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Saponin Profile of Green Asparagus Genotypes

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ABSTRACT: The main goal of this study was to determine the saponin profiles of different "triguero" asparagus genotypes and to compare them to green asparagus commercial hybrids. The samples consisted of 31 commercial hybrids and 58 genotypes from the Huétor-Tájar (HT) population variety ("triguero"). The saponin analysis by high-performance liquid chromatography−mass spectrometry allowed for the determination of 12 saponins derived from a furostan-type steroidal genin, 4 of which had never been described in the edible part of asparagus. The saponin profile of "triguero" asparagus was a combination of these new saponins and protodioscin. Although protodioscin was the major saponin found in commercial hybrids, some of these 12 saponins were detected as major components in some of the commercial hybrids. The total contents of saponins described in some of these HT genotypes reach values as high as 10−100 times higher than those found in commercial hybrids.

KEYWORDS: *"Triguero" asparagus, saponins, commercial hybrids, HPLC−MS*

INTRODUCTION

Andalusia, a southern region of Spain, is one of the leading asparagus producers in Europe.[1] Among the different types of commercial green asparagus, Andalusia produces the so-called "Triguero asparagus from Huétor-Tájar" ("Triguero" HT asparagus) also known as "Morado de Huetor" because these asparagus are characterized to have a purple coloration. This is an asparagus landrace that together with "Violetto d'Albenga", an Italian asparagus, are the only two European tetraploid cultivars,[2] although some triploid plants have also been identified using both a flow cytometry technique[3] and cytology technique,[4] at least in the Spanish landrace. In general, the tetraploid populations show a higher variation than diploid cultivars at both isoenzymatic and random amplified polymorphic DNA (RAPD) marker levels.[5,6] This high diversity can be easily observed on a morphological level from stem number, spear diameter, ramification height, plant color, or spear color, for example. Some plants in the field can also have a wild appearance with thinner, bitter spears, shorter cladodes, and slightly striated stems.[6]

Some studies have suggested that "triguero" HT asparagus could be a hybrid between cultivated diploid varieties of *Asparagus officinalis* and wild *Asparagus maritimus*,[7] although some others did not find evidence to confirm this.[2]

"Triguero" HT asparagus is well-appreciated for its organoleptic characteristics and is very well differentiated from diploid commercial hybrids derived from *A. officinalis*. Nevertheless, the cultivated area of this population has decreased because of the introduction of modern cultivars that produce higher yields than the local population. These landraces constitute a valuable genetic resource that could help to enlarge the genetic background of modern cultivars.[8] They could be used for the development of new varieties with improved organoleptic, functional, and nutritional characteristics. With this aim, the asparagus sector, together with several research groups, has created a germplasm of HT asparagus containing 65 parental genotypes selected according to their morphological characteristics.[7]

Previously, we have studied the phenolic composition of the different HT asparagus germplasm genotypes and found that HT asparagus has a distinct flavonoid profile compared to commercial diploid hybrids.[9−11]

Apart from flavonoids, the other most important group of bioactive components of asparagus is saponins, which play an important role in the organoleptic and functional properties of asparagus. Some studies have shown their important hypocholesterolemic effect in both experimental animals and humans and are beginning to be sonsidered as potential nutritional supplements in the control of dislipidemias and obesity.[12,13] On the other hand, steroidal saponins from different species of asparagus showed cytotoxic and cytostatic effects in different human cell lines.[14] However, the saponins present in edible plants of the genus *Asparagus* are not only responsible of the biological activity but, according to some authors, can also contribute in the asparagus flavor and, therefore, in their organoleptic properties.[15,16] Recently, saponins have been identified as the molecules responsible for the bitter taste of white asparagus.[17]

Previously, the application of a liquid chromatography−mass spectrometry (LC−MS) method to selected genotypes of HT asparagus revealed that, while commercial hybrids contain

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Table 1. New Saponins Identified by HPLC−MS (+/−) in Green Asparagus

			Molecular Ion (*m/z*)		Fragmentation Pathway	
Saponin	Rt (min)[a]	MW[b]	Negative Ion	Positive Ion	Negative Mode	Positive Mode
HTSAP-9	22.24	1050	1049[M-H]$^-$	1073[M+Na]$^+$	-[c]Pen917-[d]Hex755-Hex593-Hex431[g] -Hex887-Pen755	[-Na-H_2O]1033-Hex871-Pen739-Hex577-Hex415 [g]
HTSAP-10	27.05	1048	1047[M-H]$^-$	1071[M+Na]$^+$	-[e]DoHex901-DoHex755-[f]Unk595-Hex433[g]	[-Na-H_2O]1031-Hex869- DoHex723-DoHex577- Unk417[g] -DoHex885- DoHex739-Hex577
HTSAP-11	27.30	1034	1033[M-H]$^-$	1057[M+Na]$^+$	-Pen901-DoHex755-Hex593-Hex431	[-Na-H_2O]1017--Hex855-Pen723-DoHex577 -Hex415 -Pen885-DoHex739-Hex577
HTSAP-12	27.40	1050	1049[M-H]$^-$	1073[M+Na]$^+$	-DoHex903-DoHex757-Hex595-Hex433	[-Na-H_2O]1033-DoHex887-Hex725-DoHex579-Hex417

[a]Rt = retention time. [b]MW = molecular weight. [c]Pen = pentose. [d]Hex = hexose. [e]DoHex = deoxyhexose. [f]Unk = unknown. [g]Genins = 431, 433, 415, and 417.

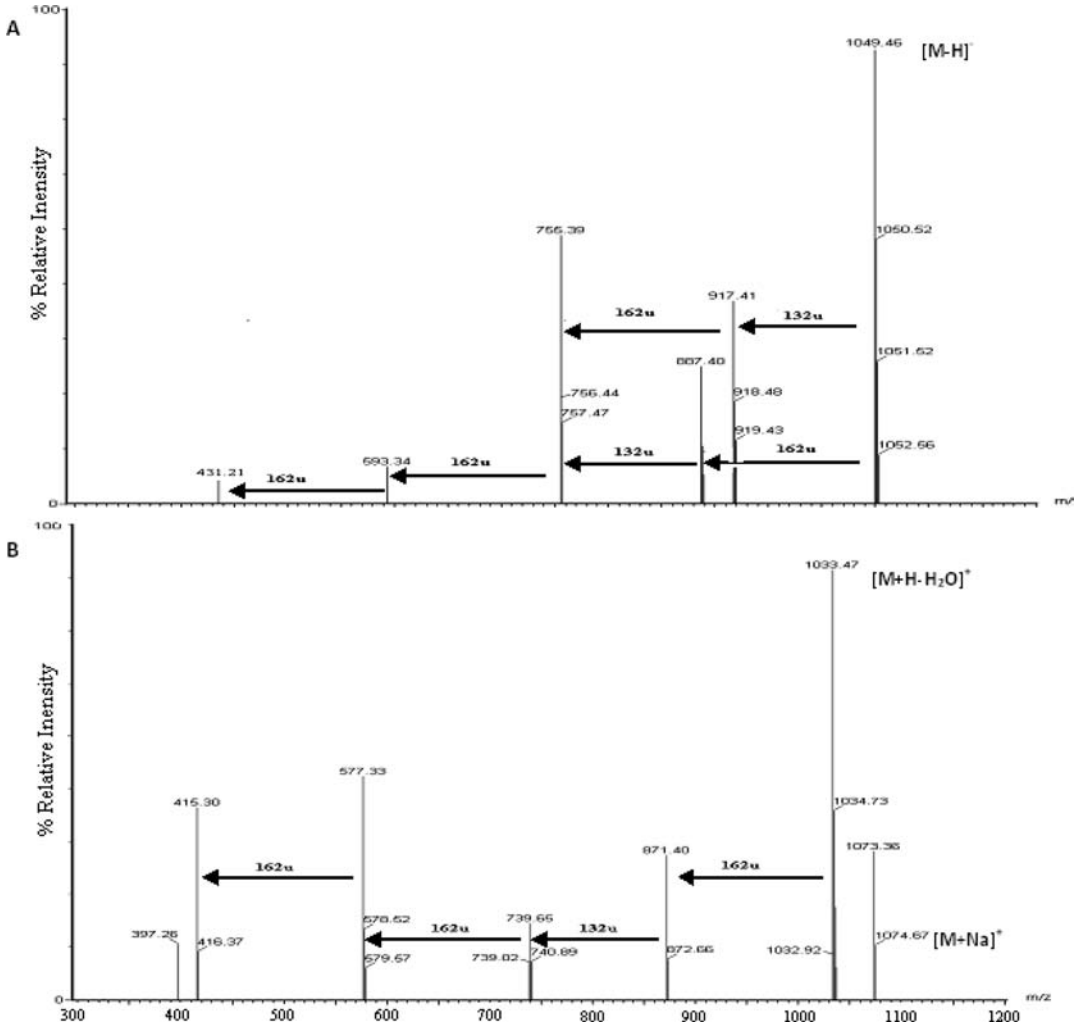

Figure 1. Electrospray ionization (ESI) mass spectra of HTSAP-12 in (A) negative (100 V−) and (B) positive (50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

protodioscin as the major saponin, HT asparagus presents a more complex saponin profile, a result of the combination of protodioscin and at least eight different new saponins derived from a furostanol-type steroidal genin with a single bond between C_5 and C_6 of the B ring.[18] In the present study, we have extended the analysis to the whole HT asparagus germplasm containing 65 different genotypes and 31 of the most relevant commercial varieties.

MATERIALS AND METHODS

Plant Material. The samples evaluated consisted of spears from 58 native lines of "triguero" asparagus from the HT landrace and the green asparagus shoots of 31 outstanding commercial hybrids from an existing collection at Las Torres Agricultural Research Center, Alcalá del Rio, Sevilla, Spain.

The spears were harvested from experimental fields and immediately transported to the laboratory, where they were trimmed to a final length of 21 cm, weighed, and frozen at −20 °C. Afterward,

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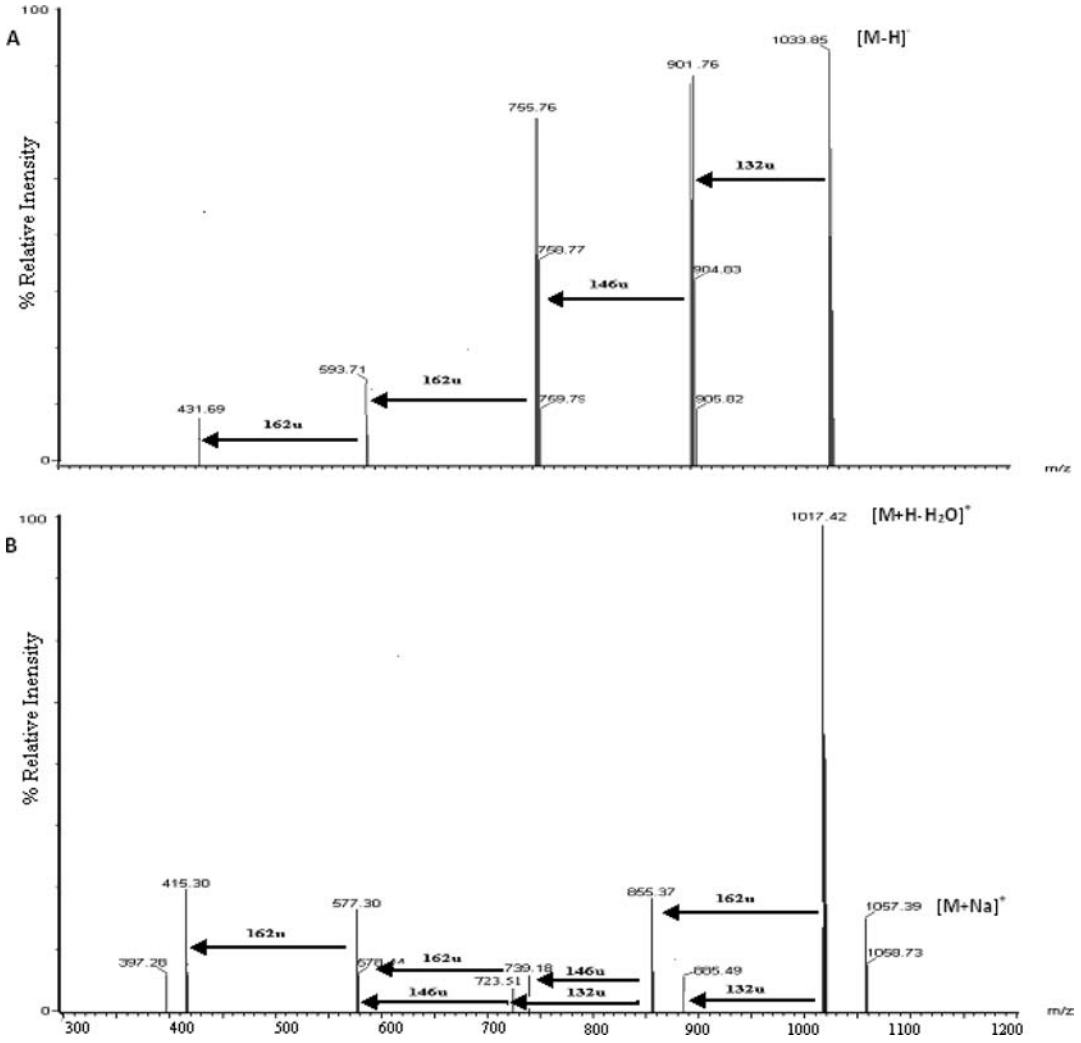

Figure 2. ESI mass spectra of HTSAP-9 in (A) negative (100 V−) and (B) positive (50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

they were freeze-dried, ground to a fine powder, and stored at −20 °C until further analysis.

These 58 native lines of "triguero" asparagus were studied in a previous study to analyze their flavonoid profile; therefore, the nomenclature used in this work was the same as that received in the previous paper.[11]

Chemicals and Reagents. Protodioscin (97%) and shatavarin (98.6%), purity checked by nuclear magnetic resonance (NMR), were purchased from Chromadex Chemical Co. (Barcelona, Spain). Ethanol, formic acid (96%), and acetonitrile, high-performance liquid chromatography (HPLC) grade, were purchased from Sigma Chemical Co. (St. Louis, MO). The C-18 cartridges (500 mg) were purchased from Varian Incorporated (Lake Forest, CA). Pure deionized water was obtained from a Milli-Q50 system (Millipore Corporation, Bedford, MA).

Saponin Extraction and Purification. Each sample, consisting of 2.5 g of lyophilized material, was extracted with 100 mL of 80% ethanol in an Ultraturrax (T25) for 1 min at maximum speed and filtered through filter paper. The residue was extracted again in the same conditions. The ethanol extracts were pooled together and evaporated to dryness at reduced pressure. All extractions were made in triplicate.

The dried ethanol extract was redissolved in 10 mL of Milli-Q water and loaded into a C-18 cartridge previously activated with 10 mL of 96% EtOH and washed with 10 mL of Milli-Q water. The cartridge was eluted with increasing percentages of ethanol in water: water (20 mL), 20% EtOH (60 mL), 40% EtOH (20 mL), and 96% EtOH (20 mL). The 40% EtOH fraction, which contains saponins, was evaporated, dissolved in 1 mL of 80% EtOH, centrifuged at 12 000 rpm for 3 min, and injected onto the HPLC−MS system.

Saponin Analysis by HPLC−MS. A HPLC Waters Alliance (Manchester, U.K.) system fitted to a mediterranea sea_{18} reverse-phase analytical column (25 cm length × 4.6 mm inner diameter, 5 μm particle size, Teknokroma, Barcelona, Spain) was used. An elution gradient was used with solvent A (water with 1% formic acid) and B (acetonitrile with 1% formic acid): 0−30 min, 20% B; 30−60 min, linear gradient to 30% B; 60−70 min, linear gradient to 100% B; and 70−80 min, linear gradient to 20% B.

The saponins were detected using an online connected quadrupole mass analyzer (ZMD4, Micromass, Waters, Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 V (negative mode) and 50 V (positive mode) with scans from m/z 200 to 1200. The capillary voltage was 3 kV; the dessolvation temperature was 200 °C; the source temperature was 100 °C; and the extractor voltage was 12 V. The flow rate was kept at 1 mL/min with a split ratio of 5:1 for each analysis.

Quantitative Analysis. The external standard method[18] was used for the quantification of asparagus saponins. Two different external standards were used: protodioscin and shatavarin IV. For each standard, 10 dilutions from 0 to 500 μg/mL were prepared and injected into the LC−MS system. For each standard, the selected ion chromatogram corresponding to its molecular ion in negative mode at 100 V was integrated and the peak area was plotted against the concentration and subjected to regression analysis.

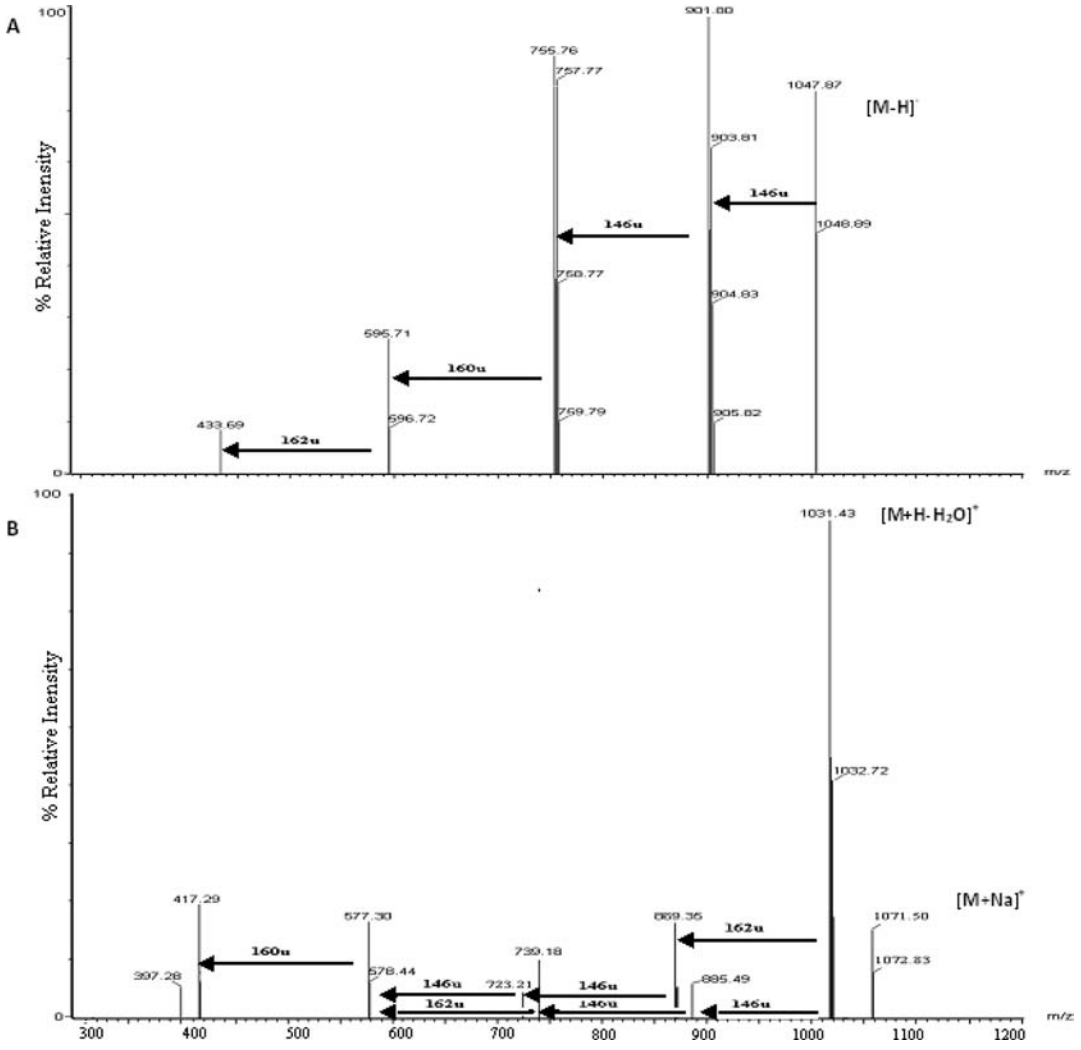

Figure 3. ESI mass spectra of HTSAP-11 in (A) negative (100 V−) and (B) positive (50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

Statistical Analysis. Results were calculated from the mean of three replicates. A cluster analysis was performed by *k*-means clustering (Stata, version 12).

RESULTS AND DISCUSSION

Saponin Profile of "Triguero" HT Asparagus. In a previous study,[18] we found at least eight different new saponins derived from a furostanol-type steroidal genin with a single bond between C_5 and C_6 of the B ring in the HT genotypes.[18] When comparing the results to the previous study, we have found four new peaks identified as novel saponins, which have not been described previously from the edible part of asparagus. These new saponins were identified by their retention time, molecular weight, and fragmentation pathway and are shown in Table 1. The fragmentation pathway has been studied through the mass spectrum obtained in negative (100 V−) and positive (50 V+) modes.

Unlike the previous study, where the eight new saponins found were derived from a furostanol genin with a single bond between C_5 and C_6 of the B ring, two of the new saponins HTSAP-9 and HTSAP-11 had a double bond between C_5 and C_6 of the B ring.

The mass spectra of HTSAP-9 (Figure 1) in negative and positive modes are compatible with a saponin containing one pentose and three hexoses. Figure 1A shows the product ions originated in negative mode from the molecular ion $[M - H]^-$ (m/z 1049) by the loss of either a pentose m/z 917 or a hexose m/z 887. The ion m/z 755 originated from the loss of a hexose from the ion at m/z 917 or a pentose from the ion at m/z 887. The ion at m/z 593 originated from the loss of a hexose, and the ion at m/z 431 (deprotonated genin) originated from another hexose loss. In the case of the positive spectrum mode (Figure 1B), it showed the ions at m/z 1073 (adduct where a molecule forms with sodium), m/z 1033 (loss of one H_2O molecule), and m/z 871, 739, 577, and 415 corresponding to the loss of a hexose, a pentose, a hexose, and a hexose, respectively.

HTSAP-11 has a sugar fragmentation order similar to HTSAP-9 (Figure 2). In the spectrum in negative mode (Figure 2A), ions can be seen at m/z 901, 755, 593, and 431 (deprotonated genin), resulting from consecutive losses of a pentose, a deoxyhexose, a hexose, and a hexose, respectively. In positive mode (Figure 2B), the ion observed at m/z 1057 corresponded to the adduct with sodium and the ion at m/z 1017 corresponded to the loss of one water molecule. From this, two different fragmentation patterns can be depicted: one based on the sequence m/z 885, 739, 577, and 415 corresponding to the loss of a pentose, a deoxyhexose, a

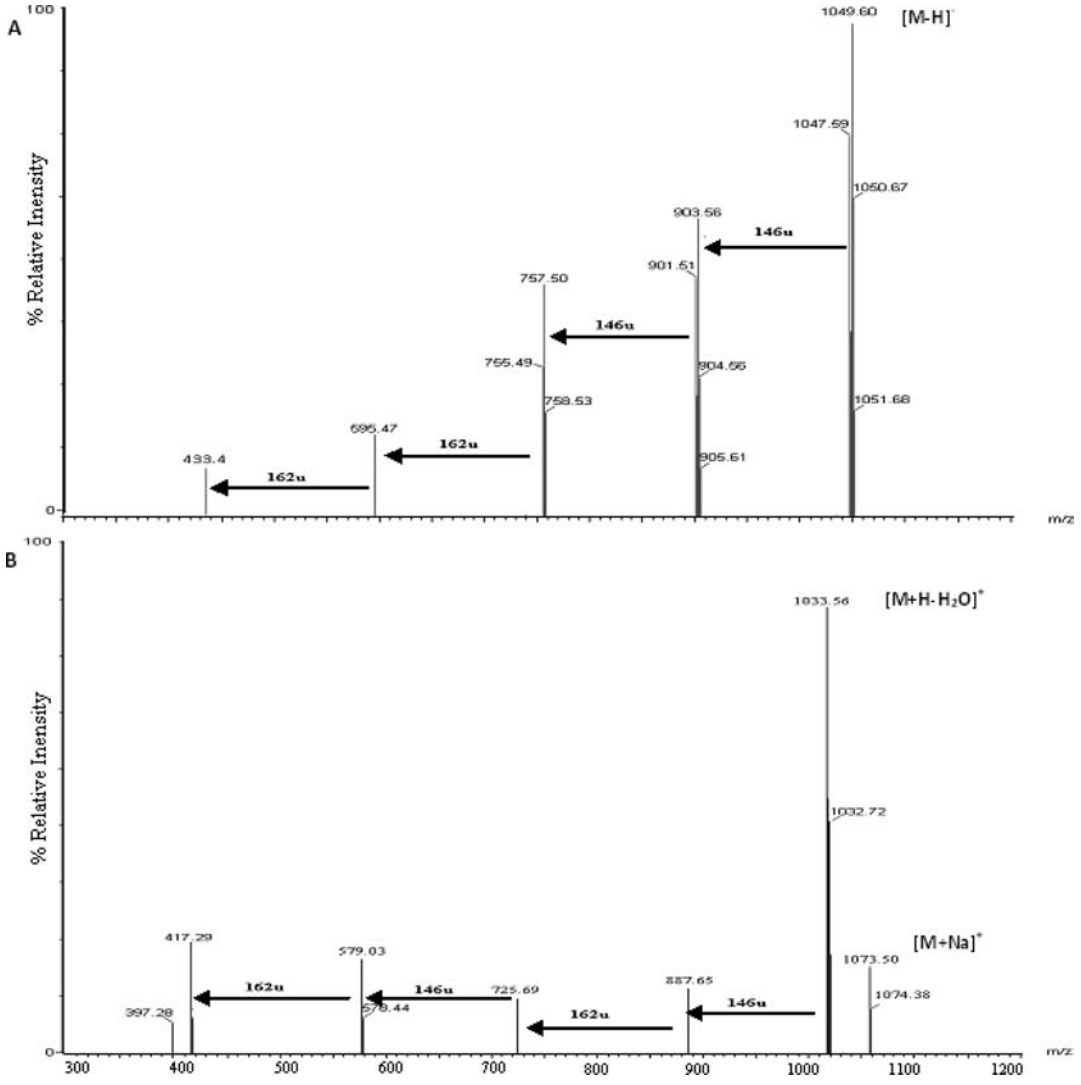

Figure 4. ESI mass spectra of HTSAP-10 in (A) negative (100 V−) and (B) positive (50 V+) modes. Arrows indicate the loss of single monosaccharide moieties.

hexose, and a hexose, respectively, and a second one based on *m*/*z* 855, 723, 577, and 415 corresponding to the loss of a hexose, a pentose, a deoxyhexose, and a hexose, respectively. Therefore, it can be concluded that HTSAP-11 is a diosgenin-like genin glycoside with a pentose, a deoxyhexose, and two hexoses.

HTSAP-10 has the same molecular weight as protodioscin and is also a furostanol saponin, as shown by the prominent $[M + H - H_2O]^+$ ion (*m*/*z* 1030) in the positive mode. However, according to the smallest ion found in the negative mode spectra (*m*/*z* 433), it has a single bond between C_5 and C_6 of the B ring. Possibly HTSAP-10 is formed by four sugars, although only three of them could be identified, because in the spectrum obtained in negative and positive modes, not all of the ions corresponding to fragmentation of sugars appear (Table 1 and Figure 3).

Finally, HTSAP-12 (Figure 4) presents a fragmentation pattern very similar to that previously described in the other eight saponins found in "triguero" HT asparagus.[18] Figure 4A shows the negative mode spectra; in addition to the molecular ion [M − H] *m*/*z* 1049, ions at *m*/*z* 903, 757, 595, and 433 appeared corresponding to the consecutive loss of two deoxyhexoses and two hexoses, respectively. Similarly, in the positive mode spectrum (Figure 4B), ions appeared at *m*/*z* 1073 (adduct molecule that forms with sodium), *m*/*z* 1033 (loss of one H_2O molecule), and ions at *m*/*z* 887, 725, 579, and 417 resulting from the loss of a deoxyhexose, a hexose, a deoxyhexose, and a hexose, respectively. Therefore, it could be deduced that HTSAP-12 is a saponin derived from a furostanol genin with a single bond between C_5 and C_6 in the B ring with two deoxyhexoses and two hexoses attached.

Further studies are necessary to clarified the sequences of the sugar moieties in the different saponins. NMR and methylation analysis could help this purpose.

Quantification of Saponins from "Triguero" HT Asparagus and Commercial Hibrids. The percent compositions of each saponin present in HT genotypes as well as the total contents were analyzed and listed in Table 2. These results show that the saponin profile in "triguero" HT asparagus consists of 12 saponins, 3 of which are present in over 60% of the samples tested: HTSAP-1 (93.10%), HTSAP-8 (81.03%), and HTSAP-11 (63.8%), which could be considered characteristic saponins of this Spanish landrace. However, unlike the previous work,[18] where protodioscin in the HT genotypes was not observed, these results show that over 39% of the genotypes analyzed contains protodioscin.

Table 2. Saponin Content in 58 Genotypes of "Triguero" Asparagus from HT (Percentage)[a]

	saponins (%)													
sample	HTSAP-1	HTSAP-2	HTSAP-3	HTSAP-4	HTSAP-5	protodioscin	HTSAP-6	HTSAP-7	HTSAP-8	HTSAP-9	HTSAP-10	HTSAP-11	HTSAP-12	mg/100 g
HT-1	-	-	-	-	-	-	-	-	-	-	-	-	-	0
HT-2	28.28	-	17.72	-	-	-	41.59	12.42	-	-	-	-	-	1.18
HT-3	36.40	8.20	17.70	-	-	-	12.60	2.20	10.50	-	6.60	5.70	-	2.11
HT-4	34.20	5.60	12.30	-	-	-	4.40	3.70	26.20	-	8.00	1.50	4.10	3.76
HT-5	-	-	72.80	-	-	-	19.30	-	4.80	-	-	-	3.10	2.41
HT-6	20.90	5.90	35.10	-	-	-	18.20	-	19.90	-	-	-	-	9.72
HT-7	17.56	7.36	35.76	-	8.17	-	3.72	-	27.45	-	-	-	-	2.72
HT-8	3.70	-	-	-	-	82.10	-	-	3.90	-	-	10.30	-	7.35
HT-9	14.30	5.20	-	-	-	-	12.40	4.20	37.60	-	13.20	5.70	7.30	15.24
HT-10	1.79	-	66.02	-	18.30	-	2.83	11.05	-	-	-	-	-	2.47
HT-11	5.50	-	4.50	-	-	74.30	-	-	5.70	-	-	10.10	-	6.15
HT-12	14.30	-	-	-	-	-	10.50	-	6.20	-	32.10	10.70	26.10	2.93
HT-13	30.40	-	-	31.10	-	-	-	-	8.70	-	29.80	-	-	1.57
HT-14	29.40	-	-	12.80	-	-	10.50	1.30	10.20	-	15.70	11.00	9.10	7.97
HT-15	16.38	-	-	-	-	63.23	-	-	7.17	-	-	13.22	-	1.64
HT-16	11.30	-	0.30	-	-	53.20	-	-	9.00	-	-	26.10	-	3.49
HT-17	8.60	-	-	-	-	29.70	20.60	-	-	-	-	9.40	31.80	4.45
HT-18	20.60	-	-	-	-	-	17.00	-	12.30	-	30.80	19.30	-	16.22
HT-19	4.70	-	-	0.60	-	82.70	-	-	1.50	-	-	10.50	-	0.91
HT-20	28.30	-	3.30	16.00	-	19.80	-	-	11.20	-	-	11.40	11.00	4.56
HT-21	39.10	-	-	3.92	-	14.35	10.25	-	28.66	-	-	3.72	-	2.09
HT-22	11.80	-	-	10.30	-	-	7.80	-	-	-	44.20	-	25.90	1.16
HT-23	not analyzed													
HT-24	7.20	-	0.30	1.70	-	52.90	-	-	3.70	-	-	23.50	10.80	10.02
HT-25	34.70	-	10.15	-	-	-	32.00	-	9.96	-	-	-	-	1.87
HT-26	3.10	-	-	6.30	-	52.80	-	-	5.00	-	-	5.80	27.00	4.04
HT-27	100	-	-	-	-	-	-	-	-	-	-	-	-	0.02
HT-28	20.33	-	66.40	-	-	-	2.98	6.33	3.97	-	-	-	-	1.83
HT-29	24.03	-	-	-	-	46.90	13.09	-	-	-	-	15.98	-	1.29
HT-30	7.10	-	21.00	-	-	-	-	-	6.70	-	35.90	5.80	23.50	1.80
HT-31	30.30	-	-	-	-	33.40	-	-	11.90	-	-	24.40	-	5.65
HT-32	not analyzed													
HT-33	22.20	-	-	-	-	-	-	-	10.90	-	48.00	18.90	-	4.31
HT-34	15.00	-	-	-	-	-	-	-	3.90	-	63.00	18.10	-	1.18
HT-35	-	-	-	-	-	74.00	-	-	-	-	-	10.50	15.40	1.45
HT-36	15.70	-	-	3.50	-	-	14.00	-	4.20	-	33.40	12.80	13.60	8.88
HT-37	13.60	-	-	-	-	30.10	6.90	-	17.70	-	-	5.90	25.90	5.31
HT-38	33.50	11.30	5.60	-	-	-	33.10	-	16.50	-	-	-	-	10.43
HT-39	13.60	-	-	-	-	30.50	5.90	-	21.40	-	-	-	28.60	4.62
HT-40	7.40	0.50	22.40	25.70	-	-	-	-	1.00	-	23.00	5.60	12.90	4.34
HT-41	20.30	-	-	-	-	30.40	-	2.60	17.10	5.10	-	13.80	10.60	10.1
HT-42	not analyzed													

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Table 2. continued

	saponins (%)													
sample	HTSAP-1	HTSAP-2	HTSAP-3	HTSAP-4	HTSAP-5	protodioscin	HTSAP-6	HTSAP-7	HTSAP-8	HTSAP-9	HTSAP-10	HTSAP-11	HTSAP-12	mg/100 g
HT-43	7.50	-	-	-	-	-	-	-	16.50	-	44.90	-	31.10	0.94
HT-44	26.10	-	15.70	-	-	-	6.70	-	-	-	-	-	51.50	0.24
HT-45	14.50	4.50	16.90	-	-	-	27.20	-	26.30	-	-	-	10.60	12.40
HT-46	8.10	0.80	-	-	-	-	-	-	14.50	-	41.20	-	35.50	2.42
HT-47	15.60	6.60	2.40	-	-	-	26.30	12.20	29.60	-	-	-	7.40	15.38
HT-48	not analyzed													
HT-49	5.40	1.20	-	2.80	-	60.00	-	-	2.10	-	-	28.40	-	4.12
HT-50	5.20	-	1.20	1.70	-	63.30	-	-	1.40	-	-	27.10	-	4.55
HT-51	32.20	4.30	0.80	1.90	-	29.60	-	-	14.20	-	-	17.00	-	11.32
HT-52	14.20	-	-	3.00	-	61.60	-	-	9.60	-	-	11.70	-	1.90
HT-53	-	-	-	-	-	98.90	-	-	1.10	-	-	-	-	0.87
HT-54	49.90	11.70	23.30	-	2.00	-	8.80	0.80	3.30	-	-	-	0.30	7.97
HT-55	-	-	-	-	-	56.40	-	-	-	-	-	43.60	-	3.78
HT-56	51.00	7.20	7.70	-	-	-	11.80	6.40	13.60	-	-	2.30	-	6.56
HT-57	55.95	28.57	3.59	-	-	-	5.61	-	6.29	-	-	-	-	2.49
HT-58	23.50	-	-	-	-	52.40	-	-	15.90	-	-	8.30	-	1.33
HT-59	16.80	3.40	29.70	-	-	-	-	5.10	6.30	-	10.20	28.40	-	11.73
HT-60	33.10	22.80	7.70	-	-	-	18.00	-	6.90	-	7.50	4.00	-	4.74
HT-61	7.80	-	-	25.60	-	-	3.20	-	17.20	-	19.90	3.70	22.60	1.79
HT-62	17.10	1.20	-	-	-	-	12.70	-	-	-	58.50	10.50	-	0.69
HT-63	not analyzed													
HT-64	not analyzed													
HT-65	not analyzed													

[a]Data represent the relative percent. Data (mg/100 g) are the mean of three replicates. Standard derivation was <5%. - = not detected.The HT nomenclature used is the same as these genotypes received in a previous study.[11]

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Table 3. Saponin Content in 31 Commercial Hybrids of Green Asparagus (Percentage)[a]

		saponins (%)													
sample	commercial name	HTSAP-1	HTSAP-2	HTSAP-3	HTSAP-4	HTSAP-5	protodioscin	HTSAP-6	HTSAP-7	HTSAP-8	HTSAP-9	HTSAP-10	HTSAP-11	HTSAP-12	mg/100 g
CH-1	Apollo	-	-	-	-	-	100	-	-	-	-	-	-	-	0.22
CH-2	Juic-1	-	-	-	-	-	100	-	-	-	-	-	-	-	0.03
CH-3	Plaverd	-	-	-	-	-	100	-	-	-	-	-	-	-	0.03
CH-4	Solar	-	-	-	-	-	100	-	-	-	-	-	-	-	1.22
CH-5	Supreme	4.00	-	-	-	-	96.00	-	-	-	-	-	-	-	5.43
CH-6	Ercole	-	-	-	-	-	100	-	-	-	-	-	-	-	0.26
CH-7	Ravel	-	-	-	-	-	100	-	-	-	-	-	-	-	0.80
CH-8	NJ-956	-	-	-	-	-	99.00	-	-	1	-	-	-	-	3.19
CH-9	G-Millenium	-	-	-	-	-	100	-	-	-	-	-	-	-	0.04
CH-10	A-1978	-	-	-	-	-	100	-	-	-	-	-	-	-	0.26
CH-11	Atlas	-	-	-	-	-	100	-	-	-	-	-	-	-	1.25
CH-12	Deluxe	-	-	17.40	-	47.80	12.50	-	-	-	-	-	-	22.40	0.76
CH-13	Dulce V	8.70	-	78.60	-	6.10	-	-	6.60	-	-	-	-	-	4.38
CH-14	Fileas	-	-	-	-	-	100	-	-	-	-	-	-	-	0.14
CH-15	Grande	-	-	42.80	-	-	33.20	-	-	-	-	24.00	-	-	0.11
CH-16	Italo	11.60	-	66.10	-	14.20	-	-	-	-	-	-	-	-	7.90
CH-17	Jking	-	-	-	-	-	100	-	-	-	-	-	-	-	1.94
CH-18	Jnight	1.50	20.70	-	8.50	-	47.70	-	-	21.60	-	-	-	-	0.54
CH-19	NJ-953	-	-	-	-	-	100	-	-	-	-	-	-	-	0.17
CH-20	NJ-977	-	-	-	-	-	100	-	-	-	-	-	-	-	0.21
CH-21	PuPass	5.70	-	-	-	-	87.50	-	-	6.80	-	-	-	-	1.25
CH-22	Rally	5.60	-	88.50	-	-	5.90	-	-	-	-	-	-	-	0.65
CH-23	Rambo	6.20	-	76.80	-	9.90	-	-	7.10	-	-	-	-	-	4.60
CH-24	Rapsody	-	-	-	-	-	100	-	-	-	-	-	-	-	0.10
CH-25	UC-115	-	-	-	-	-	100	-	-	-	-	-	-	-	0.28
CH-26	Ramada	-	-	-	-	-	-	-	-	-	-	-	-	-	0
CH-27	G. Welph	-	-	-	-	-	-	-	-	-	-	-	-	-	0
CH-28	J. Giant	-	-	-	-	-	-	-	-	-	-	-	-	-	0
CH-29	NJ1016	-	-	-	-	-	-	-	-	-	-	-	-	-	0
CH-30	P. purple	-	-	-	-	-	-	-	-	-	-	-	-	-	0
CH-31	UC-157	-	-	-	-	-	-	-	-	-	-	-	-	-	0

[a]Data represent the relative percent. Data (mg/100 g) are the mean of three replicates. Standard derivation was <5%. - = not detected.

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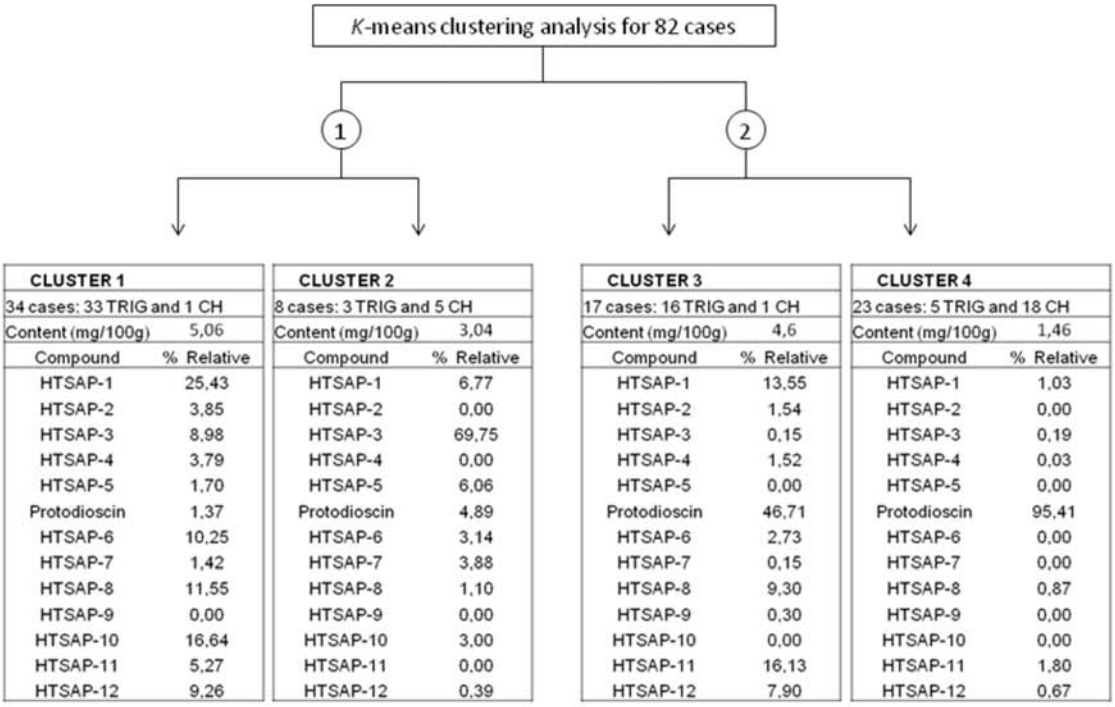

CLUSTER 1	
34 cases: 33 TRIG and 1 CH	
Content (mg/100g)	5,06
Compound	% Relative
HTSAP-1	25,43
HTSAP-2	3,85
HTSAP-3	8,98
HTSAP-4	3,79
HTSAP-5	1,70
Protodioscin	1,37
HTSAP-6	10,25
HTSAP-7	1,42
HTSAP-8	11,55
HTSAP-9	0,00
HTSAP-10	16,64
HTSAP-11	5,27
HTSAP-12	9,26

CLUSTER 2	
8 cases: 3 TRIG and 5 CH	
Content (mg/100g)	3,04
Compound	% Relative
HTSAP-1	6,77
HTSAP-2	0,00
HTSAP-3	69,75
HTSAP-4	0,00
HTSAP-5	6,06
Protodioscin	4,89
HTSAP-6	3,14
HTSAP-7	3,88
HTSAP-8	1,10
HTSAP-9	0,00
HTSAP-10	3,00
HTSAP-11	0,00
HTSAP-12	0,39

CLUSTER 3	
17 cases: 16 TRIG and 1 CH	
Content (mg/100g)	4,6
Compound	% Relative
HTSAP-1	13,55
HTSAP-2	1,54
HTSAP-3	0,15
HTSAP-4	1,52
HTSAP-5	0,00
Protodioscin	46,71
HTSAP-6	2,73
HTSAP-7	0,15
HTSAP-8	9,30
HTSAP-9	0,30
HTSAP-10	0,00
HTSAP-11	16,13
HTSAP-12	7,90

CLUSTER 4	
23 cases: 5 TRIG and 18 CH	
Content (mg/100g)	1,46
Compound	% Relative
HTSAP-1	1,03
HTSAP-2	0,00
HTSAP-3	0,19
HTSAP-4	0,03
HTSAP-5	0,00
Protodioscin	95,41
HTSAP-6	0,00
HTSAP-7	0,00
HTSAP-8	0,87
HTSAP-9	0,00
HTSAP-10	0,00
HTSAP-11	1,80
HTSAP-12	0,67

CLUSTER 1:
33 *triguero* genotypes (HT-2, HT-3, HT-4, HT-6, HT-7, HT-9, HT12, HT-13, HT-14, HT-18, HT-20, HT-21, HT-22, HT-25, HT-27, HT-30, HT-33, HT-34, HT-36, HT-38, HT-40, HT-43, HT-44, HT-45, HT-46, HT-47, HT-54, HT-56, HT-57, HT-59, HT-60, HT-61 and HT-62)
1 commercial hybrid (CH-12)
CLUSTER 2:
3 *triguero* genotypes (HT-5, HT-10 and HT-28)
5 commercial hybrids (CH-13, CH-15, CH-16, CH-22 and CH-23)
CLUSTER 3:
16 *triguero* genotypes (HT-15, HT-16, HT-17, HT-24, HT-26, HT-29, HT-31, HT-37, HT39, HT-41, HT-49, HT-50, HT-51, HT-52, HT-55 and HT-58)
1 commercial hybrid (CM-18)
CLUSTER 4:
5 *triguero* genotypes (HT-8, HT-11, HT-19, HT-35 and HT-53)
18 commercial hybrids (CH-1, CH-2, CH-3, CH-4, CH-5, CH-6, CH-7, CH-8, CH-9, CH-10, CH-11, CH-14, CH-17, CH-19, CH-20, CH-21, CH-24 and CH-25).

Figure 5. Classification of genotypes of green asparagus in four clusters obtained by the application of a *k*-means clustering analysis.

With respect to the total saponin content of the 58 tested "triguero" HT genotypes, 31.1% presents a concentration higher than 5 mg/100 g (with 50% of these higher than 10 mg/100 g), which is similar to those described for white asparagus.[17,19] Most of the samples (59.6%) have a concentration of 1−5 mg/100 g, and only 12% has lower than 1 mg/100 g.

Similarly, the 31 commercial hybrid asparagus were analyzed, and the results are presented in Table 3, showing the percent compositions of each saponin and the total saponin contents. The results showed protodioscin as the main saponin present in commercial hybrids, present in 70.97% of samples and in most of them at 100%. Note that 6 commercial hybrids of the 31 analyzed did not contain any saponins. Similarly, the presence in some commercial hybrids of saponins different from protodioscin (HTSAP-1, HTSAP-2, HTSAP-3, HTSAP-4, HTSAP-5, HTSAP-7, HTSAP-8, HTSAP-10, and HTSAP-12) is also remarkable. Previously, the presence of these saponins in asparagus has been suggested but always as minor components.[19,20] To our knowledge, this is the first time that some of these saponins HTSAP-3 and HTSAP-5 have been found as major components of some commercial hybrids, such as CH-13, CH-15, CH-16, CH-22, CH-23, and CH-12.

With regards to the total saponin content studied in these commercial hybrids, most samples (70.97%) have concentrations below 1 mg/100 g, 22.58% contains between 1 and 5 mg/100 g, and only 6.45% has a higher concentration than 5 mg/100 g. When these results were compared to the samples of "triguero" asparagus, it was observed that the saponin concentrations of "triguero" asparagus are higher (in most cases, 70%) than commercial hybrids, with 16% of them reaching saponin concentrations 100 times greater than those found in commercial hybrids.

Previously, we classified the different genotypes according to their flavonoid content[11] into three groups. Two of these groups contained both commercial hybrids and "triguero" HT asparagus, and the other group consisted of only "triguero" HT asparagus (21 genotypes). In this study, we have performed a similar analysis and classified the different samples based on the total content of saponins and the relative percent of each of the 12 individual saponins and protodioscin identified. Using the *k*-means clustering analysis technique, we have classified the saponin-containing samples (82 genotypes) into four different clusters. The distribution of the 82 genotypes containing saponins of green asparagus and the average composition of each group are shown in Figure 5. Taking into account only the

percentage of protodioscin, the clusters could be classified into two groups. The first group joins clusters 1 and 2 and includes 36 "triguero" genotypes and only 6 commercial hybrids. The average protodioscin level of this group is below 5%. On the other hand, the second group comprises clusters 3 and 4. This group includes 21 "triguero" genotypes and 19 commercial hybrids and has a protodioscin level above 45%.

Cluster 1 mainly consists of "triguero" genotypes (33) and only 1 commercial hybrid. Most of the "triguero" genotypes have no protodioscin, only 2 of them (HT20 and HT21) and the commercial hybrid contain it. It is interesting to note that this cluster contains the 21 genotypes previously classified on the basis of their low rutin content.[11] These results suggest that, within the HT asparagus germplasm, there is a group of genotypes with a very distinct chemical profile, characterized by lacking protodioscin and having a low rutin percentage.

Cluster 2 is integrated by three "triguero" genotypes and five commercial hybrids. This cluster is very similar to cluster 1 with very low levels of protodioscin (about 5%), but the most important difference is that all samples show HTSAP-3 as the major saponin. These five commercial hybrids (CH-13, CH-15, CH-16, CH-22, and CH-23) together with CH-12 (cluster 1) were mentioned above to present a very different saponin profile within the commercial hybrid groups (Table 3). It is interesting to note that, despite having little protodioscin, they contain high percentages of rutin.[11]

Cluster 3 consists of 16 "triguero" genotypes and 1 commercial hybrid, with values of protodioscin at about 50%. In contrast, cluster 4 is formed mainly by commercial hybrids (18) and only 5 "triguero" genotypes; most of these samples have values of protodioscin close to 100%. This cluster 4 represents a saponin profile, which is characteristic of commercial hybrids of green asparagus.[20]

The characteristics typical of wild asparagus, thinner and bitter spears, shorter cladodes, slightly and striated stems,[6] are more evident for genotypes included in cluster 1 and appear less evident when we go from cluster 2 to cluster 4. The classification of the different HT germplasm genotypes might be related to the fact, pointed out for some authors, that the HT landrace could be a hybrid between cultivated diploid varieties of *A. officinalis* and wild *A. maritimus*.[7]

As previous results suggested,[18] this study shows very clearly that, among the different genotypes of the "triguero" asparagus germplasm, there are some genotypes with a saponin profile very different from that of the commercial hybrids. Partially, the organoleptic characteristics of asparagus depend upon the saponin profile.[15−17] On the other hand, small structural variations in the saponins lead to substantial variations in physical, chemical, and biological properties.[21] For instance, some authors[22−24] concluded that the structure of the sugar in steroid saponins plays an important role in the specific cytotoxicity against tumor cells. In addition, studies have shown that the aglycone type also influences the cytotoxic effect.[25−29] Therefore, the saponin profile may have a relevant impact on the functional properties of asparagus. Furthermore, we have previously shown that the flavonoid profile as well as the antioxidant properties are also genotype-dependent;[7,8] therefore, it is reasonable to think that, on the basis of the "triguero" asparagus germplasm, it is possible to develop new asparagus varieties with distinct organoleptic and functional properties. In this respect, further research is necessary to establish the functional and organoleptic characteristics of individual saponins.

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Notes

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ABBREVIATIONS USED

HT, Huétor-Tájar; HPLC, high-perfomance liquid chromatography; LC−MS, liquid chromatography−mass spectrometry; DAD, diode array detector

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