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Organic Acid Contents in Onion Cultivars (*Allium cepa* L.)

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The following organic acids (glutamic, oxalic, pyruvic, malic, tartaric, citric, and fumaric), pungency, Brix degree, acidity, and pH were determined in onion cultivars (Texas, Guayonje, San Juan de la Rambla, Carrizal Alto, Carrizal Bajo, and Masca) harvested in the same agroclimatic conditions. Glutamic acid was the most abundant organic acid (325 ± 133 mg/100 g) followed by citric acid (48.5 ± 24.1 mg/100 g) and malic acid (43.6 ± 10.4 mg/100 g). There were significant differences between the onion cultivars in the mean concentrations of all of the analyzed parameters. The San Juan de la Rambla and Masca cultivars presented, in general, higher concentrations of the organic acids than the other cultivars. Significant differences in most of the analyzed parameters were observed between the two seed origins for the Masca and San Juan de la Rambla cultivars. The onion samples tended to be classified according to the cultivar and, in the case of San Juan de la Rambla cultivar, according to the precedence of the seeds after applying discriminant analysis.

KEYWORDS: Onion (*Allium cepa* L.); HPLC; organic acid; pungency; discriminant analysis

INTRODUCTION

The onion (*Allium cepa* L.) is a species of the Alliaceae family of great economic importance and is the second most cultivated vegetable crop in the world, especially in Asia and Europe. Besides making a significant contribution to the nutritional value in the human diet, they also have medicinal and functional properties. They are also mainly consumed for their unique flavor or for their ability to enhance the flavor of other foods (*1*).

With regard to the pharmacological activities attributed to the onion and garlic species, there have been a number of chemical and pharmacological reports testifying to the effectiveness of extracts, such as antioxidant, antimicrobial, anti-asthmatic, antitumor, and cancer-preventing agents, as a platelet-anti-aggregant agent, for reducing hypercholesterolemia, and bacteriostatic activity against *Helicobacter pylori*, which is responsible for gastric ulcers and gastric cancer (*2*).

The flavor of onions and other allium vegetables (e.g., garlic and chives) is dominated by organosulfur stemming from the enzymatic decomposition of *S*-alk(en)yl-L-cysteine *S*-oxide flavor precursors. Besides, chemical compounds related to the flavor are generated from precursors, such as pyruvic acid, as well as ammonia and chemically unstable sulfenic acids (*3*). The amount of enzymatically generated pyruvic acid on onion homogenization is therefore a good measure of the allinase action on the flavor precursors and has been shown to be correlated with perceived onion pungency (*4–6*). Pungency, measured as pyruvate, varies between genotypes and growing conditions (*7*). Other compounds, such as sugars and organic acids, can contribute to the organoleptic experience. Organic acids are involved in multiple metabolic pathways, among which the main one is the Cycle of Krebs (*8*). All these organic acids contribute to a larger or smaller degree, depending upon their concentration and their pK_a, to the acidity and pH of the onion juice.

The literature about the chemical composition of the onion is scarce, particularly regarding the organic acids, except for pyruvic acid. In this paper, we determined the organic acid contents and pungency in different cultivars of onions cultivated in the same agroclimatic conditions to establish differences between cultivars. A correlation study and discriminant analysis was carried out to investigate relationships between the analyzed variables classified according to cultivar.

MATERIALS AND METHODS

Onion Sampling. A total of 90 samples of onion were provided for analysis by the Agricultural by Biodiversity Conservation Center of Tenerife (CCBAT), within the Interreg IIIB "Germobanco Agrícola de la Macaronesia" European Project. The description of the onion samples, including cultivar, mean weight, number of samples, and precedence of the seeds is shown in **Table 1**. The onions were cultivated

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Table 1. Description of the Onion Samples[a]

cultivar	seed origin	number assays	mean weight[b] (g/onion)	total number of analyzed samples
Texas	commercial Z-Seeds	1	104 ± 29 a	6
Masca	El Lomo	1	206 ± 49 ab	6
Masca	El Turrón	3	181 ± 56 cd	18
Guayonje	Pto. de la Madera	3	203 ± 52 bcd	18
Guayonje	Juan Fernández	2	252 ± 67 bcd	12
San Juan de La Rambla	El Rosario	1	143 ± 33 cd	6
San Juan de La Rambla	Las Aguas	1	211 ± 36 d	6
Carrizal Bajo	Carrizal Bajo	2	435 ± 93 e	12
Carrizal Alto	Carrizal Alto	1	611 ± 63 f	6
	total	15	253 ± 143	90

[a] All data were expressed as fresh weight. [b] Results in the same row with the same letter were not statistically significant ($p < 0.05$) according to the classification obtained by the Duncan test.

in March 2005 on a farm belonging to the CCBAT in the municipality of Tacoronte. Six onion cultivars were used in the present study: five of them were traditional cultivars from Tenerife (Guayonje, San Juan de la Rambla, Carrizal Alto, Carrizal Bajo, and Masca) and the other was a commercial cultivar (Texas Early Grano 502). The seed origin of the Masca, Guayonje, and San Juan de la Rambla cultivars proceeded from two zones of the same municipality. Between 1 and 3 assays were carried out on each cultivar, considering the different seed origins; therefore, a total of 15 accesions were analyzed. These 15 accesions were experimentally planted in random blocks with three repetitions. Two onion samples containing 3−5 onion bulbs (approximately 1.5 kg) were collected from each experimental block between June and July 2005; therefore, 6 onion samples by accession were analyzed. All of the samples were cultivated using the same environmental and agronomic conditions. A dose of 3.5 meq/L of sulfur in the nutritious solution was applied during the first 2 months of culture. The onion samples were collected at the same maturation stage, when between 50 and 80% of the plants had their leaves flattened (*9, 10*), which is the optimal moment for harvesting. After harvesting, the onions were stored at an average temperature of 20−25 °C for 1 week.

Sample Preparation Method. Three onions were selected from each onion sample. The outer leaves and top and bottom of onions were discarded, and after the onions were hand-rinsed with ultra pure water, they were shaken to remove any excess water and gently blotted with a paper towel. Each onion was cut horizontally into two equal parts. An horizontal slice (approximately 3 g of weight) of each onion was cut and introduced into an Erlenmeyer flask containing 10 mL of metaphosphoric acid 3%, and then they were frozen at $T = -80$ °C. This was carried out in triplicate. The ascorbic acid was determined in these three subsamples. After this, the onions were mixed and homogenized (Solac, Spain) to obtain a homogeneous and representative puree. Two subsamples were taken from this puree to measure pH, acidity, and Brix degree. The puree was stored in a polyethylene tube at $T = -80$ °C temperature to measure the rest of the organic acids (oxalic, glutamic, tartaric, pyruvic, malic, citric, maleic, fumaric, and succinic acids). Thus, 90 onions samples were available, and a total of 180 assays for each parameter were carried out, except for ascorbic acid (270 assays).

Routine Analysis. The pH was determined by potentiometric measurement at $T = 20$ °C with a pH meter (*11*). The acidity was determined by means of titration with 0.1 mol/L NaOH until pH 8.1, expressing the results in grams of anhydrous citric acid/100 g fresh weight (FW) (*11*). The onion juice was used to measure the Brix degree and the refractive index in a refractometer set at $T = 20$ °C (*11*). The ascorbic acid was determined by the 2,6-dichlorophenol-indophenol titration procedure (*11*). In this determination, a pink color in the aqueous extract of many onion samples was observed, which made difficult the observation of the end point of the titration. Therefore, a titration curve representing the absorbance (520 nm) against the volume of DIP added was built for each sample. Before the end point, the absorbance did not change with the volume of DIP added, but after the end point, a lineal increase of the absorbance was observed because of the excess of DIP (pink color). The intersection of both defined lines indicates the end point of the titration. A quality control measure was used in the determination of ascorbic acid. Several onion samples were spiked and checked with standards of ascorbic acid from Sigma. Recoveries within ±3% of their true value and precision of 3.5% were obtained.

Determination of Organic Acids. The organic acids were determined using a high-performance liquid chromatography (HPLC) method with a diode array detector described by Hernández Suárez et al. (*12*).

Equipment. The analytical HPLC system was comprised of a Waters 2690 high-performance liquid chromatograph equipped with a Waters 996 photodiode array detector (Water, Milford, MA). The separation was performed using a Waters Atlantis dC18 steel cartridge (150 × 4.6 mm i.d.) with a particle diameter of 3 μm, and a Waters Atlantis (20 × 4.6 mm) dC18 guard column was used to protect the analytical column. The temperature of the column oven was set at $T = 25$ °C during all of the experiments. The use of online connected diode array increases selectivity and sensitivity for the determination of these compounds. The HPLC pumps, autosampler, column oven, and diode-array system were monitored and controlled using the Millennium (*32*) system. A wavelength of 210 nm was used for the detection of the organic acids.

Reagents and Standards. Methanol of HPLC-gradient grade, sodium dihydrogen phosphate (NaH_2PO_4), and phosphoric acid were purchased from Merck (Darmstadt, Germany). Ethanol was purchased from Scharlau (Barcelona, Spain). Standards of *cis*-aconitic, fumaric, (−)-quinic, L-(+)-tartaric acids, and sodium pyruvate came from Sigma (St. Louis, MO); L-glutamic, maleic, L-(−)-malic, ascorbic, and oxalic acids came from Fluka (Buchs, Switzerland); and citric, shikimic, and succinic acids came from Aldrich (Milwaukee, WI). Stock solutions (5 g/L) were prepared in ultrapure water (Millipore, Bedford, MA) and stored in darkness at $T = 5$ °C. Deionized water was purified with a Milli-Q water system (Millipore Corporation, MA).

Procedure. About 1 g of the frozen homogenized onion puree was weighed directly in polypropylene tubes and mixed with 2 mL of ethanol 80%. After this, the tubes were put into an ultrasound bath for 5 min and then centrifuged for 5 min at 1090*g*. The supernatant was carefully recovered to prevent contamination with the homogenized onion puree pellet. Another 2 mL of 80% ethanol were then added to the pellet, placed in an ultrasound bath, and centrifuged as above. The two supernatants were recovered in the same tube. This liquid phase was concentrated with nitrogen stream until elimination of all of the ethanol. The residue was adjusted to 5 mL with ultrapure water (Milli-Q water system) and stored at $T = -80$ °C in the freezer.

This dissolution was passed through a 0.45 μm filter GHP (Waters, Millford, MA) and through a Sep-Pak Accell Plus QMA cartridge (Waters, Millford, MA), which was previously preconditioned with 3 mL of ultrapure water (Milli-Q water system). The compounds were eluted by washing with 2 mL of 20 mmol/L sodium dihydrogen phosphate to pH 1. Duplicate injections were performed, and average peak areas were used for the quantification.

The mobile phase was composed of 20 mmol/L sodium dihydrogen phosphate to pH 2.7. The injection volumes were 10 μL for both the standard solutions and sample extracts, and a flow rate of 0.7 mL/min was used.

Pungency Determination. One bulb of each onion sample was sliced in half longitudinally, and one portion was homogenized using a model T-25 Basic Turmix (Ika-Werke, Staufen, Germany) at a ratio of 1 mL of added water per gram of onion. The homogenate was left to stand for 10 min at room temperature and then filtered. Colorimetric determination of pyruvic acid was performed using a modified Schwimmer and Weston method (*13*). An 25 μL aliquot of the onion filtrate was added to 1 mL of water in a test tube. One mL of 0.25 g/L 2,4-dinitrophenylhydrazine (DNPH) in 1 M HCl was added to this, and the sample was placed in a 37 °C water bath. After 10 min, the samples were removed from the water bath and 1 mL of 1.5 M NaOH was added. The absorbance at 515 nm was then determined. A blank and standards were prepared by adding 25 μL of sodium pyruvate solution, ranging in concentration from 0 to 6 mM, in place of the onion sample.

Table 2. Results [Mean ± Standard Deviation (Minimum−Maximum)] of the Analyzed Parameters Expressed in Overall Terms and According to Individual Cultivars[a]

cultivar	pH	acidity (g/100 g)	Brix degree
Texas	5.61 ± 0.05 b (5.54−5.68)	0.109 ± 0.009 ab (0.094−0.118)	4.9 ± 0.6 a (3.8−5.2)
Masca	5.56 ± 0.12 ab (5.33−5.78)	0.110 ± 0.011 ab (0.089−0.132)	4.9 ± 1.4 a (2.3−7.4)
Guayonje	5.47 ± 0.15 a (5.14−5.72)	0.115 ± 0.015 b (0.090−0.149)	6.8 ± 0.6 b (5.9−8.1)
San Juan de la Rambla	5.60 ± 0.13 b (5.39−5.84)	0.130 ± 0.015 c (0.100−0.156)	8.6 ± 2.3 c (6.2−11.6)
Carrizal Bajo	5.61 ± 0.11 b (5.46−5.79)	0.099 ± 0.015 a (0.075−0.130)	4.9 ± 1.4 a (3.1−6.4)
Carrizal Alto	5.67 ± 0.79 b (5.57−5.78)	0.108 ± 0.010 ab (0.091−0.118)	5.4 ± 0.3 a (5.0−6.0)
total	5.55 ± 0.14 (5.14−5.84)	0.113 ± 0.016 (0.075−0.156)	6.0 ± 1.8 (2.3−11.6)

[a] All data were expressed as fresh weight. Results in the same column with the same letter were not statistically significant ($p < 0.05$) according to the classification obtained by the Duncan test.

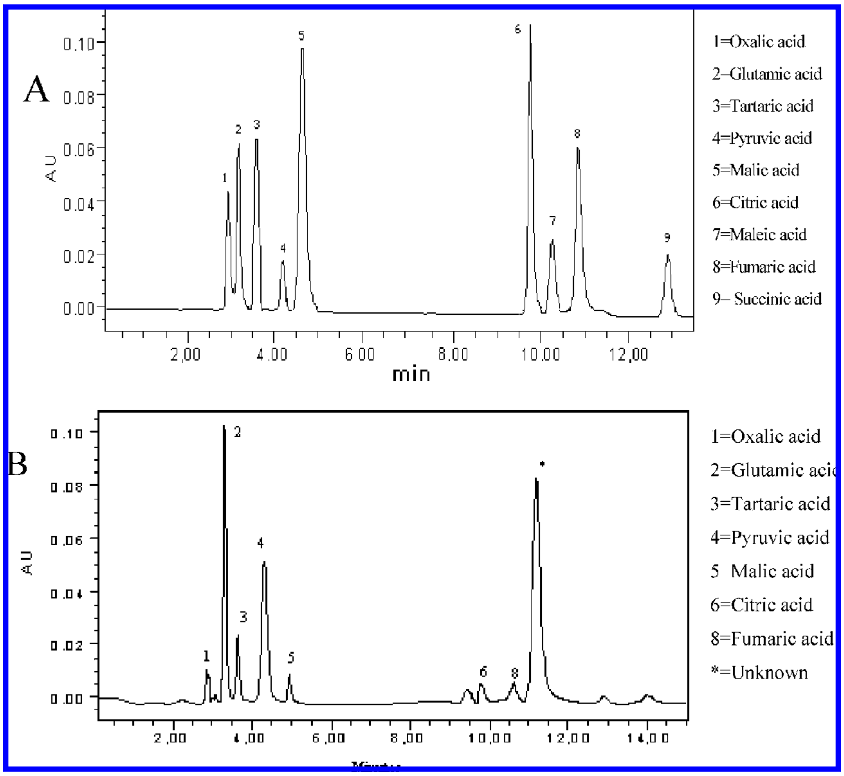

Figure 1. Chromatograms of an eight-component standard (A) and an onion sample (B).

Statistics. All of the statistics were performed by means of the SPSS version 14 software for Windows (SPSS, Inc., Chicago, IL). The Kolmogorov−Smirnov test was applied to verify whether the distribution of the variables was normal ($p < 0.05$). When the statistical distribution was not normal, the variables were transformed by applying neperian logarithms to convert them into normal distributions. The Levene test was applied to verify the homogeneity of the variances. Mean values obtained for the variables studied in the different groups were compared by one-way analysis of variation (ANOVA) (Duncan's multiple range) assuming there were significant differences among them when the statistical comparison gave $p < 0.05$. Simple linear and logarithmic correlation analysis was used to indicate a measure of the correlation and the strength of the relationship between two variables. Discriminant analysis (DA) is based on the extraction of linear discriminant functions of the independent variables by means of a qualitative dependent variable and several quantitative independent variables. Two processes were applied in DA: (1) stepwise DA that selected the quantitative variables that enhance discrimination of the groups established by the dependent variable and (2) introduction of all independent variables. The objective of this process is not to lose information, although the system obtained is more complex.

RESULTS AND DISCUSSION

Table 2 shows the results obtained for pH, acidity, and Brix degree in all of the onion samples and differentiates the onion samples according to the cultivars considered. The results of the variance analysis of the comparison among the mean values are also shown in this table. Significant differences were observed for all these parameters in the mean concentrations or values among the cultivars of onion. The onion juice was slightly acidic. The mean pH spread over a relatively short range, between 5.14 and 5.84, which agrees with other data in the literature (*14–16*). Pozzo Ardizzi et al. (*15*) did not find differences in the pH values among the five studied cultivars. However, in this study, the Guayonje cultivar showed the lowest mean pH value, with significant differences in relation to the mean values obtained for the rest of the cultivars, except for the Masca cultivar. The Guayonje cultivar had the most intensely red color, which is due to the presence of a specific group of phenolic compounds, the anthocyanins (*17, 18*). The anthocyanins have their maximum expression of red color to acid pH, and its shape is produced colorless neutral or alkaline pH. The lowest pH value found in the Guayonje cultivar could explain the high intensity of the red color in this cultivar, because the colored form anthocyanins are stable in acid pH (*19*).

Acidity presented a higher variation than pH in all of the onion samples, with a coefficient of variation of 14.1% against 2.5% for the pH, which is due to the fact that pH is defined as

Table 3. Results [Mean ± Standard Deviation (Minimum−Maximum)] of the Analyzed Parameters Expressed in Overall Terms and According to Individual Cultivars[a]

cultivar	ascorbic acid (mg/100 g)	glutamic acid (mg/100 g)	citric acid (mg/100 g)	malic acid (mg/100 g)	tartaric acid (mg/100 g)	oxalic acid (mg/100 g)	fumaric acid (mg/100 g)	pungency (μmol of pyruvic acid/g)
Texas	4.15 ± 0.082 b (2.99−5.13)	252 ± 61 ab (157−299)	39.4 ± 6.6 ab (29.5−46.7)	47.8 ± 4.6 c (41.4−53.5)	8.9 ± 1.4 a (7.2−11.3)	6.6 ± 3.5 a (3.2−10.5)	0.47 ± 0.14 c (0.24−0.61)	3.91 ± 1.64 c (2.62−6.80)
Masca	4.84 ± 0.83 c (3.23−5.97)	433 ± 85 c (273−565)	54.3 ± 12.2 b (33.4−77.1)	49.3 ± 8.2 c (26.9−66.2)	23.3 ± 9.2 c (13.9−42.6)	13.0 ± 2.1 bc (9.3−16.5)	0.20 ± 0.07 ab (0.10−0.37)	4.26 ± 2.16 c (1.09−8.14)
Guayonje	2.80 ± 0.86 a (1.11−5.04)	346 ± 34 bc (117−696)	44.0 ± 13.8 ab (14.5−75.9)	31.1 ± 8.1 ab (19.9−56.6)	19.2 ± 7.3 bc (8.4−34.4)	11.2 ± 3.0 b (5.7−16.6)	0.16 ± 0.11 a (0.04−0.42)	3.63 ± 1.71 bc (0.99−6.18)
San Juan de La Rambla	4.52 ± 0.43 bc (3.51−5.03)	288 ± 16 ab (102−493)	75.3 ± 47.5 c (16.4−145.7)	50.3 ± 6.2 c (40.6−62.2)	15.5 ± 4.1 b (7.6−20.7)	14.6 ± 4.2 c (10.0−22.1)	0.27 ± 0.16 b (0.08−0.52)	2.32 ± 1.02 ab (0.74−3.60)
Carrizal Bajo	3.43 ± 0.37 a (2.92−4.09)	195 ± 68 ab (91−307)	34.6 ± 13.8 ab (13.2−62.5)	34.3 ± 9.3 a (19.5−53.7)	14.0 ± 5.0 b (6.3−23.4)	8.6 ± 2.6 a (5.3−12.3)	0.39 ± 0.11 c (0.24−0.59)	3.59 ± 0.77 bc (2.24−4.88)
Carrizal Alto	2.93 ± 0.41 a (2.17−3.37)	192 ± 65 a (105−254)	31.1 ± 13.3 a (17.6−49.5)	44.7 ± 17.8 bc (25.9−72.5)	25.2 ± 13.9 c (11.1−44.6)	7.8 ± 3.4 a (4.0−12.8)	0.28 ± 0.08 b (0.17−0.36)	1.68 ± 0.44 a (1.04−2.24)
total	3.75 ± 1.13 (1.11−5.97)	325 ± 133 (91−696)	48.5 ± 24.1 (13.2−145.7)	43.6 ± 10.4 (19.5−72.5)	18.8 ± 8.6 (6.3−44.6)	11.3 ± 3.7 (3.2−22.1)	0.24 ± 0.14 (0.04−0.61)	3.51 ± 1.76 (0.74−8.14)

[a] Results in the same column with the same letter were not statistically significant ($p < 0.05$) according to the classification obtained by the Duncan test.

a logarithmic scale of acidity. Acidity data found in the bibliography for other cultivars were higher than the results obtained in this paper. Roberts and Kidds (*16*) reported acidity levels (expressed as lactic acid) before fermentation within the interval 0.14−0.22 g/100 mL (0.10−0.16 g citric acid/100 mL). Zambrano, Ramírez, and Manzano (*20*) reported values (expressed as pyruvic acid) in the interval 0.177 and 0.218 g/100 g FW (0.129−0.159 g citric acid/100 mg), and Rodríguez et al. (*14*) described an interval of 0.120 and 0.190 g/100 g FW in the same cultivar collected at different periods of the year. Therefore, similar results were obtained when these data were expressed in the same units as our results, grams of citric acid/100 g FW. The San Juan de la Rambla cultivar had a higher mean acidity ($p < 0.05$) than the rest of the cultivars, and the Carrizal Bajo cultivar showed the lowest mean acidity with significant differences with regard to the San Juan de la Rambla and Guayonje cultivars. The pH and acidity levels of the Texas cultivar were in the middle of the range of the cultivars produced in Tenerife.

The Brix degree concentration for fresh onions reported in the literature (*14, 20–24*) also varies over a wide range (4−8 Brix degree), and our data fell well within this range. In contrast, Jaime et al. (*25*), Kopsell and Randle (*1*), and Pozzo Ardizzi et al. (*26*) found values of the Brix degree higher than our data. The San Juan de la Rambla cultivar showed a higher ($p < 0.05$) mean Brix degree concentration than the Guayonje cultivar, which in turn was higher ($p < 0.05$) than the mean Brix degree concentration for the rest of the cultivars.

Figure 1 shows a chromatogram corresponding to an eight-component mixture standard (A) and a chromatogram of an onion sample (B). One can observe the good resolution and separation of the identified organic acids in a real sample of onion using the isocratic elution described in the Materials and Methods. Additional organic acids, such as ascorbic, shikimic, aconitic, and quinic acids, were used to try to identify the unknown chromatographic peaks in the real samples. The identification of the observed peaks was carried out by checking the retention time and the absorption spectra of each organic acid of both real onion samples and the standards in the range between 190 and 400 nm. Also, the onion samples were spiked with standards to verify the identity of the chromatographic peaks. The following seven organic acids were separated and identified in all of the onion samples: glutamic, oxalic, pyruvic, malic, tartaric, citric, and fumaric acids. There was a well-resolved and high peak at a retention time of 11.57 min, which was observed in all of the analyzed onion samples. This peak could be an organic acid on the basis of the absorption spectrum; however, the retention time did not match any of the standards and identification was not possible.

Table 3 shows the results corresponding to the organic acids detected by HPLC, pungency, and ascorbic acid. Ascorbic acid was not detected by HPLC. However, this acid was determined using the 2,6-dichlorophenol-indophenol titration procedure as indicated in the Materials and Methods. The contribution to the daily vitamin C intake from the consumption of a serving (100 g) of onion is relatively low, representing 6.2% of the recommended dietary intake (*27*). The mean ascorbic acid concentration found by us was higher than other data reported in the literature, such as by Gökmen, Kahraman, Demir, and Acar (*28*) (1.5 and 1.8 mg/100 g), Pellegrini et al. (*29*) (1.98 mg/100 g FW), and Adam et al. (*30*) (19.7 mg/100 g dry matter) and similar than obtained by Franke et al. (*31*) (3.7−8.4 mg/100 g FW) and Proteggente et al. (*32*) (6 mg of vitamin C/100 g FW). The Guayonje, Carrizal Alto, and Carrizal Bajo cultivars showed

Table 4. Results [Mean ± Standard Deviation] in the Masca, Guayonje, and San Juan de la Rambla Cultivars According to Seed Origin[a]

parameter	Masca			Guayonje			San Juan de la Rambla		
seed origin	El Turrón	El Lomo	*p*	Juan Fernández	Pto. de la Madera	*p*	El Rosario	Las Aguas	*p*
pH	5.56 ± 0.12	5.55 ± 0.13	NS[b]	5.39 ± 0.15	5.52 ± 0.12	*c*	5.51 ± 0.08	5.70 ± 0.11	*c*
acidity	0.11 ± 0.01	0.11 ± 0.01	NS	0.11 ± 0.02	0.12 ± 0.01	NS	0.14 ± 0.01	0.12 ± 0.01	*c*
Brix degree	4.4 ± 1.2	6.2 ± 1.1	*d*	6.4 ± 0.4	7.0 ± 0.6	*d*	10.7 ± 0.6	6.4 ± 0.2	*e*
ascorbic acid	5.0 ± 0.7	4.5 ± 1.1	NS	2.6 ± 0.4	2.9 ± 1.1	NS	4.7 ± 0.3	4.4 ± 0.5	NS
glutamic acid	440 ± 89	411 ± 78	NS	298 ± 171	378 ± 94	NS	347 ± 95	229 ± 111	NS
citric acid	50.6 ± 10.3	65.2 ± 11.4	*d*	42.2 ± 19.1	45.2 ± 9.2	NS	118 ± 21	32.8 ± 13.8	*e*
malic acid	48.1 ± 8.9	53.0 ± 4.3	NS	37.3 ± 8.7	40.4 ± 7.7	NS	54.1 ± 4.3	46.4 ± 5.5	*c*
tartaric acid	20.9 ± 7.8	30.4 ± 10.2	*c*	19.9 ± 7.4	18.7 ± 7.4	NS	16.0 ± 2.7	15.1 ± 5.4	NS
oxalic acid	12.7 ± 1.8	13.8 ± 2.7	NS	10.4 ± 2.9	11.8 ± 3.0	NS	18.0 ± 3.3	11.2 ± 1.0	*e*
fumaric acid	0.22 ± 0.07	0.15 ± 0.03	*c*	0.17 ± 0.14	0.14 ± 0.08	NS	0.13 ± 0.04	0.41 ± 0.08	*e*
pyruvic acid	14.6 ± 3.2	15.4 ± 2.5	NS	10.9 ± 2.7	13.2 ± 3.3	NS	17.8 ± 2.4	10.6 ± 2.7	*d*
pungency	5.2 ± 1.7	1.6 ± 0.5	*e*	3.2 ± 1.6	3.9 ± 1.8	NS	3.0 ± 0.6	1.6 ± 0.8	*d*

[a] All data were expressed as mg/100 g FW, except pungency, which was expressed as μmol of pyruvic acid/g, and acidity, which was expressed as g/100 g. [b] NS = not significant $p > 0.05$. [c] $0.05 > p > 0.01$. [d] $0.01 > p > 0.001$. [e] $p < 0.001$.

Table 5. Matrix Correlation for All of the Samples[a]

	pH	acidity	Brix degree	ascorbic acid	glutamic acid	citric acid	malic acid	tartaric acid	oxalic acid	fumaric acid	pyruvic acid	pungency
pH	1.000	−0.380								0.434	−0.215	
acidity		1.000	0.501		0.233	0.448			0.433	−0.338	0.318	
Brix degree			1.000			0.555			0.400	−0.316	0.297	
ascorbic acid				1.000	0.257	0.301	0.424		0.215		0.321	
glutamic acid					1.000	0.380	0.305	0.348	0.553	−0.446	0.497	0.296
citric acid						1.000	0.463		0.646	−0.314	0.620	
malic acid							1.000	0.468	0.464		0.714	
tartaric acid								1.000	0.411	−0.327	0.346	
oxalic acid									1.000	−0.314	0.658	
fumaric acid										1.000	−0.254	
pyruvic acid											1.000	
pungency												1.000

[a] Only the significant ($p < 0.05$) correlations are shown as Pearson's coefficient correlation.

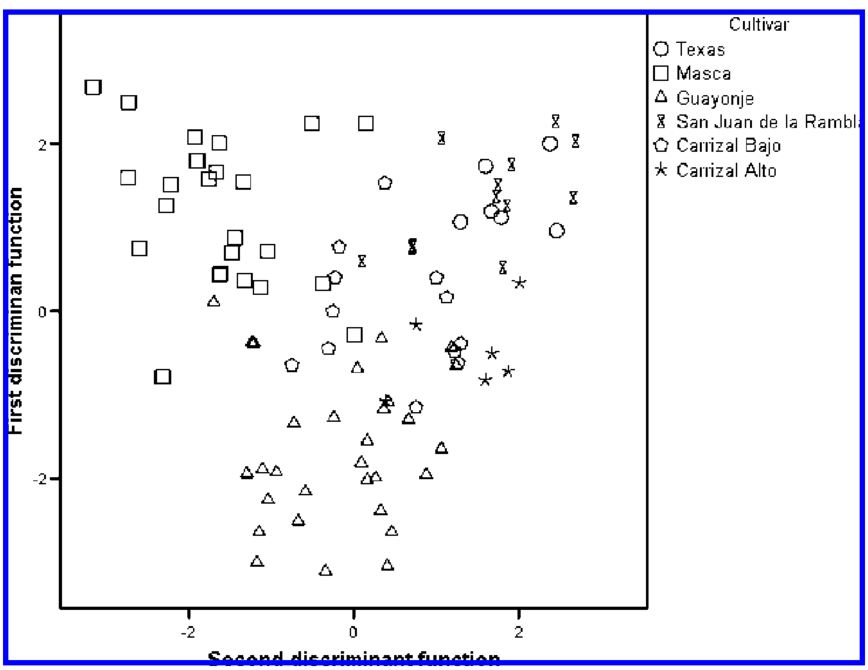

Figure 2. Scatter diagram on the axes representing the first two discriminant functions according to cultivar.

lower mean ascorbic acid contents than those found for the Texas, San Juan de la Rambla, and Masca cultivars. The Masca cultivar had significantly higher ascorbic acid content than the mean value for other cultivars examined, except the San Juan de la Rambla.

Glutamic acid was the major organic acid followed by citric acid and malic acid in all of the onion samples, followed by tartaric, oxalic, and pyruvic acids, which were present in medium amounts, and fumaric acid was the least abundant. There are few suitable data on organic acids in onions. Benkeblia and Varoquax (*21*) detected oxalic, citric, succinic, malic, and fumaric acids in a study to investigate the effect of nitrous oxide on the respiration rate, soluble sugars, and quality attributes of onion bulbs. Those data agree with the results obtained by us,

Table 6. Results of the Discriminant Analysis with All of the Variables To Differentiate the Onion According to the Cultivar and Seed Origin[a]

	cultivars and seed origin	predicted group: Masca (El Turrón)	Masca (El Lomo)	Guayonje (Pto. de la Madera)	Guayonje (Juan Fernández)	San Juan de la Rambla (El Rosario)	San Juan de la Rambla (Las Aguas)	Carrizal Alto	Carrizal Bajo
initial group	Masca (El Turrón)	94.4%	5.6%						
	Masca (El Lomo)		100%						
	Guayonje (Pto. de la Madera)		5.6%	66.7%	27.8%				
	Guayonje (Juan Fernández)			25%	66.7%				8.3%
	San Juan de la Rambla (El Rosario)					100%			
	San Juan de la Rambla (Las Aguas)						100%		
	Carrizal Alto							100%	
	Carrizal Bajo								100%

[a] Global correct classification 82.1% (69% after cross-validation).

except that we did not detect succinic acid. We also detected other organic acids, such as glutamic, tartaric, and pyruvic acids. The concentrations found by Benkeblia and Varoquax (*21*) were higher for oxalic (37 mg/100 g FW), malic (102 mg/100 g FW), and fumaric (0.68 mg/100 g FW) acids and lower for citric acid (18.9 mg/100 g FW) than the results obtained in this study.

Many significant differences were observed between the mean organic acid concentrations. The common synthetic pathways of these organic acids could explain this high number of significant correlations. Besides, the organic acids are involved in multiple metabolic pathways, among which the main one is the Cycle of Krebs (*8*). In general, the San Juan de la Rambla and Masca cultivars presented higher mean concentrations of the organic acids than the other three cultivars, except for fumaric acid. The Masca cultivar showed the highest mean glutamic acid concentration with significant differences with respect to the rest of the cultivars, except the Guayonje cultivar. In contrast, the Carrizal Alto had a lower mean glutamic acid concentration than the Masca and Guayonje cultivars. The San Juan de la Rambla cultivar had the highest ($p < 0.05$) mean citric acid concentration. Furthermore, the Masca cultivar had a higher ($p < 0.05$) mean citric acid concentration than that found for the Carrizal Alto cultivar. The Carrizal Bajo and Guayonje cultivars presented a lower ($p < 0.05$) mean malic acid concentration than those found in the rest of the cultivars, except the Guayonje and Carrizal Alto cultivars. The lowest ($p < 0.05$) mean tartaric acid concentration was observed in the Texas cultivar, and the Carrizal Alto cultivar had a higher ($p < 0.05$) mean concentration than the Texas, San Juan de la Rambla, and Carrizal Bajo cultivars. The San Juan de la Rambla, Masca, and Guayonje cultivars presented higher ($p < 0.05$) mean oxalic acid concentrations than the other three cultivars. The Guayonje had the lowest mean fumaric acid concentration, with significant differences with respect to the rest of the organic acids, except the Masca cultivar, while the Texas cultivar showed the highest mean fumaric acid concentration, with significant differences with respect to the rest, except the Carrizal Bajo cultivar.

Pyruvic acid was determined by HPLC in the same way as the other organic acids. As in the other organic acids, except fumaric acid, the mean pyruvic acid concentration in the Masca and San Juan de la Rambla cultivars was higher ($p < 0.05$) than those mean concentrations found in the Texas, Carrizal Bajo, and Carrizal Alto cultivars. The Guayonje cultivar had medium quantities of pyruvic acid, with significant differences only in relation to the Carrizal Alto cultivar. Pungency of onion can be measured indirectly, as pyruvic acid content, which is a product of the enzymatic breakdown of *S*-alk(en)yl-cysteine sulfoxides. However, the time and conditions necessary for the action of allinase were not optimized in the HPLC systems used here. Therefore, the determination of the pungency (μmol of pyruvic acid/g) using pyruvic acid determined by HPLC is not suitable because the synthesis of this acid by the action of allinase is not complete. Therefore, pungency was determined in the onion samples using the colorimetric and enzymatic method (*13*) described in the Materials and Methods. There is an important variation in pungency levels among commercial onion cultivars. Therefore, a classification of onions according to pungency has been proposed as follows (*26*): low, 0−3 μmol of pyruvic acid/g; moderate, 3−7 μmol of pyruvic acid/g; and high pungency, >7 μmol of pyruvic acid/g. The mean pungency values varied significantly among the onion cultivars, which is in accordance with that found by other authors (*13, 22, 24, 33–35*). Therefore, the Carrizal Alto cultivar had the lowest mean pungency value, with significant differences in relation to the rest of the onion cultivars, except the San Juan de la Rambla cultivar. All of the onion samples belonging to the Carrizal Alto cultivar had low pungency (0−3). The Masca cultivar (18% of the onion samples with high pungency), followed by the Texas cultivar, showed the highest pungency values.

A subsequent study was conducted to discover the influence of the seed origin in any individual cultivar on the pH, acidity, Brix degree, organic acids, and pungency (**Table 4**). This study was performed on three cultivars: Masca, Guayonje, and San Juan de la Rambla, which were produced from seeds of different precedence. The onions from the San Juan de la Rambla cultivar showed a significant difference in the mean concentrations obtained between the two seed origins for all of the analyzed parameters, except ascorbic, glutamic, and tartaric acids. In addition, significant differences were obtained in the mean concentrations of Brix degree, citric, tartaric, and fumaric acids, and pungency between both seed origins considered in the Masca cultivar. In contrast, only the mean values of pH and Brix degree showed significant differences between the two seed origins of the Guayonje cultivar. Thus, these results suggest that, in some cases, environmental conditions, such as climatic conditions or soil type, could influence the genetic information of the onion cultivars, thereby producing variations in their organic acids and related parameters.

A statistical study of the correlation between all of the analyzed parameters was previously conducted to discover associations between measured pairs of these parameters. In **Table 5**, one can see a high number of significant ($p < 0.05$) correlations, which are due to the common and complex interactions between the organic acids and also between the organic acids, with parameters related to acidity. Acidity is inversely correlated with pH and positively correlated with Brix degree. The acidity presented significant and positive correlations with glutamic, citric, oxalic, and pyruvic acids. This finding suggests a relevant role of these organic acids and particularly of the major glutamic and citric acids in the acidity of the onion. The Brix degree showed positive correlations with citric, oxalic, and pyruvic acids. The common metabolic routes between these organic acids and the metabolism of sugars, the main component responsible for the Brix degree, could be related to these correlations. Fumaric acid presented inverse correlations with glutamic, citric, tartaric, oxalic, and pyruvic acids and positive correlations with pH. Ascorbic acid is positively correlated with glutamic, citric, malic, oxalic, and pyruvic acids. All of the organic acids analyzed by HPLC presented significant and positive correlations, except fumaric acid versus malic acid and tartaric acid versus citric acid. Among these correlations, the pyruvic acid−malic acid ($r = 0.714$), oxalic acid−citric acid ($r = 0.646$), and oxalic acid−pyruvic acid ($r = 0.658$) should be emphasized because of their high correlation coefficients.

Lineal discriminant analysis (LDA) was performed on all of the quantitative parameters studied to differentiate the onion samples according to cultivar. After application of stepwise LDA, moderate (83.3 and 73.3% after cross-validation) percentages of correct classification of the onion samples according to the cultivar were obtained. The following variables were selected: Brix degree and tartaric, malic, fumaric, pyruvic, ascorbic, and oxalic acids. When all of the variables were introduced, the classification improved to 91.1% (75.6% after cross-validation). A tendency to differentiate the onion samples according to the cultivar was observed as can be seen in the representation of the first two discriminant functions obtained using LDA with all of the variables (**Figure 2**). All of the onion samples belonging to the Texas, San Juan de la Rambla, and Carrizal Bajo cultivars were well-classified.

A correct classification of 82.1% (69.0% after cross-validation) was obtained selecting Brix degree, pungency, and pyruvic, malic, citric, fumaric, and ascorbic acids after applying stepwise LDA to differentiate the traditional onion samples according to the cultivar and seed origin. **Table 6** shows the classifications obtained when all of the variables were introduced. The global classification improved to 86.9% (69.0% after cross-validation), and a complete classification (100%) was observed in the onion samples belonging to the Masca (El Lomo), Carrizal Alto, and Carrizal Bajo cultivars and in those belonging to cultivar San Juan de la Rambla proceeding from the two zones: El Rosario and Las Aguas. This confirms the results previously described (**Table 4**), which suggest that the onions belonging to San Juan de la Rambla from both zones considered could be different cultivars.

A new LDA was performed to classify the onion samples according to the considered accessions. When all of the variables were introduced, a total classification of the onion sample reached 95.2% (81.0% after cross-validation). All of the onion samples were correctly classified within their accessions, except for four onion samples belonging to the Masca (Turrón), Guayonje (Puerto Madera), Guayonje (Juan Fernández), and Carrizal Bajo cultivars, respectively. Thus, the place in the plantation and/or the onion sampling carried out at the time of harvesting seems to be an important factor in the organic acid concentrations and Brix degree, acidity, and pH in onions.

The following concluding remarks can be drawn. Seven organic acids (glutamic, oxalic, pyruvic, malic, tartaric, citric, and fumaric acids) were identified and quantified in onions. Succinic, shikimic, aconitic, and quinic acids were not detected. Glutamic acid followed by citric acid and malic acid were the major organic acids present in the onions. Most of the analyzed onion samples showed moderate to low pungency. There are considerable differences in Brix degree, acidity, and organic acid contents among the onions cultivars. There were differences in the Brix degree, acidity, and organic acids, as well as parameters related to some onion cultivars associated with the seed origin. Therefore, the environment and agronomic practices may affect the expression of genes of the seeds, thereby determining changes of the organic acid contents. LDA is a useful tool to differentiate the onion samples according to the cultivar and the seed origin of the cultivars. The Texas, San Juan de la Rambla, and Carrizal Alto cultivars showed the most differentiated composition of organic acids. The San Juan de la Rambla cultivar onion samples were differentiated according to their seed origin.

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