. The contribution of the genotype−environment interaction to the total variation was high (47%) for GSPC, whereas a

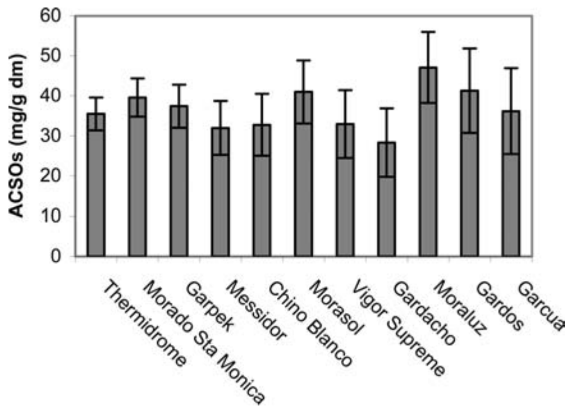

Figure 1. Average content of total ACSOs, except cycloalliin, of garlic cultivars grown in Cordoba, Santaella, Granada, and Mengíbar in order of increasing variation among locations. The error bars represent standard error.

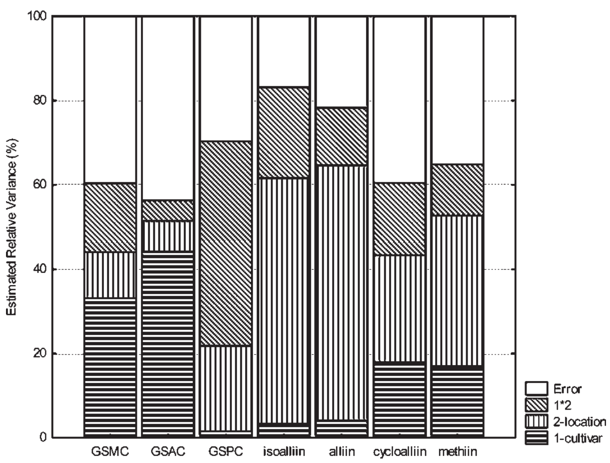

Figure 2. Contribution of cultivar, location, and the interaction term between cultivar and location to the variation of the levels of organosulfur compounds in garlic samples.

negligible contribution (5%) of this interaction was found for GSAC. This is supported by a significantly high correlation between the GSAC levels in garlic from different locations (data not shown). For the remaining compounds, the contribution of the interaction term was <20%. These findings suggest that GSAC may be a target for selection in plant breeding to develop new garlic cultivars with enhanced health properties. GSAC is the precursor compound of *S*-allylcysteine (SAC), which is generated through hydrolysis in the processing of garlic and has been reported to have antioxidant, anti-cancer, anti-hepatopathic, and neurotrophic activities.[26] However, the error term measuring the residual variation that cannot be explained by genotype, location, or their interaction was relatively high for this compound (Figure 2).

Effects of Planting Date. The planting date had a significant effect on the content of alliin and isoalliin in the four garlic cultivars investigated (Table 6). A substantial increase in isoalliin and alliin was observed between early planting in November and the normal planting date of December. The changes in the bioactive compounds between the two early planting dates of October and November as shown in the Gardos cultivar were not significant, with the exception of the GSAC content. GSMC and methiin levels were affected by the planting date in the Moraluz cultivar, but the effect was not significant for the remaining cultivars. The cycloalliin content was affected by the planting date in the Morado de Santa Monica and Moraluz cultivars. The GSPC content increased markedly from November to December in the Gardos cultivar, but the opposite effect was observed in the Morado de Santa Monica cultivar.

The above results indicated the positive effect of the normal planting date of December for higher levels of organosulfur compounds in garlic. However, the early planting dates would result in a higher garlic yield.[7]

Chemometric Study of Organosulfur Compounds in Garlic. PCA is a well-known technique for reducing the dimensionality of the data, by calculating a number of components that best describe the differences between the samples and allowing visualization of clusters and outliers. In the present study, PCA was used to derive the first two principal components from the data set and to examine the possible grouping of samples. The first principal component (PC1) explained 45.41%, and the second principal component (PC2) explained 23.21% of the total variance in the data set. The first two principal components thus accounted for 68.6% of the total variance. However, the score plot of the samples as a function of PC1 versus PC2 did not show a clear discrimination between cases based on the growing location, garlic type, or cultivar (data not shown).

DA was also applied to the data set. Table 7 shows the DA classification rates (percentage classification) of garlic samples according to the growing location. The model produced an overall correct classification rate of 81%. These results showed that differences existing between garlic samples from the different locations could be captured by composition in organosulfur compounds and revealed by DA. Incorrect answers within the Córdoba group were mainly due to their classification as belonging to the Santaella group and vice versa. Similarly, incorrectly classified samples from Granada were mostly assigned to the Mengíbar group and vice versa. A canonical analysis was also carried out to study the contribution of each variable to the discrimination among groups. The procedure also determined the corresponding canonical variables. Plotting the loads of the samples on the plane defined by the first two canonical variables made it possible to visualize the tendency of the points to separate into four groups (Figure 3). The variables that most contributed to discrimination in the canonical function 1 were alliin, cycloalliin, and isoalliin. In function 2, the most outstanding contributions were from methiin and GSAC.

The composition of organosulfur compounds was also used to discriminate among garlic ecotypes. In this case, the DA classification matrix showed 86, 85, and 86% correct answers for purple, white, and Chinese types, respectively, with a total of 86% correct answers for all types (data not shown). As result, the score plot of the garlic samples in the space defined by the two canonical functions (Figure 4) showed a better tendency of the samples to separate into groups than that exhibited in Figure 3. The most outstanding contributions were due to methiin and alliin (function 1) and methiin and GSAC (function 2).

Finally, DA was also used to discriminate among garlic cultivars, but results were not satisfactory. An overall rate of only 51% correct classification was obtained, and no evident segregation between cases according to cultivars was observed (data not shown).

In summary, the content of health-related organosulfur compounds was determined in garlic from different cultivars grown

Table 6. Mean Values[a] of the Contents of Organosulfur Compounds (mg/g dm) in Different Garlic Cultivars from the Same Location (Cordoba) According to the Planting Date

cultivar	planting date	GSMC	GSAC	GSPC	isoalliin	alliin	cycloalliin	methiin	total ACSOs (pungency)[b]
Gardos									
	Oct 2007	2.17 a	17.60 a	35.54 a	0.15 a	34.30 a	2.72 a	3.44 a	37.90 a
	Nov 2007	2.37 a	22.73 b	34.85 a	0.11 a	33.26 a	3.06 ab	3.72 a	37.09 a
	Dec 2007	2.18 a	25.99 b	63.12 b	0.48 b	49.86 b	3.51 a	3.89 a	54.23 b
Morado de Santa Monica									
	Nov 2007	2.11 a	17.13 a	37.87 b	0.12 a	33.93 a	2.44 a	3.36 a	37.41 a
	Dec 2007	1.91 a	19.84 a	24.70 a	0.42 b	42.86 b	3.10 b	3.30 a	46.58 b
Moraluz									
	Nov 2007	1.38 a	25.33 a	36.54 a	0.14 a	29.18 a	1.97 a	2.40 a	31.73 a
	Dec 2007	2.60 b	35.13 a	39.81 a	0.46 b	52.71 b	3.19 b	4.21 b	57.37 b
Morasol									
	Nov 2007	2.93 a	24.13 a	38.15 a	0.13 a	36.22 a	2.66 a	4.12 a	40.47 a
	Dec 2007	2.74 a	29.08 a	38.57 a	0.44 b	47.03 b	2.82 a	4.02 a	51.48 b

[a] Mean values with different letters within a column for each cultivar are significantly different according to the Student−Newman−Keuls test ($p < 0.05$). [b] Total cysteine sulfoxides, except cycloalliin.

Table 7. DA Classification Matrix of Garlic Samples According to Their Growing Location

	predicted group membership				
actual group	Cordoba	Granada	Santaella	Mengíbar	percent correct (%)
Cordoba	56	0	7	6	81
Granada	1	53	4	10	78
Santaella	11	4	56	2	77
Mengíbar	0	6	2	61	88
total	68	63	69	79	81

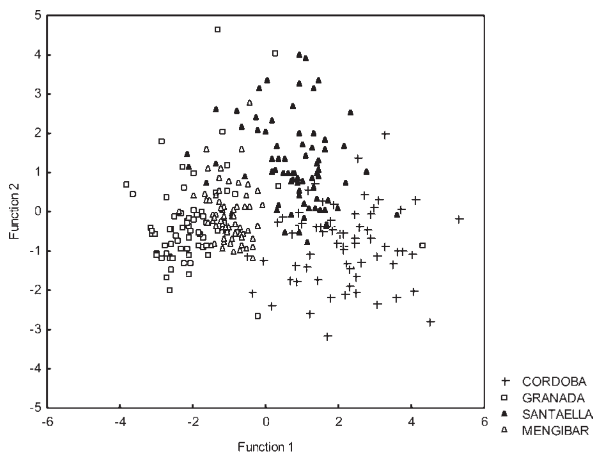

Figure 3. Plot of garlic sample scores as a function of the two first canonical discriminant functions, according to growing locations.

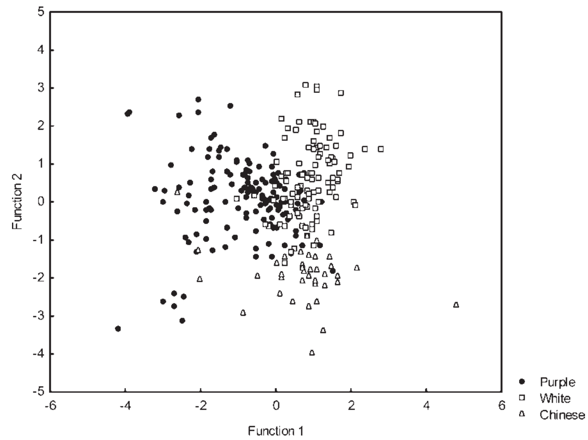

Figure 4. Plot of garlic sample scores as a function of the two canonical discriminant functions, according to garlic types.

in different locations. The levels of individual organosulfur compounds were affected by cultivar and location. The impact of genotype was high but the impact of the environment and the interaction between genotype and environment was low for GSAC, indicating that this compound may be a target for selection in plant breeding. However, the possibility to completely control the level of this compound in garlic is limited because of residual variation as a result of variation between bulbs. The impact of genotype was low for alliin and isoalliin, which are the most important compounds for the food-processing industry, because they are the main precursors of the pungent flavor and greening discoloration, respectively. Nevertheless, the purple-type cultivars showed on average more favorable characteristics than the white or Chinese types, regarding higher levels of GSAC and alliin and lower levels of isoalliin. Our study demonstrated for the first time that the planting date had a clear influence on alliin and isoalliin levels, being higher in the normal (December) planting dates; however, the influence on the other organosulfur compounds appeared to depend upon the garlic cultivar. The analysis of organosulfur compounds combined with chemometrics (DA) achieved 86% accuracy in the distinction between the three different ecotypes.

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Funding Sources

This work was supported by the Junta de Andalusia (Spain) through Excellence Project AGR02495. The Junta de Andalusia is also thanked for the research contract of Victor Manuel Beato.

REFERENCES

(1) Iciek, M.; Kwiecien, I.; Wlodek, L. Biological properties of garlic and garlic-derived organosulfur compounds. *Environ. Mol. Mutagen.* **2009**, *50*, 247–265.

(2) Matsuura, H.; Inagaki, M.; Maeshige, K.; Ide, N.; Kajimura, Y.; Itakura, Y. Changes in contents of γ-glutamyl peptides and fructan during growth of *Allium sativum*. *Planta Med.* **1996**, *62*, 70–71.

(3) Ichikawa, M.; Ide, N.; Yoshida, J.; Yamaguchi, H.; Ono, K. Determination of seven organosulfur compounds in garlic by high-performance liquid chromatography. *J. Agric. Food. Chem.* **2006**, *54*, 1535–1540.

(4) Block, E. The organosulfur chemistry of the genus *Allium*. Implications for the organic chemistry of sulfur. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1135–1178.

(5) Huchete, O.; Kahane, R.; Auger, J.; Arnault, I.; Bellamy, C. Influence of environmental and genetic factors on the alliin content of garlic bulbs. *Acta Hortic.* **2005**, *688*, 93–99.

(6) Hong, G. H.; Lee, S. K.; Moon, W. Alliin and fructan contents in garlics, by cultivars and cultivating areas. *J. Korean Soc. Hortic. Sci.* **1997**, *38*, 483–488.

(7) Kilgori, M. J.; Magaji, M. D.; Yakubu, A. I. Effect of plant spacing and date of planting on yield of two garlic (*Allium sativum* L.) cultiuvars in Sokoto, Nigeria. *Am.-Eurasian J. Agric. Environ. Sci.* **2007**, *2*, 153–157.

(8) Ministry of the Environment and Rural and Marine Affairs (MARM). *Surface Area and Crop Production Forecasts*; MARM: Madrid, Spain, 2010.

(9) Shen, C.; Parkin, K. L. In vitro biogeneration of pure thiosulfinates and propanethial-*S*-oxide. *J. Agric. Food. Chem.* **2000**, *48*, 6254–6260.

(10) Carson, J. F.; Lundin, R. E.; Lukes, T. M. The configuration of (+)-*S*-(1-propenyl)-L-cysteine *S*-oxide from *Allium cepa*. *J. Org. Chem.* **1966**, *31*, 1634–1635.

(11) Lawson, L. D.; Wang, Z.-Y. L.; Hughes, B. G. γ-Glutamyl-*S*-alkylcysteines in garlic and other *Allium* spp.: Precursors of age-dependent *trans*-1-propenyl thiosulfinates. *J. Nat. Prod.* **1991**, *54*, 436–444.

(12) Lawson, L. D.; Wood, S. G.; Hughes, B. G. HPLC analysis of allicin and other thiosulfinates in garlic clove homogenates. *Plant Med.* **1991**, *57*, 263–270.

(13) Hughes, J.; Collin, H. A.; Tregova, A.; Tomsett, A. B.; Cosstick, R.; Jones, M. G. Effect of low storage temperature on some of the flavour precursors in garlic (*Allium sativum*). *Plant Foods Hum. Nutr.* **2006**, *61*, 81–85.

(14) Mütsch-Eckner, M.; Sticher, O. Reversed-phase high-performance liquid chromatography of *S*-alk(en)yl-L-cysteine derivatives in *Allium sativum* including the determination of (+)-*S*-allyl-L-cysteine sulphoxide, γ-L-glutamyl-*S*-allyl-L-cysteine and γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine. *J. Chromatogr.* **1992**, *625*, 183–190.

(15) Kubec, R.; Dadáková, E. Chromatographic methods for determination of S-substituted cysteine derivatives—A comparative study. *J. Chromatogr., A* **2009**, *1216*, 6957–6963.

(16) Agricultural Research Service (ARS), United States Department of Agriculture (USDA). USDA Nutrient Database for Standard Reference, Release 23. *Nutrient Data Laboratory Home Page*; USDA: Washington, D.C., 2010 (http://www.nal.usda.gov/fnic/foodcomp).

(17) González, R. E.; Soto, V. C.; Sance, M. M.; Camargo, A. B.; Galmarini, C. R. Variability of solids, organosulfur compounds, pungency and health-enhancing traits in garlic (*Allium sativum* L.) cultivars belonging to different ecophysiological groups. *J. Agric. Food. Chem.* **2009**, *57*, 10282–10288.

(18) Yoo, K. S.; Pike, L. M. Determination of flavour precursor compound *S*-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci. Hortic.* **1998**, *75*, 1–10.

(19) Krest, I.; Glodek, J.; Keusgen, M. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food. Chem.* **2000**, *48*, 3753–3760.

(20) Hornícková, J.; Kubec, R.; Cejpek, K.; Velísek, J.; Ovesná, J.; Stavelíková, H. Profiles of *S*-alk(en)ylcysteine sulfoxides in various garlic genotypes. *Czech J. Food Sci.* **2010**, *28*, 298–308.

(21) Ichikawa, M.; Ide, N.; Ono, K. Changes in organosulfur compounds in garlic cloves during storage. *J. Agric. Food. Chem.* **2006**, *54*, 4849–4854.

(22) Lukes, T. M. Factors governing the greening of garlic puree. *J. Food Sci.* **1986**, *51* (1577), 1582.

(23) Imai, S.; Akita, K.; Tomotake, M.; Sawada, H. Model studies on precursor system generating blue pigment in onion and garlic. *J. Agric. Food. Chem.* **2006**, *54*, 848–852.

(24) Kubec, R.; Velísek, J. *Allium* discoloration: The color-forming potential of individual thiosulfinates and amino acids: Structural requirements for the color-developing precursors. *J. Agric. Food. Chem.* **2007**, *55*, 3491–3497.

(25) Pardo, J. E.; Escribano, J.; Gómez, R.; Alvarruiz, A. Physical—chemical and sensory quality evaluation of garlic cultivars. *J. Food Qual.* **2007**, *30*, 609–622.

(26) Kodera, Y.; Suzuki, A.; Imada, O.; Kasuga, S.; Sumioka, I.; Kanezawa, A.; Taru, N.; Fujikawa, M.; Nagae, S.; Masamoto, K.; Maeshige, K.; Ono, K. Physical, chemical, and biological properties of *S*-allylcysteine, an amino acid derived from garlic. *J. Agric. Food. Chem.* **2002**, *50*, 622–632.