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Free Amino Acid and Cysteine Sulfoxide Composition of 11 Garlic (*Allium sativum* L.) Cultivars by Gas Chromatography with Flame Ionization and Mass Selective Detection

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Two garlic subspecies ($n = 11$), *Allium sativum* L. var. *opioscorodon* (hardneck) and *Allium sativum* L. var. *sativum* (softneck), were evaluated for their free amino acid composition. The free amino acid content of garlic samples analyzed ranged from 1121.7 to 3106.1 mg/100 g of fresh weight (mean = 2130.7 ± 681.5 mg/100 g). Hardneck garlic had greater methiin, alliin, and total free amino acids contents compared to softneck garlic. The major free amino acid present in all but one subspecies was glutamine (cv. Mother of Pearl had aspartic acid as the major free amino acid). Cv. Music Pink garlic (a rocambole hardneck variety) contained the most methiin, alliin, and total free amino acids. The solid-phase extraction, alkylchloroformate derivatization, GC-FID, and GC-MS methods used in this study were simple and rapid, allowing 18 free amino acids in garlic to be separated within 10 min.

KEYWORDS: ***Allium*; garlic; alliin; free amino acid**

INTRODUCTION

Garlic (genus *Allium*, family Alliaceae) is one of the oldest cultivated plants to have been an integral part of human health and diet (*1*). There are over 600 *Allium* species distributed worldwide. There are three main subspecies of *Allium sativum*, with varieties *ophioscorodon* (hardneck) and *sativum* (softneck) being the most common (*2*). Health-beneficial properties of garlic have been attributed to several sulfur-containing compounds derived from amino acid secondary metabolites (*3*). Alliin is one of the most important secondary metabolites present in garlic, and it is also a precursor to the flavor compounds that are unique to *Allium* (*4*).

There are numerous chromatographic methods published for determining the free and total amino acids of a given sample (*5*, *6*). Fountoulakis and Lahm (*5*) and Molnár-Perl (*6*) provided a comprehensive review of the advantages and disadvantages regarding the different chromatographic methods historically used. This paper addresses a fairly new free amino acid analysis method and evaluates the method with different garlic cultivars. A rapid derivatization and gas chromatographic method with flame ionization detection (GC-FID) based on a method developed by Hušek (*8*) has been recently used in the analysis of honey (*7*). Aqueous amino acid solutions were treated with ethylchloroformate in a pyridine and ethanol reaction medium, resulting in *N*-ethoxycarbonyl ethyl esters (*9*, *10*). This method is easily and rapidly implemented with a reagent kit from Phenomenex (Torrance, CA) and can be used with GC-FID or GC−mass spectrometry (GC-MS). The method has been used for common amino acids (*7*, *8*) as well as sulfur-containing amino acids. A noted disadvantage of this GC method is the inability to determine arginine content due to the adsorption of arginine onto the GC column (*10*, *11*). The guanidine group (the imino group of the guanidine group does not derivatize) of the arginine irreversibly adsorbs onto the column (*10*, *11*).

Four studies have examined the free amino acid composition of garlic quantitatively (*4*, *12*−*14*) using three different analytical methods (butylthiol-isoindole derivatization for HPLC, paper chromatography, and amino acid analyzer). Lawson (*4*) pointed out that the wide range of free amino acid values reported for garlic may be due to the different methods used and/or variation in garlic samples. The objective of this study was to evaluate alkylchloroformate derivatization with GC-FID and GC-MS for the determination of free amino acids in a variety of garlic samples with special emphasis on the ability of the method to detect secondary amino acid metabolites.

MATERIALS AND METHODS

Plant Material. A total of 11 commercially available varieties of garlic were purchased from Seeds of Change (Santa Fe, NM). One additional garlic sample was purchased from a local supermarket (Beltsville, MD), for preliminary work to obtain the proper GC conditions. The 11 garlic samples were harvested in July 2004 (from

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Table 1. Garlic Samples Used in This Study

common name	abbreviation	Latin name	subspecies, varieties
Spanish Roja	SRHN	*Allium sativum* L. var. *opioscorodon*	hardneck, rocambole
German White	GWHN	*Allium sativum* L. var. *opioscorodon*	hardneck, porcelain
Purple Italian Easy Peel	PIHN	*Allium sativum* L. var. *opioscorodon*	hardneck, rocambole
Chesnok Red	CRHN	*Allium sativum* L. var. *opioscorodon*	hardneck, purple stripe
Music Pink	MPHN	*Allium sativum* L. var. *opioscorodon*	hardneck, rocambole
Persian Star	PSHN	*Allium sativum* L. var. *opioscorodon*	hardneck, purple stripe
Mother of Pearl	MOPSN	*Allium sativum* L. var. *sativum*	softneck, silverskin
Inchelium Red	IRSN	*Allium sativum* L. var. *sativum*	softneck, artichoke
Kettle River Giant	KRSN	*Allium sativum* L. var. *sativum*	softneck, artichoke
Shantung Purple	SPSN	*Allium sativum* L. var. *sativum*	softneck, turban
Chilean Silver	CSSN	*Allium sativum* L. var. *sativum*	softneck, silverskin

a commercial field in northern New Mexico), cured (complete drying of the outer skins and stems for storage purposes), and then stored until shipment in October 2004 and immediately extracted upon arrival. Six hardneck (*Allium sativum* L. var. *opioscorodon*) and five softneck (*Allium sativum* L. var. *sativum*) garlic samples were examined in this study (**Table 1**). No wild garlic species were included in this study.

Reagents and Standards. L-(+)-*S*-Allylcysteine sulfoxide (= alliin) was purchased from LKT Laboratories (St. Paul, MN). *S*-Methyl-L-cysteine sulfoxide (= methiin) was purchased from Research Organics (Cleveland, OH). Solid-phase extraction (SPE) sorbent tips, amino acid standards, and all reagents used in the SPE and derivatization steps were included in "EZ:faast GC-FID for free amino acid analysis" and "EZ:faast GC-MS for free amino acid analysis" kits purchased from Phenomenex. Compositional information of the reagents from the kit was proprietary. All gases used in this study were purchased from Airgas (Hyattsville, MD) and were of chromatographic purity. All other chemicals were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

Extraction. Garlic cloves were carefully manually peeled. Samples were then boiled for 5 min and immediately chilled in an ice bath for 15 min. The weight of the garlic before and after blanching was recorded and then used to express the final amino acid concentration as milligrams of amino acids per 100 g of fresh garlic. Chilled-blanched garlic cloves were then pressed through a garlic press, before being made into a paste by a Potter-Elvehjem tissue grinder [composed of a serrated PTFE pestle and graduated tubes (Kontes Glass Co., Vineland, NJ), connected to a Caframo Stirrer Type RZR1 tissue grinder motor (Wiarton, ON, Canada)]. Blanched garlic paste (200 mg) was placed in 6 mL of distilled water and sonicated for 30 min with a Branson Ultrasonicator (Branson Cleaning Equipment Co., Shelton, CT). The extract was then filtered through a 0.45 μm pore size PP filter membrane Whatman filter (Whatman International, Clifton, NJ). Filtered extracts were stored at −70 °C until further analysis. Each type of garlic was subsampled, and extraction was conducted on each subsample, so replications were conducted at the subsample level.

SPE and Derivatization. Two hundred microliters of filtered garlic extract and 200 μL of internal standard (norvaline at a concentration of 200 μmol/L) were placed in a glass vial and slowly absorbed through a 40 μL resin-packed sorbent tip (*15*) by a 1.5 mL syringe. The resin was then rinsed with 200 μL of washing solution. Residual liquid was removed by pulling air through the sorbent tip. The sorbent particles were collected in the glass vial by 200 μL of eluting medium. Fifty microliters of reagent 4 was added to the glass vial and vortexed for 5 s. The reaction was allowed to proceed for 2 min, and the liquid was then re-emulsified by vortexing for 5 s and allowed to proceed for another 1 min. One hundred microliters of reagent 5 was added and then vortexed for 5 s, and the reaction was left to proceed for another 1 min. FID reagent 6 (100 μL) was added and vortexed for 5 s, and then the top organic layer was transferred into a GC vial containing an insert for the FID analysis.

In the case of MS analysis, the organic layer was removed without the addition of FID reagent 6 and placed in a GC vial with insert. The organic layer was slowly evaporated under a gentle stream of nitrogen by an N-EVAP analytical evaporator (Organomation Associates, Inc., Berlin, MA). The residue was then reconstituted with 100 μL of MS reagent 6. Cleaned and derivatized samples were immediately analyzed by GC-FID or GC-MS.

***S*-Alk(en)ylcysteine Sulfoxide Determination.** Additional reduction steps were taken after the derivatization process to determine the presence of *S*-alk(en)ylcysteine sulfoxide in the garlic samples by GC-FID and GC-MS. The reduction step was performed as described by Kubec et al. (*16*). Briefly, the derivatized sulfoxide-containing amino acids in the garlic samples were reduced by the addition of 200 μL of an aqueous sodium iodide (NaI) solution (1 g of sodium iodide/mL) and 50 μL of acetyl chloride. The vessel containing this mixture was allowed to stand for 2 h at room temperature. The excess iodine was removed with the addition of stannous chloride crystals. The reduced−derivatized sulfoxide amino acids were then extracted with 400 μL of dichloromethane. Reduced samples were analyzed using the same GC-FID and GC-MS condition as the free amino acid determination described in the next section.

Determination of Free Amino Acid Composition. A Zebron ZB-PAAC-MS 10 m × 0.25 mm column (stationary phase information was proprietary) from Phenomenex was used for both FID and MS detection. Each extracted sample was analyzed once.

GC-FID Conditions. The derivatized free amino acids were separated by using a Hewlett-Packard (HP) 6890 Network GC system (Wilmington, DE) equipped with a HP 7683 series injector and a flame ionization detector. The helium carrier gas flow was constant (1.8 mL/min) during the run, and the column head pressure was 9.33 psi. The GC oven temperature was initially held at 90 °C for 1 min, then raised to 140 °C (15 °C/min), and finally increased to 320 °C (35 °C/min) and held for 1 min. The inlet temperature was 250 °C. The detector was set at 320 °C. A 2 μL sample was injected in split mode (15:1, v/v).

GC-MS Conditions. MS was used to confirm the identification of the free amino acids present in the standards and samples. A Hewlett-Packard (HP) 6890 Network GC system (Wilmington, DE) coupled to a HP 5973 mass spectrometer was used. The helium carrier gas was constant at 1.8 mL/min. The oven temperature program was the same as the GC-FID oven program. The injection port temperature was 250 °C. The MS temperatures were set at 250 °C for the ion source, 180 °C for the quadrupole, and 310 °C for the auxiliary. The scan range was set to 30−500 (3.15 scans/s). The injection volume was 2 μL. Injection was done in split mode (15:1, v/v).

Statistical Analysis. Statistica for Windows version 7.0 (StatSoft, Inc., Tulsa, OK) was used for statistical analyses. Differences among the garlic samples were tested using the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) at the $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

A description of the garlic samples used in this study is summarized in **Table 1**. Two subspecies (hardneck and softneck) and six different varieties (rocambole, porcelain, purple stripe, silver skin, artichoke, and turban) were sampled. Amino acid profiles of the standards are shown in **Figure 1**. The original GC-FID analysis method had to be altered to resolve peak 1, a degradation product of *S*-allylcysteine sulfoxide (alliin), and peak 2, alanine (data not shown). The 28 standards were separated in <10 min. All of the unlabeled peaks were carefully examined by MS and found to be byproducts of the sample

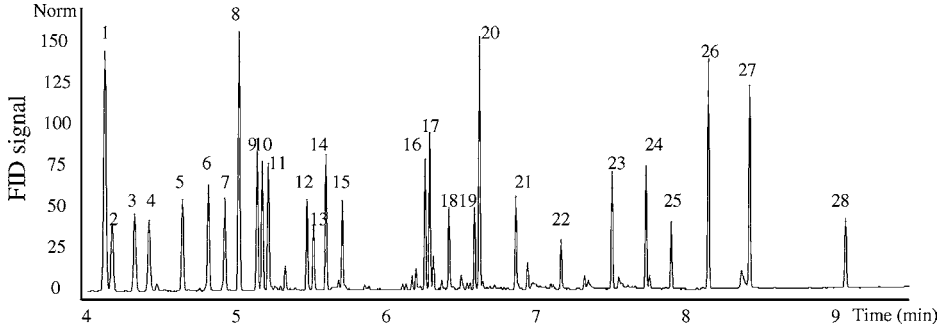

Figure 1. Amino acid chromatogram of the 28 standards obtained by GC-FID. Peaks: 1, alliin and methiin degradation product; 2, alanine (Ala); 3, sarcosine (Sar); 4, glycine (Gly); 5, α-aminobutyric acid (ABA); 6, valine (Val); 7, β-aminobutyric acid (BAIB); 8, internal standard (IS) = norvaline; 9, leucine (Leu); 10, allo-isoleucine (alle); 11, isoleucine (Ile); 12, threonine (Thr); 13, serine (Ser); 14, proline (Pro); 15, asparagine (Asn); 16, aspartic acid (Asp); 17, methionine (Met); 18, 4-hydroxyproline (Hyp); 19, glutamic acid (Glu); 20, phenylalanine (Phe); 21, α-aminoadipic acid (AAA); 22, glutamine (Gln); 23, ornithine (Orn); 24, lysine (Lys); 25, histidine (His); 26, tyrosine (Tyr); 27, tryptophan (Trp); 28, cystine (C–C).

Table 2. Amino Acid Abbreviations and Mass Fragment Ions of Their Derivatives Presented in the Order of Elution[a]

elution order	chemical name	abbreviation	mass fragment ions
1	alliin and methiin degradation product		215, 173, 114
2	*alanine*	*Ala*	*130, 88*
3	sarcosine	Sar	130, 217
4	*glycine*	*Gly*	*116, 207*
5	α-aminobutyric acid	Aba	144, 102
6	*valine*	*Val*	*158, 116*
7	β-aminobutyric acid	BAIB	158, 116
8	internal standard (norvaline)	IS	158, 72
9	*leucine*	*Leu*	*172, 86*
10	allo-iosleucine	alle	172, 130
11	*isoleucine*	*Ile*	*172, 130*
12	*threonine*	*Thr*	*160, 101*
13	*serine*	*Ser*	*146, 203*
14	*proline*	*Pro*	*156, 243*
15	*asparagine*	*Asn*	*155, 69*
16	*aspartic acid*	*Asp*	*216, 130*
17	methionine	Met	203, 277
18	4-hydroxyproline	Hyp	172, 86
19	*glutamic acid*	*Glu*	*230, 170*
20	*phenylalanine*	*Phe*	*206, 190*
21	α-aminoadipic acid	Aaa	244, 98
22	glutamine	Gln	84, 187
23	ornthine	Orn	156, 70
24	*lysine*	*Lys*	*170, 128*
25	*histidine*	*His*	*282, 168*
26	*tyrosine*	*Tyr*	*206, 107*
27	*trptophan*	*Trp*	*130*
28	*cystine*	*C–C*	*248, 216*

[a] Compounds in italic type were present in garlic.

preparation process (cleanup and derivatization). The corresponding mass fragment ions of the derivatized common free amino acid standards, used to confirm the identification of the amino acid peaks, are listed in **Table 2**.

Figure 2 shows typical free amino acid chromatograms of fresh and blanched garlic samples. Eighteen free amino acids were identified in the 11 garlic cultivars (**Table 3**). The difference between the two chromatograms is the striking appearance of peak 1 (alliin degradation product) in chromatogram B (**Figure 2**). Blanching the garlic samples before extraction inhibited the native enzyme alliinase, which would have converted alliin to allicin (diallylthiosulfinate), which cannot be derivatized by alkylchloroformate. Thus, the alliin (converted to allicin by the native enzyme alliinase) peak is not visible in chromatogram A (**Figure 2**).

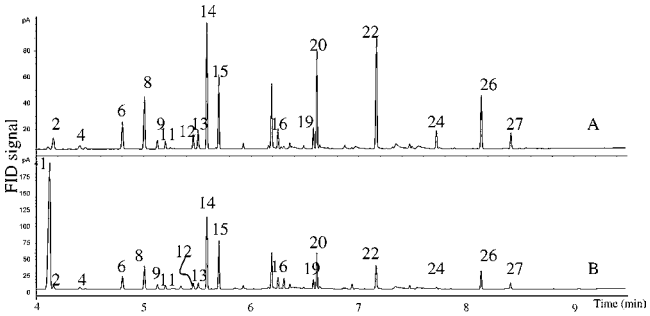

Figure 2. Free amino acid profiles of fresh (**A**) and blanched (**B**) garlic (sample obtained from a local supermarket) samples obtained by GC-FID. Peaks: 1, alliin degradation product; 2, Ala; 4, Gly; 6, Val; 8, IS (norvaline); 9, Leu; 11, Ile; 12, Thr; 13, Ser; 14, Pro; 15, Asn; 16, Asp; 19, Glu; 20, Phe; 22, Gln; 24, Lys; 26, Tyr; 27, Trp. His and C–C were not detected in this sample.

Peak 1 (**Figure 2**) was in fact composed of the degradation products of alliin and methiin (*S*-methylcysteine sulfoxide) on the basis of the mass spectra information. Derivatized alliin and methiin were found to decompose and coelute under the GC condition reported by Kubec et al. (*16*). They reduced the amino acids using NaI (*16*) after the derivatization step to obtain *S*-allylcysteine and *S*-methylcysteine (sulfoxide groups were removed). The reduction process was reproducible, and the reduced amino acids were stable under the GC conditions. The chromatograms for reduced alliin and methiin standards are shown in **Figure 3**, parts **B** and **A**, respectively, as well as a chromatogram of a reduced blanched garlic sample (**Figure 3C**). Kubec et al. (*16*) synthesized butylcysteine sulfoxide for use as an internal standard for quantifying the efficiency of the reduction of the alkylcysteine sulfoxides. Unfortunately, it is not commercially available. Consequently, there was no quantification of these compounds in this study. The corresponding mass fragment ion for the reduced form of methiin was 176 and for alliin it was 202 and 289.

Although methiin and alliin were not quantified, a general comparison can be made on the basis of peak areas if it is assumed that the reduction efficiency was the same for both compounds. The peak area for methiin ranged from 1.0 to 10.6, and the area for alliin ranged from 54.9 to 192.2 (**Table 3**). MPHN garlic (a rocambole hardneck variety) contained the most methiin and alliin, and GWHN garlic (a porcelain hardneck variety) contained the least. Kubec et al. (*13*) reported methiin and alliin contents of nine different garlic samples to vary from 50 to 126 mg/100 g of fresh weight and from 481 to 1140 mg/100 g, respectively.

Table 3. Free Amino Acid Composition of the 11 Blanched Garlic Samples Obtained by GC-FID[a]

amino acid	garlic samples												
	SRHN	GWHN	PIHN	CRHN	MPHN	PSHN	MOPSN	IRSN	KRSN	SPSN	CSSN	mean	SD
Ala	42.0	25.8	18.0	31.6	33.3	26.6	22.6	23.7	20.2	44.5	20.0	28.0	8.9
Gly	n.d.	3.1	2.4	n.d.	n.d.	0.9	1.1	2.0	n.d.	2.8	n.d.	1.1	1.2
Val	22.5	50.7	29.0	48.8	66.4	29.1	34.6	53.8	18.3	63.5	17.9	39.5	17.8
Leu	9.8	15.7	17.9	16.1	21.4	11.0	6.8	16.0	7.9	13.9	5.9	12.9	5.0
Ile	6.1	16.9	10.5	11.9	25.4	9.9	6.0	3.1	4.5	11.5	4.6	10.0	6.5
Thr	1.3	27.7	4.2	39.6	42.9	4.9	66.8	25.8	1.4	71.0	11.2	27.0	25.5
Ser	111.9	145.4	40.3	143.2	156.0	100.2	146.8	100.9	47.7	125.1	61.5	107.2	41.5
Pro	202.6	121.5	80.2	232.8	170.0	199.2	23.7	100.7	74.7	101.2	49.0	123.2	68.5
Asn	53.1	108.4	298.9	386.4	311.1	81.4	604.8	196.0	34.3	415.6	140.7	239.2	180.4
Asp	39.3	95.1	79.0	103.0	121.9	74.9	153.1	68.1	45.8	180.8	98.6	96.3	42.9
Glu	220.7	91.9	232.9	289.7	261.5	194.9	259.3	227.7	150.8	389.6	192.3	228.3	76.8
Phe	30.3	61.1	40.6	40.9	76.6	32.0	n.d.	14.8	12.6	25.8	19.6	32.2	22.1
Gln	471.1	402.1	549.9	730.4	1004.0	541.3	549.3	754.7	416.7	693.8	400.8	592.2	187.1
Lys	141.1	160.9	125.3	277.8	180.7	238.2	313.9	125.0	49.5	151.8	160.7	175.0	75.3
His	52.5	50.5	48.4	92.6	78.9	71.3	103.2	50.2	20.7	68.1	34.1	61.0	24.6
Tyr	42.1	112.1	54.5	102.1	119.1	60.4	83.1	46.9	44.8	60.2	38.3	69.4	29.6
Trp	50.3	51.3	66.8	103.1	105.9	57.1	96.9	79.8	87.3	68.1	73.2	76.3	20.0
C–C	118.5	54.9	373.3	354.6	331.0	332.2	111.5	118.1	84.7	397.9	52.8	211.8	142.6
total[b]	1615.2 abc	1595.0 abc	2072.1 abcd	3004.7 cde	3106.1 d	2065.8 abcd	2583.5 acde	2007.3 abc	1121.7 ab	2884.9 cde	1381.2 abc	2130.7	681.5
r-methiin	*1.3*	*1.0*	*3.2*	*1.2*	*10.6*	*9.3*	*2.3*	*2.9*	*1.4*	*4.2*	*6.3*	*4.0*	*3.4*
r-alliin	*177.2*	*54.9*	*147.2*	*118.7*	*192.2*	*167.8*	*135.8*	*163.8*	*90.5*	*100.3*	*186.0*	*139.5*	*44.0*

[a] Amino acid concentrations expressed as mg/100 g of fresh weight. Values for reduced (r) methiin and alliin (italicized) are peak area (SD, standard deviation; nd, not detected, < 0.1 mg/100 g fresh weight. [b] Totals with different letters are significantly different ($p < 0.05$).

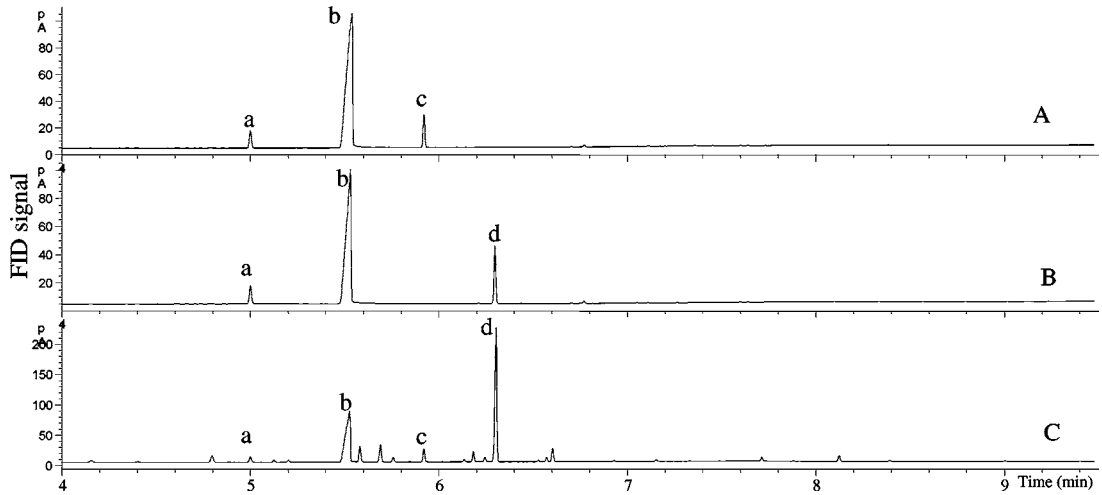

Figure 3. GC-FID profile of derivatized and then reduced methiin standard (**A**), alliin standard (**B**), and blanched garlic sample (**C**, sample obtained from a local supermarket): (a) IS (norvaline); (b) reduction byproduct (present in water blank that underwent the same derivatization and reduction steps); (c) methiin reduced; (d) alliin reduced.

The common free amino acid standards were also subjected to this reduction step in hopes that one method could be used for the determination of all free amino acids present in garlic. However, later eluting free amino acids (AAA, Gln, Orn, Lys, His, Tyr, Trp, and C−C) were significantly degraded and diluted from this reduction step (data not shown), making it difficult to determine all of the free amino acids (common free amino acids and alkylcysteine sulfoxides) in a given sample by a single method. Thus, two chromatographic runs are necessary, one after derivatization and one after reduction. Two runs are also necessary if the samples contain both alkylcysteine sulfoxides and alkylcysteines. Analysis after derivatization would quantify the alkylcysteines, and analysis after reduction would quantify the sum of the two.

Table 3 summarizes the free amino acid composition of the blanched garlic samples. The 11 garlic samples had total free amino acid contents ranging from 1121.7 to 3106.1 mg/100 g of fresh weight (mean = 2130.7 mg/100 g). Among the hardneck garlic samples, MPHN (3106.1 mg/100 g) contained the most free amino acids, and GWHN contained the least (1595.0 mg/100 g). The softneck garlic samples free amino acid contents ranged from 1121.7 mg/100 g (KRSN) to 2884.9 mg/100 g (SPSN). MPHN had significantly more free amino acids than SRHN, GWHN, IRSN, KRSN, and CSSN ($p < 0.05$). The mean of free amino acids of the hardneck garlic samples (2243.1 mg/100 g) was higher than the mean of softneck garlic samples (1995.7 mg/100 g), although no statistically significant difference was found (p value = 0.362). There was no significant difference of total free amino acid content when different varieties (p value = 0.192) were compared as well. There are no recent reports on the free amino acid content of garlic. In 1996, Lawson (*4*) reported 26 mg of free amino acids/g of dry weight from the six strains of garlic they analyzed by HPLC after butylthiol-isoinodole derivatization.

Overall, the main free amino acids present in the 11 garlic samples were Gln, Asn, Glu, C−C, and Lys (in decreasing

order). The most abundant free amino acid in all but one sample was Gln. The exception was MOPSN, in which Asn was most prevalent. Lawson (*4*) reported arginine as the most abundant amino acid present in the samples they examined. No arginine was reported in this study due to the permanent absorption of arginine onto the column (*10*). Val, Leu, Ile, Thr, Phe, Lys, and Trp were the essential amino acids found in these garlic samples. The three most abundant essential amino acids present overall were Lys, Trp, and Val (in decreasing order). Seventeen percent of the free amino acids identified in this study were essential amino acids. CRHN contained the most essential amino acids when compared to the others, 538.2 mg/100 g (17.9% of the free amino acid). There was no clear distinction in free amino acid composition between softneck and hardneck garlics. There was a wide range of free amino acids present in the 11 garlic samples examined.

All garlic samples used in this study were from a single growing season, and one would expect variation between growing seasons and varieties. Sampling was not large enough to observe trends. For future research, a larger number of garlic samples should be analyzed. It would also be interesting to include wild garlics and garlics from across the nation. This study used cultivated garlic from only a single geographic source that was stored for a known length of time.

Despite one common amino acid (arginine) being undetectable by this method, the procedure remains a simple and quick way to analyze free amino acids present in garlic. The entire analysis takes <20 min for sample cleanup, derivatization, and GC-FID or GC-MS analysis. The free amino acid composition of garlic differed considerably among the different varieties. There was no apparent distinction between the two subspecies used in this study. The reduction step proposed by Kubec et al. (*16*) is simple, but the need for an internal standard (butylcysteine sulfoxide) prevents the use of this method to quantify methiin and alliin.

ABBREVIATIONS USED

SPE, solid-phase extraction; GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography with mass spectrometer; HPLC, high-performance liquid chromatography.

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