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ARTICLE

Variability of Solids, Organosulfur Compounds, Pungency and Health-Enhancing Traits in Garlic (*Allium sativum* L.) Cultivars Belonging to Different Ecophysiological Groups

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Garlic is a vegetable mainly agamically propagated, and it has been dispersed all around the world. Garlic cultivars have been classified in different ecophysiological groups (EG) according to their bulbing requirements. The variability in organosulfur composition (ACSOs), solids content (SC), pungency (PC) and antiplatelet activity (IAA) and the correlation among these traits in garlic clones belonging to three EG was studied. We found variability for ACSOs, SC, PC and IAA between clones belonging to different EG and also among clones belonging to the same EG. Cutivars EG III presented more variability than EG IV for ACSOs, thiosulfinates, allicin and PC, while for SC, EG IV was the most variable. The correlations found suggested that IAA observed was mainly due to organosulfur composition. Finally recommendations about the most suitable cultivars for fresh consumption, pharmaceutical and dehydration industry are made.

KEYWORDS: Garlic; organosulfur composition; flavor attributes; health benefits; antiplatelet activity

INTRODUCTION

Garlic (*Allium sativum* L.) is a vegetable originated from Central Asia (*1*). Since it is mainly agamically propagated, the variability of this crop is high and arises from mutations. Nevertheless the crop has been dispersed all around the world and is grown in tropical, subtropical and temperate areas. Garlic clones have been classified according to their requirements for bulbing (*2*,*3*). These classifications consider the adaptation of garlic clones to different climates which is correlated with the genetic variability.

The classification proposed by Burba (*2*) includes four ecophysiological groups (EG) based on their low-temperature and day-length requirements for bulbing. EG I includes clones of tropical climates that have very short postharvest conservation and low requirements of day length for bulbing. EG II includes clones adapted to subtropical or warm-temperature climates, which have relatively short postharvest conservation. EG III includes clones adapted to temperate climates, which normally have intermediate postharvest conservation, and EG IV includes clones adapted to cold-temperature climates, which have very long postharvest conservation (*4*).

There is an increasing demand for natural food with high health value added for worldwide human consumption. Garlic has been used as a spice, food and folklore medicine for over 4,000 years, and is one of the most widely researched medicinal plants (*5*). There are several published studies that relate garlic with health attributes (*6*−*9*), but the variability of garlic clones for health related traits has not been explored in a collection of germplasm based on ecophysiological requirements.

On the other hand Argentina is the second garlic exporter of the world. In the past decade, several garlic cultivars belonging to three different EG have been released by INTA (Instituto Nacional de Tecnología Agropecuaria), giving a wider range of options to garlic growers (*10*).

Volatile organosulfur compounds are responsible for the characteristic smell and taste of *Allium*. These compounds are alk(en)yl-thiosulfinates formed by the action of alliinase on odorless *S*-alk(en)yl-L-cysteine sulfoxides, as a result of plant material disruption (*11*). These compounds are pharmacologically active substances that exhibit antibiotic (*9*, *12*), lipid-lowering effects (*6*), antioxidant (*13*)and antitumor activities (*14*), and inhibition of thrombocyte aggregation (*7*, *8*, *15*). Furthermore, platelet aggregation is an important cause of thrombosis leading to cardiovascular diseases (*16*). Inhibitors of aggregation can provide protection against this syndrome that affects millions of people worldwide (*15*). Aqueous and organic garlic extracts inhibit platelet aggregation induced by a number of physiologically important aggregating agents, such as collagen and adrenaline (*5*). Platelet inhibition has been demonstrated *in vitro* and *in vivo* with fresh garlic cloves (*17*), ajoene (*18*), garlic oil (*19*) and aged garlic extract (AGE) (*20*). Worldwide most of the garlic is not consumed in these forms, instead it is usually cooked before consumption. Only a few reports have investigated the antithrombotic properties of cooked *Allium* (*21*−*23*). More recently, Cavagnaro et al. (*24*, *25*) have studied the effect of cooking on garlic and onion antiplatelet activity and other related traits.

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Their results suggest that, in order to obtain the maximum health benefits, garlic and onions should be eaten raw or moderately cooked.

Also, antiplatelet activity of vegetables is affected by genotype, environment and storage duration (*12*). It has been reported that, in both garlic and onions, the platelet activity is related to the native concentration of organosulfur compounds which are genotypically determined by the sulfur content of the bulb (*26*).

Pungency (an indicator of the strength of flavor) and solids content are important attributes of onion bulb quality for processing and storage (*27*). Furthermore, in raw onions significant correlations have been reported among *in vitro* antiplatelet activity, pungency and solids content (*26*−*28*); also common QTL (quantitative trait loci) for these properties have been detected (*29*). The correlations for these traits are not well established for raw garlic.

The objective of this work was to evaluate simultaneously the differences in flavor precursor levels, thiosulfinates, solids content, pungency and antiplatelet activity and the correlation among these traits in nine garlic clones belonging to different ecophysiological groups which were grown in the same location under field controlled conditions.

The characterization of Argentinean garlic germplasm according to its functional properties would help the selection of cultivars with enhanced flavor and defined medicinal benefits.

MATERIALS AND METHODS

Chemicals. (+)-*S*-Methyl-L-cysteine sulfoxide (methiin), (+)-*S*-propyl-L-cysteine sulfoxide (propiin), (+)-*S*-*trans*-1-propenyl-L-cysteine sulfoxide (isoalliin) and diallyl thiosulfinate (allicin) were synthesized as previously described (*30*−*32*). Alliin, (+)-*S*-allylcysteine sulfoxide, was purchased from Extrasynthese (Lyon-Nord, France). Pyruvic acid, 2,4-dinitro-phenylhydrazine (2,4-DNPH), cysteine, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) and Hepes buffer were purchased from Sigma (St. Louis, MO). All solvents for high-performance liquid chromatography (HPLC) and other chemicals of reagent grade were purchased from Interchemistry (Buenos Aires, Argentina). Collagen was purchased from Chrono-log Corp.

Equipment. A Virtis-Freeze equipment was used to freeze-dry garlic for powder preparation. A Decalab magnetic stirrer (Argentina), an Osterizer liquefier-blender (Union Comp Corporation, Canway, AR), and an Eppendorf MiniSpin plus microcentrifuge (Germany) were used for sample preparation. A Konik KNK-500-series system liquid chromatographer coupled with a UV−vis 200 detector (scan 190−380 nm) was used for cysteine sulfoxide and allicin determination. A Beckman DU 520 spectrophotometer (general purpose UV/vis) was used for total thiosulfinates and pungency determination. Antiplatelet activity was measured using an electrical impedance aggregometer (Chrono-log, Havertown, PA). Solids content was measured using an Atago refractometer (Japan) and a Dalvo (Argentina) stove.

Garlic Samples. Nine garlic cultivars were chosen from the germplasm collection of INTA La Consulta: Morado INTA, Nieve INTA, Perla INTA, Unión, Norteño INTA, Sureño INTA, Fuego INTA, Gostoso INTA and Castaño INTA. These clones were classified according to Burba et al. (*2*) into three ecophysiological groups (**Table 1**). These cultivars were grown at INTA's experimental field located in La Consulta, Mendoza, Argentina (33° 44′ S, 69° 07′ W) during 2005−2006, using a complete randomized design. Bulbs were harvested from November to December, and then they were fully cured. During postharvest at 0 ± 3 °C, when most of the unsprouted bulbs reach 40−60% visual index overcame (VIDO) (*2*), ten bulbs were sampled. VIDO represents the relationship percentual between length of sprouting leaf and storage leaf (*2*).

For the organosulfur determination garlic powder was prepared as follows: 500 g of freshly peeled garlic cloves were frozen in liquid nitrogen, and freeze-dried at −80 °C for 72 h in a vacuum. The resulting lyophilizate was ground into powder with a mortar and stored at −80 °C.

HPLC Analysis of Flavor Precursors (ACSOs). Sample preparation for analysis of flavor precursors was performed according to refs *32*, *33* with slight modifications. Garlic powder (1 g) was added to 30 mL of 90% methanol solution containing 0.01 N HCl, and the mixture was shaken for 30 min using a magnetic stirrer. Additional 90% methanol solution containing 0.01 HCl was added to the mixture to obtain 50 mL. The resulting mixture was centrifuged at 14000 rpm for 5 min. The supernatant was analyzed by HPLC. The HPLC conditions were the following: column, Waters Spherisorb ODS2 (5 μm, 250 × 4.6 mm); column temperature, 25 °C; flow, 0.9 mL min^{-1}; phase Movil, 0.03 M HCl; UV, 210 nm; injection volume, 10 μL.

Table 1. Argentine Classification of Garlic Cultivars

ecophysiological group	cultivars	origin
II	Morado INTA	Morado Asiático
III	Perla INTA	Blanco Americano
	Unión	Blanco Mendoza
	Norteño INTA	Blanco Mendoza
	Nieve INTA	Blanco Mendoza
IV	Gostoso INTA	Colorado Bonaerense
	Fuego INTA	Colorado Mendoza
	Sureño INTA	Colorado Mendoza
	Castaño INTA	Ajo Ruso

Spectrophotometric Analysis of Thiosulfinates (TS). A colorimetric procedure based on the reaction of DTNB was used to measure the concentration of thiosulfinates (*34*). One gram of freeze-dried garlic powder was shaken for 30 min with 30 mL of distilled water at room temperature and filtered, and distilled water was added to the mixture to obtain 100 mL exactly. Aliquots of 625 μL of 0.8 mM cysteine solution were added to both an aliquot of 375 μL of diluted garlic extract and a similar aliquot of distilled water (reference test tube). After shaking they were kept for 10 min at room temperature. An aliquot of 200 μL of garlic/cysteine solution or water/cysteine solution was added to the 800 μL of 200 μM DTNB which was prepared with 50 mM Hepes buffer (pH: 7.5). In order to produce a blank, 200 μL of water was added to the DTNB tube. After shaking, test tubes were left 10 min to allow color development. Absorbance was measured at 412 nm, and the thiosulfinate concentrations were calculated according to ref *35*.

Allicin Quantification by HPLC. This analysis was performed as previously described (*36*). Distilled water was added to 1 g of garlic powder (30 mL per g), mixed, kept 10 min at room temperature and then centrifuged at 14000 rpm for 5 min. Then 600 μL of supernatant was added to methanol (1:1 v/v) according to ref *36*. The HPLC conditions were the following: column, Waters Spherisorb ODS2 (5 μm, 250 × 4.6 mm); column temperature, 25 °C; flow, 0.8 mL/min; phase Movil, methanol: water (50% v/v); UV, 254 nm; injection volume, 10 μL. Dried garlic powder, with a standardized quantity of allicin, was used as secondary standard for allicin quantification by ref *36*.

Pyruvate Analysis (PC). Pyruvate concentration was analyzed according to ref *37*. Garlic cloves were blended for 1 min in distilled water (1:10 w/v). The juice was collected, filtered and kept at room temperature for 15 min to allow enzymatic hydrolysis of the flavor precursors to occur. A juice aliquot was added to an equal volume of 5% trichloroacetic acid and centrifuged for 10 min at 10000 rpm. One milliliter of 0.0125% 2,4-DNPH in 2 N HCl was added to 2 mL of juice/TCA diluted (1:20 v/v). The tubes were incubated at 37 °C during 10 min in a thermostatized bath, and then 5 mL of NaOH 0.6 N was added. The absorbance was measured at 420 nm. The pyruvic acid concentration of the garlic juice was determined using as a reference a standard curve developed with known concentrations of pyruvate. Values were expressed as μmol g^{-1} of fresh weight.

Soluble Solids Content (SSC). Ten garlic cloves were blended for a minute, and the resulting juice was squeezed through cheesecloth. Several drops of juice were applied to a hand refractometer to measure soluble solids content (expressed as °Brix), (*38*).

Total Solids Content (TSC). Fresh garlic cloves were crushed and dried in a stove at 80 °C until constant weight. Values were expressed as g%.

Measurement of Antiplatelet Activity (IAA). Platelet aggregation was measured *in vitro* using an electrical impedance aggregometer of whole blood (*39*). Blood was collected from three healthy donors by venipuncture (Hematology Service Central Hospital, Mendoza, Argentina) into tubes containing sodium citrate anticoagulant (3.8%, 1 vol of anticoagulant per

Table 2. Content and Relative Proportion of ACSOs in Garlic Cultivars[a]

		alliin		methiin		isoalliin		
EG[b]	cultivars	content (mg·g^{-1} fw)	rel proportion (%)	content (mg·g^{-1} fw)	rel proportion (%)	content (mg·g^{-1} fw)	rel proportion (%)	total
II	Morado INTA	15.23 ± 0.26[c] b[d]	72	0.81 ± 0.03 e	4	5.15 ± 0.14 c	24	21.19 c
III	Perla INTA	21.83 ± 1.33 a	72	1.53 ± 0.09 c	5	6.87 ± 0.32 b	23	30.22 a
	Unión	15.08 ± 0.49 b	74	1.44 ± 0.03 c	7	3.85 ± 0.15 d	19	20.38 cd
	Norteño INTA	15.64 ± 0.59 b	64	0.78 ± 0.02 ef	3	8.19 ± 0.21 a	33	24.61 b
	Nieve INTA	9.86 ± 0.47 d	58	0.54 ± 0.03 g	3	6.74 ± 0.17 b	39	17.14 e
IV	Gostoso INTA	12.25 ± 0.43 c	66	2.69 ± 0.18 a	15	3.49 ± 0.05 d	19	18.43 de
	Fuego INTA	14.08 ± 0.60 b	69	1.04 ± 0.01 d	5	5.32 ± 0.03 c	26	20.44 c
	Sureño INTA	8.08 ± 0.01 e	77	0.58 ± 0.01 fg	6	1.88 ± 0.11 f	18	10.55 f
	Castaño INTA	11.80 ± 0.26 c	58	1.84 ± 0.04 b	9	6.76 ± 0.38 b	33	20.40 c

[a] Propiin content was not detected. [b] EG: ecophysiological group. [c] Each value is the mean ± SD of fresh material (n = 5). [d] Values followed by the same letter were not significantly different according to the Tukey test (P < 0.05).

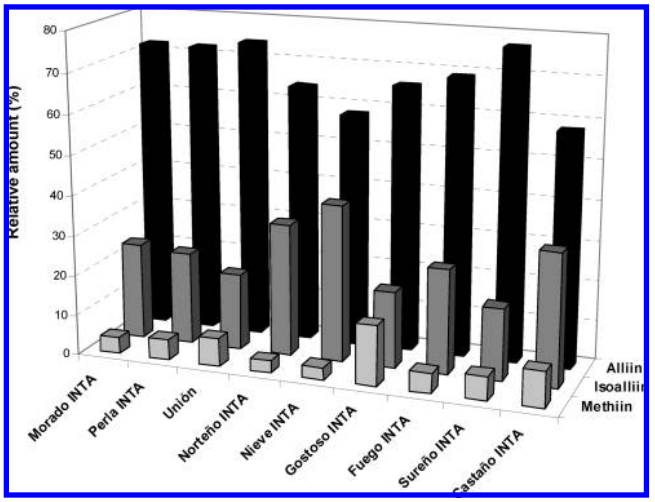

Figure 1. Relative amounts of cysteine sulfoxides of different garlic cultivars.

9 vol of blood). The blood samples were diluted in equal volume of TRIS-buffered saline (TBS, pH 7.4) and vortexed. The diluted blood was maintained at room temperature during the experiment and used within 2 h of extraction.

Aliquots of 1 mL of blood/TBS were incubated for 3 min at 37 °C, after a dose of garlic juice was added and platelet aggregation was induced by the addition of 2.5 μL of collagen (1 mg mL^{-1}). Change in the impedance was recorded over 6 min; this change is proportional to platelet aggregation. Antiplatelet activity *in vitro* was expressed as percentage of inhibition of platelet aggregation, compared to control samples prepared in the same way but without adding garlic juice.

A comparative study of antiplatelet activity of chosen garlic extracts and aspirin (known for its antithrombotic action) was performed. The platelet inhibitory effect of aspirin (acetylsalicylic acid) was measured at 0.36 mM (*15*). Aspirin was dissolved in TBS and platelet aggregation was compared to non-aspirin controls.

Statistical Analysis. Data were analyzed by ANOVA procedure using the software STATGRAPHIS Plus 4.0. Means of each group were compared by Tukey HSD test. A correlation analysis among antiplatelet activity, organosulfur content, solids content and pungency was performed using the same software.

RESULTS

Variability of Flavor Precursors Content. The total content of ACSOs ranged between 10.55 and 30.22 mg g^{-1} fw (**Table 2**). We found alliin to be the most abundant ACSO in garlic, representing more than 50% of the total content of ACSOs; meanwhile methiin content represented less than 10%. Taking into account the average of all cultivars from different ecophysiological groups (EG), there were differences on total ACSO content between EG III and IV but less between EG II and EG III. Cultivars of EG III presented less variability (22.4%) than cultivars of EG IV (27.5%) on total content of ACSOs. Methiin and propiin showed higher interclonal variability than alliin content, with an average coefficient of variation of 56.19%, 37.34% and 28%, respectively. The relative composition of cysteine sulfoxides is shown in **Figure 1**.

Table 3. Average Relative Contents of Thiosulfinates in Garlic Cultivars

EG[a]	cultivars	total thiosulfinate content (mM %g fw)
II	Morado INTA	3.23 ± 0.02[b] bc[c]
III	Perla INTA	4.59 ± 0.07 a
	Unión	3.58 ± 0.01 b
	Norteño INTA	2.96 ± 0.10 bcd
	Nieve INTA	3.19 ± 0.36 d
IV	Gostoso INTA	3.01 ± 0.15 bcd
	Fuego INTA	3.44 ± 0.01 b
	Sureño INTA	2.65 ± 0.51 cd
	Castaño INTA	3.19 ± 0.31 bc

[a] EG: ecophysiological group. [b] Each value is the mean ± SD of fresh material (n = 5). [c] Values followed by the same letter were not significantly different according to the Tukey test (P < 0.05).

Table 4. Allicin Content of Fresh Garlic Cultivars

EG[a]	cultivars	allicin content (mg·g^{-1} fw)
II	Morado INTA	3.66 ± 0.02[b] bc[c]
III	Perla INTA	5.21 ± 0.08 a
	Unión	4.05 ± 0.01 b
	Norteño INTA	3.35 ± 0.11 bcd
	Nieve INTA	2.70 ± 0.40 d
IV	Gostoso INTA	3.41 ± 0.16 bcd
	Fuego INTA	3.90 ± 0.01 b
	Sureño INTA	3.01 ± 0.57 cd
	Castaño INTA	3.61 ± 0.35 bc

[a] EG: ecophysiological group. [b] Each value is the mean ± SD of fresh material (n = 5). [c] Values followed by the same letter were not significantly different according to the Tukey test (P < 0.05).

Thiosulfinate Content. The levels of TS in fresh garlic were within a broad range, 2.45−4.59 mM %g (**Table 3**). For this variable there were differences between cultivars regarding the EG where they belong (P < 0.05). Perla INTA showed the highest thiosulfinate content, while Nieve INTA showed the lowest between the studied materials.

Allicin content. Allicin levels in garlic cultivars range 2.70−5.21 mg g^{-1} fw (**Table 4**). Differences were not observed among EG for this traits. Nevertheless, garlic cultivars from EG III showed more interclonal variability for allicin content than garlic cultivars from EG IV. Perla INTA had the highest

Table 5. Pungency (PC), Soluble Solids (SSC), Total Solids Content (TSC) and Antiplatelet Activity (IAA) of Garlic Cultivars

EG[a]	cultivars	PC (μmol g^{-1} fw)	SSC (Brix)	TSC (%)	IAA (%)
II	Morado INTA	52.32 ± 2.44[b] e[c]	32.06 ± 0.12 c	32.95 ± 1.28 d	68.4 ± 3.75 b
III	Perla INTA	88.27 ± 1.11 a	29.22 ± 1.31 d	35.06 ± 0.11 cd	93.7 ± 2.08 a
	Unión	77.87 ± 1.17 ab	31.96 ± 0.15 c	34.27 ± 1.51 cd	72.7 ± 3.39 b
	Norteño INTA	60.90 ± 0.21 de	34.71 ± 0.40 b	35.20 ± 0.30 c	47.66 ± 2.15 c
	Nieve INTA	65.35 ± 5.08 cd	33.50 ± 0.50 bc	36.06 ± 0.27 c	42.19 ± 6.11 c
IV	Gostoso INTA	70.37 ± 5.67 bcd	34.9 ± 0.17 b	36.07 ± 0.13 c	27.10 ± 1.90 d
	Fuego INTA	76.26 ± 1.92 bc	34.78 ± 0.18 b	38.86 ± 0,23 b	86.7 ± 1.86 a
	Sureño INTA	69.78 ± 6.41 bcd	32.03 ± 0.06 c	35.12 ± 0.75 cd	75.3 ± 3.07 b
	Castaño INTA	68.73 ± 4.96 bcd	39.3 ± 0.66 a	41.76 ± 0.68 a	51.53 ± 6.61 c

[a] EG: ecophysiological group. [b] Each value is the mean ± SD of fresh material (n = 5). [c] Values followed by the same letter were not significantly different according to the Tukey test (P < 0.05).

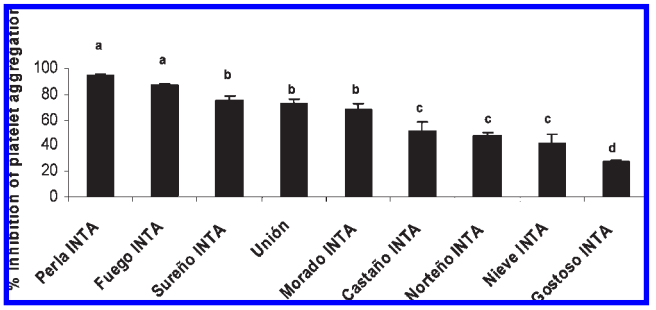

Figure 2. Effect of garlic juice on collagen-induced aggregation. Bars represent mean values for % inhibition of platelet aggregation ± SD (n = 3) as compared with a control without addition of garlic juice. Values followed by the same letter were not significantly different according to the Tukey test (P < 0.05).

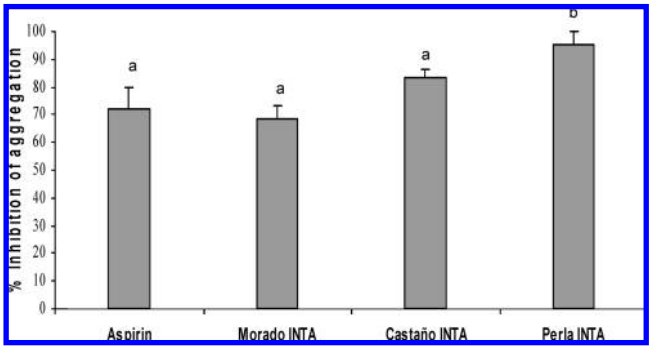

Figure 3. Inhibition of platelet aggregation by garlic extracts and aspirin. Each value is the mean ± SD (n = 3). Values followed by the same letter were not significantly different according to the Tukey test (P < 0.05).

allicin content whereas the cultivar Nieve INTA showed the lowest content.

Pungency. We found differences between cultivars from EG II compared with EG III and IV. Cultivars from EG III had the highest pungency levels. The lowest level of pungency was observed in Morado INTA (**Table 5**). Perla INTA exhibited the highest pungency, 1.67-fold when compared to Morado INTA.

Soluble Solids and Total Content. Soluble solids content varied significantly (P < 0.05) from 29°Brix for Morado INTA, to 39°Brix for Castaño INTA, with an average of 33.61°Brix. Significant variation in SSC and TSC was found between EG III and IV. The highest total solids content and soluble solids content was observed in cultivar Castaño INTA from EG IV (**Table 5**).

***In Vitro* Antiplatelet Activity.** The antiaggregatory effect of garlic extracts (1−16 mg mL^{-1}) was examined using whole human blood. Total percentage aggregation of platelet varied from 10 to 100%. Based on these results a concentration of 7 mg of garlic extract per mL of blood was chosen for platelet aggregation reactions. Significant differences were observed between cultivars (P < 0.05), regardless of the EG where they belong. Perla INTA and Fuego INTA showed the highest antiaggregatory effects (**Table 5** and **Figure 2**).

A comparative study of antiplatelet activity of aspirin and garlic extracts showed that Perla INTA inhibited platelet aggregation significantly more than aspirin (**Figure 3**, P < 0.05). Castaño INTA and Morado INTA garlic extracts were somewhat more potent but not as significant inhibitors as aspirin ($P \geq 0.05$).

Relationships between Antiplatelet Activity and Other Traits. Garlic induced antiplatelet activity was correlated with alliin, thiosulfinates, allicin content and pungency (**Table 6**). Also, significant correlations were observed between pungency, thiosulfinates and allicin content. Soluble solids content was highly correlated with total dry matter, but was not correlated with the other studied traits (**Table 6**).

Table 6. Correlation Values (r) among *in Vitro* Antiplatelet Activity (IAA), Cysteine Sulfoxides (ACSOs), Thiosulfinates (TS), Allicin, Pungency (PC) and Soluble (SSC) and Total Solids Content (TSC) of Garlic Cultivars[a]

	ACSOs								
variable	alliin	methiin	isoalliin	TS	allicin	PC	SSC	TSC	IAA
alliin		0.17	0.46*	0.84**	0.84**	0.40*	0.49	0.15	0.47*
methiin			0.14	0.30	0.30	0.33	0.29	0.29	0.33
isoalliin				0.19	0.19	0.08	0.22	0.23	0.09
TS					0.90**	0.55*	0.40	0.07	0.63**
allicin						0.54**	0.00	0.00	0.79***
pungency							0.29	0.19	0.43*
SS								0.77**	0.54
ST									0.13

[a] The symbols *, **, *** denote significance at P < 0.05, 0.01, and 0.001 respectively.

DISCUSSION

Different studies regarding the variability existing among species belonging to the family *Alliaceae* have been carried out (*11*, *15*, *28*, *29*). In all of them the profile of organosulfur compounds and several biological activities have been separately analyzed. This is the first study in which the principal organosulfur compounds, pungency, antiplatelet activity and solids content were analyzed at the same time in garlic cultivars belonging to different ecophysiological groups under controlled field conditions.

The composition of organosulfur compounds showed significant differences for the profile of cysteine sulfoxides, thiosulfinates and allicin content. For total ACSO content, cultivars of EG III had less interclonal variability than those of EG IV.

We found that alliin was the most abundant flavor precursor and was detected in all garlic cultivars. However, methiin and isoalliin were found in smaller amounts than alliin. We did not detect any compound in garlic extracts with the same retention time of synthetic propiin. These results are in agreement with those of Ziegler and Sticher (*40*) and Thomas and Parkin (*30*), who reported propiin to be absent in garlic. Lawson et al. (*36*) noted that the relative absence of n-propylated species may be due to the absence of endogenous propiin. On the other hand, we reported the presence of isoalliin. Some authors reported that garlic does not contain isoalliin (*30*, *41*), but our study showed that a significant amount of isoalliin was present, in agreement with Yoo and Pike (*42*). In addition Lawson et al. (*36*) and Block et al. (*43*) have reported the presence of 1-propenyl species in homogenates of garlic cloves probably derived from isoalliin. The level of alliin in fresh garlic ranges between 808 and 2.183 mg/100 g fw with an average of 1.376 mg/100 g fw. In general, the values determined are in agreement with those reported in the literature (*30*, *40*, *42*, *44*). The total amount of cysteine sulfoxides was relatively high in all garlic cultivars tested, with the exception of Sureño INTA. It is known that the distribution of cysteine sulfoxides in *Allium* species is predominantly controlled by genetic factors. Plant breeding has the potential to increase yields of the volatile sulfur substances (*11*). Species with a high content of cysteine sulfoxides, especially with a high content of alliin, are also suitable for medicinal purposes.

The values for thiosulfinates differed in all garlic cultivars, ranging between 2.65 and 4.59 mM %g fw. These values were higher than those previously reported by Block (*43*) of 1.4−3.6 mM %g fw. In general cultivars of EG III showed more variability than cultivars of EG IV. On the other hand, the cultivars tested recorded allicin yields in the range 2.70−5.21 mg g^{-1} fw. Perla INTA and Nieve INTA showed the highest and lowest allicin content, respectively. According to British Herbal Pharmacopoeia (*46*), the minimum allicin content to ensure pharmaceutical and economical viability of garlic powder products should be 4.5 mg g^{-1}. Perla INTA has allicin yields over 4.5 mg g^{-1}. The higher level of allicin present in this cultivar represents an opportunity to market fresh garlic to consumers based on its health-benefit quality.

Other important variables analyzed in this work were solids content and pungency. Solids content and pungency are important attributes of bulb quality for processing and storage. Significant differences were found between all the cultivars for solids content. For SSC significant variation was found between EG III and IV. Morado INTA showed the lowest TSC while Castaño INTA showed the highest. Portela et al. (*4*) reported that Morado INTA has a short period of storage while Castaño INTA exhibited the longest period of storage. These factors may be associated with the solids content present in both cultivars. In addition, TSC is an important factor for the dehydration industry because it has a direct impact on the energy required for drying. According to that, Castaño INTA would be the most suitable clone for dehydration.

For pungency, all garlic cultivars differed in the content of pyruvic acid, ranging between 52.32 and 88.28 μmol g^{-1}. These values are consistent with those reported by Freeman (*47*) of 98.9 μmol g^{-1} and Yoo and Pike (*48*) of 66.24 μmol g^{-1}. Cultivars of EG III showed higher values of PC than those of EG II and EG IV. The significant differences observed among cultivars of EG III can be due to the origin of these cultivars. Perla INTA originated from "Blanco Americano or Blanco Californiano" populations showed the highest value of pyruvic acid while the rest of the cultivars belonging of this EG, originated from "Blanco Mendoza" populations, showed lower values of PC.

Regarding antiplatelet activity, all the studied cultivars significantly inhibited platelet aggregation but to different degrees. The concentrations of the garlic extracts tested, between 1 and 16 mg mL^{-1}, produced a dose-dependent inhibition. Predicted IC_{50} (the concentration required to inhibit platelet aggregation by 50%) was determined for each cultivar. We found the IC_{50} was 5.7 mg to 11.3 mg per mL of blood (date not shown). Analyzing all cultivars at the same doses, Perla INTA and Fuego INTA were the most effective inhibitors, without difference between them. On the other hand, Perla INTA was a more potent inhibitor of platelet aggregation than aspirin *in vitro*. For Castaño INTA and Morado INTA garlic extracts were not different from aspirin. Previous studies have shown that extracts from both garlic and onion inhibit platelet aggregation in human blood *in vitro*. Ali et al. (*8*) compared the effect of raw garlic versus boiled garlic aqueous extract and onion on collagen induced platelet aggregation using rabbit and human platelet-rich plasma. The authors concluded that garlic was about 13 times more potent than onion and suggested that garlic and onion could be more potent inhibitors of platelet aggregation consumed raw than in cooked or boiled preparations. Also, in this study was reported an IC_{50} of 8.8 mg mL^{-1}. Recently, Cavagnaro et al. (*24*) demonstrated that processing of garlic and the conditions used for cooking can strongly influence its effectiveness as a platelet inhibitor. In another study, Lawson et al. (*7*) determined the inhibitory effects of adenosine and 16 quantitatively determined organosulfur compounds derived from garlic cloves or commercial garlic preparations on collagen stimulated *in vitro* platelet aggregation in whole blood. They found that allicin and other thiosulfinates provided all the antiplatelet activity of raw garlic extract in whole blood aggregometry, and reported an IC_{50} for this preparation of 2.6 mg mL^{-1}. The differences observed on IC_{50} values from our study and those previously reported could be due to the differences in the organosulfur compound content, agonist concentrations used, and garlic extract preparation.

We found positive correlations between antiplatelet activity and alliin, thiosulfinates, allicin and pungency, indicating that garlic cultivars with higher alliin content, thiosulfinates, allicin and pungency exhibit greater antiplatelet activity. Pyruvate produced by allinase upon crushing raw garlic and onion is considered as a good predictor of antiplatelet strength due to the significant correlation between them (*26*, *29*). Thiosulfinates and allicin content demonstrated significant positive correlation with both PC and IAA. Thus, the concentration of TS and allicin can be used as estimators of IAA of raw garlic extracts. In onion, previous studies have evaluated the relationship between SSC and total dry matter with PC indicating a positive correlation among these variables (*27*). This correlation was not observed among the garlic cultivars evaluated. In onion the correlation between solids content, antiplatelet activity and pungency is attributed to pleiotropic effects of genes involved in the carbohydrate pathway (*28*, *29*). Onions with a high degree of fructose polymerization into fructans have high soluble solids and more pungency due to the low presence of free fructose that restricts the plants' capacity to osmoregulate and as a consequence reduce water uptake. On the other hand, cultivars with low soluble solids have more free fructose and as result more water content that could be responsible for diluting the compounds of sulfur pathway related to PC and IAA (*28*) In garlic even in cultivars with low soluble solids the percentage of free fructose is low, so the pleiotropic effects in garlic are not as strong as in onion. That may explain the lack of positive correlation between soluble solids, pungency and antiplatelet activity observed in garlic.

In summary, the present study provides evidence that there is great variability for organosulfur composition, solids, pungency

and antiplatelet activity between garlic clones belonging to different ecophysiological groups and also among cultivars belonging to the same ecophysiological group. In general, EG III presented more variability than EG IV for total ACSO content, thiosulfinates, allicin and pungency. For solids content EG IV was the most variable. These differences probably are related to their postharvest behavior. For IAA, a comparative study realized with aspirin showed that Perla INTA has higher activity than this common antithrombotic drug.

The correlations found strongly suggest that the garlic-induced antiplatelet activity in whole blood is mainly due to the organosulfur compound profile, in particular allicin content. Furthermore, this study allowed choosing cultivars for different purposes. Perla INTA would be suitable to obtain pharmacological products with high bioactive content. Castaño INTA would be suitable for dehydration industry, and Morado INTA would be suitable for fresh consumption.

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