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Profiling of Organosulfur Compounds in Onions: A Comparative Study between LC-HRMS and DTD-GC-MS

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Abstract: Onions are known not only for their culinary importance but also for their nutritional and health-promoting properties. Both properties are closely linked to their content of organosulfur compounds, which account for up to 5% of the dry weight of an onion. Given the importance of these compounds, suitable analytical methods are required for their study. Two techniques should be highlighted in this context: gas chromatography coupled to mass spectrometry (GC-MS), and liquid chromatography coupled to mass spectrometry (LC-MS). In this study, eight different onion varieties were analyzed using two distinct analytical techniques: direct thermal desorption–gas chromatography–mass spectrometry (DTD-GC-MS) and high-resolution mass spectrometry (HRMS) on an LC-ESI-QqTOF instrument. Each method identified different organosulfur compounds, with LC-HRMS targeting 15 non-volatile compounds, such as cysteine sulfoxides, and GC-MS targeting 18 volatiles, such as disulfides and trisulfides. The results obtained were studied using Pearson correlations and principal component analysis. No precise correlation was found between the initial organosulfur compounds in onions and their hydrolysates. Consequently, although GC is one of the most employed techniques in the scientific literature, the use of LC-HRMS or a combination of both techniques may offer a more comprehensive and accurate description of the metabolomic profile of onions.

Keywords: *Allium cepa* L.; cysteine sulfoxides; gas chromatography; high-resolution mass spectrometry; onion; organosulfur compounds

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1. Introduction

The genus *Allium* includes some of the most widely consumed and cultivated vegetables such as garlic, onion, and leek. In addition to their culinary importance [1], these vegetables have medicinal properties that contribute to consumers' health [2]. In particular, the onion, recognized by the FAO as one of the 15 most produced foodstuffs (tons) worldwide [3], has been the subject of numerous studies for its preventive effects on various diseases. Research suggests that onion consumption can help prevent inflammatory diseases [4], cancer [5], diabetes [6], and neurological disorders [7]. These properties, both sensory (aromas and flavors) and medicinal, are closely linked to its content of sulfur compounds, which account for up to 5% of the dry weight of an onion [8].

Although the aromatic organosulfur compounds responsible for the onion sensory aspects constitute a diverse amalgam of compounds with varying structures, numerous studies have revealed that they are mainly derived from three non-volatile and odorless precursors. These three compounds, belonging to the family S-alk(en)yl cysteine sulfoxides (CSOs), are the following: S-methyl cysteine sulfoxide (methiin), S-(1-propenyl) cysteine sulfoxide (isoalliin), and S-propyl cysteine sulfoxide (propiin) [9]. Figure 1 illustrates the main pathway proposed for the synthesis of these CSOs. This pathway shows the

involvement of glutathione, which is alkylated and then undergoes glycine loss, oxidation, and, finally, loss of the γ-glutamyl group, converting it to alkyl cysteine sulfoxide. It should be noted that the last two steps, oxidation and loss of the γ-glutamyl group, can occur in reverse order. The formation of the three main CSOs follows the same metabolic pathway but starts with different alkylations: for isoalliin, glutathione acquires a 1-propenyl group, for methiin, glutathione acquires a methyl group, and for propiin, it acquires a propyl group [10].

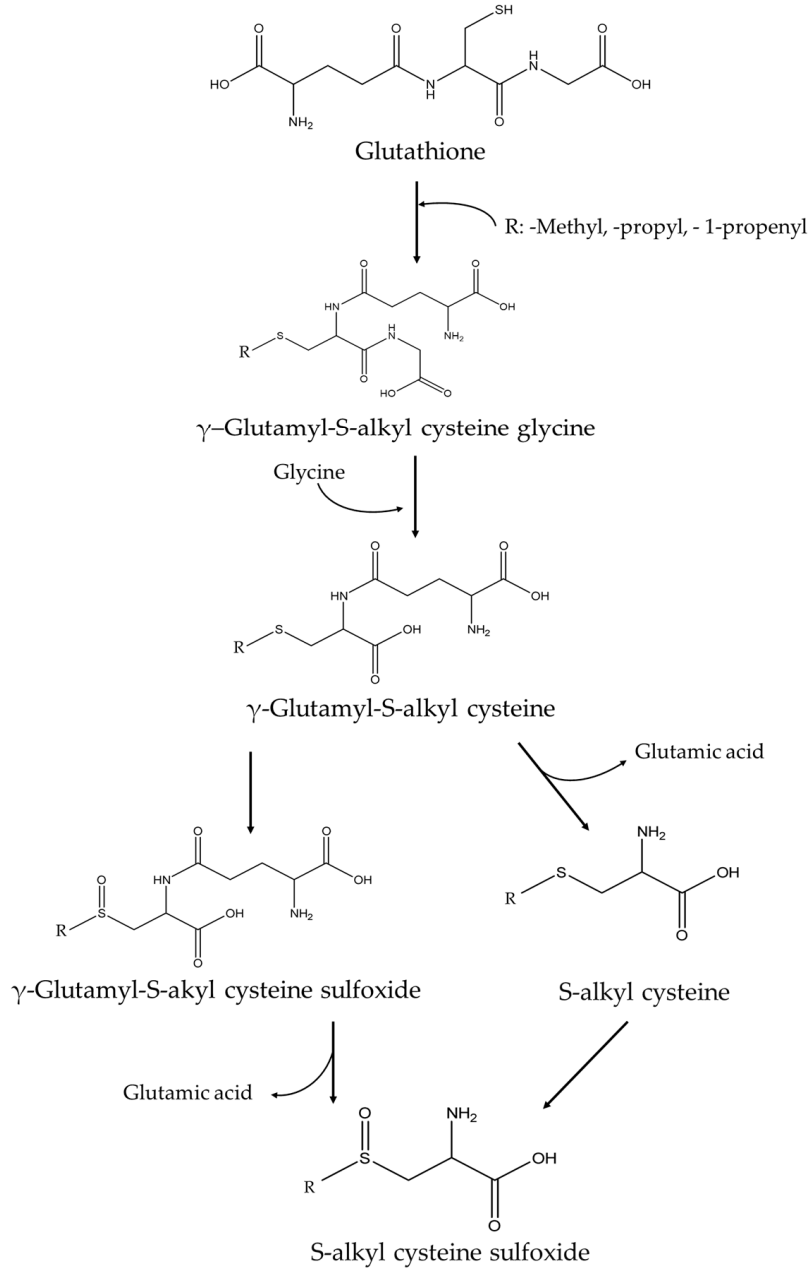

Figure 1. Metabolic pathways for the synthesis of main CSOs in onions.

Then, these CSOs form the aromatic and volatile compounds characteristic of onions. This process begins with the enzyme alliinase [11]. In an intact onion bulb, alliinase and the CSOs, are compartmentalized and kept separate. This separation prevents the premature reactions that would lead to the formation of volatile compounds. When the onion is damaged by cutting, crushing, or chewing, the cellular compartments are broken, allowing alliinase to come into contact with CSOs. The enzyme alliinase cleaves the three CSOs

giving pyruvate, ammonia, and sulfenic acids, which, being highly reactive, finally generate the variety of aromatic compounds that characterize onion aroma [12]. Figure 2 illustrates this process of aroma compound formation.

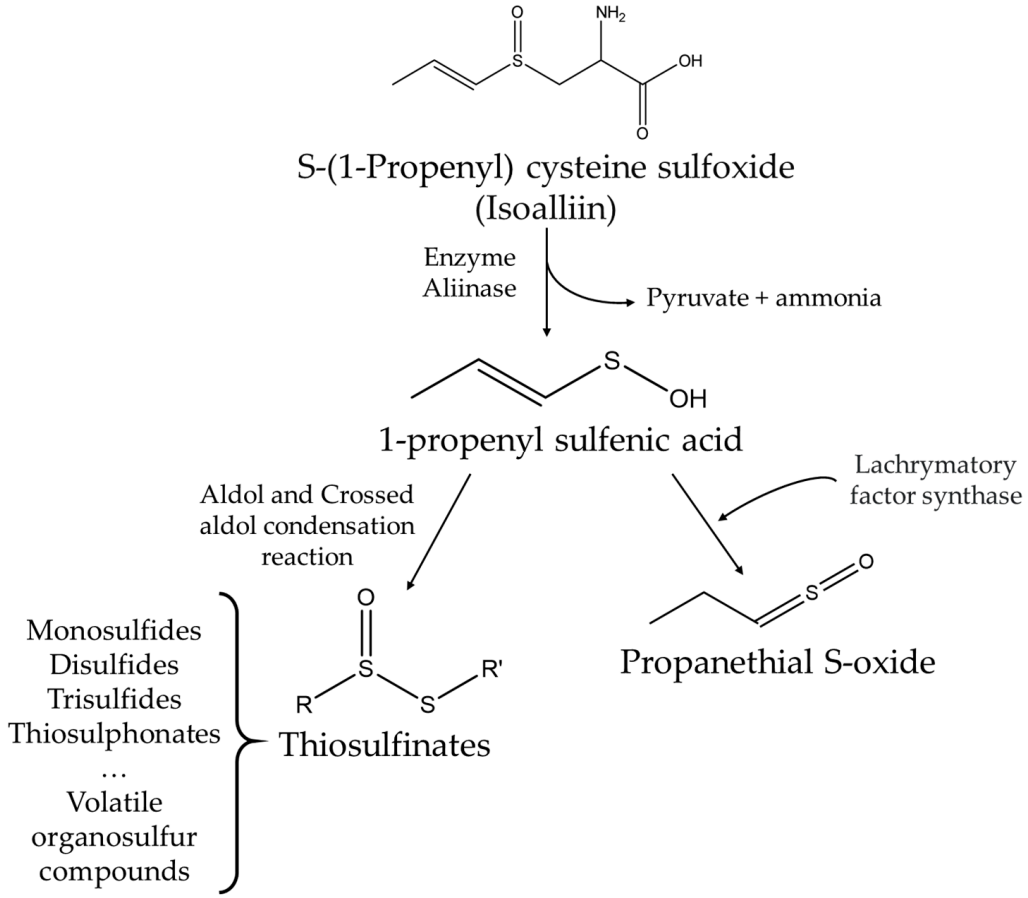

Figure 2. Metabolic pathways for the synthesis of aromatic compounds using isoalliin as a precursor example.

Therefore, the metabolomic profile of onions is extensive, and having a comprehensive understanding of these pathways is crucial. This knowledge is particularly valuable due to the significant role of CSOs in the nutraceutical and sensory qualities of onions. Additionally, the composition of CSOs is influenced by various factors, including environmental, genetic, and cultivation variables [13]. Research on these compounds provides valuable insights into these complex interactions. Consequently, developing analytical methods that enable the detection and quantification of CSOs is an essential tool.

Numerous techniques for the determination of organosulfur compounds have been described in the literature [14–17], and these are classified into direct and indirect methods. Direct methods evaluate non-volatile organosulfur compounds before enzymatic decomposition (the metabolic pathway of Figure 1). In contrast, indirect methods are based on the estimation of various products generated after the enzymatic conversion of the precursors (such as thiosulfinates, pyruvic acid, disulfides, etc.), as shown in the metabolic pathway of Figure 2. Although indirect methods have been widely used to measure onion pungency, establishing an accurate stoichiometric ratio between hydrolysates and significant CSOs can be challenging, often resulting in an underestimation of the CSO content [18].

Specifically, two techniques can be highlighted to study organosulfur compounds in onions: gas chromatography coupled to mass spectrometry (GC-MS) [19–21], and liquid chromatography coupled to mass spectrometry (LC-MS) [22–25]. In the context of the GC-MS methods, most studies focus on identifying aromatic and volatile organosulfur

compounds. When it comes to studying CSOs, their detection through GC is limited to laborious and time-consuming derivatization processes [14,26,27]. In addition, considering the thermolability of CSOs, the application of this type of analysis, where high temperatures are often applied to volatilize the compounds, may lead to erroneous estimates of CSOs [28]. In the case of LC-MS, the milder analytical conditions allow the analysis of CSOs and their precursors, providing a probably more realistic analysis of the organosulfur compound content in onion bulbs.

Despite the differences between the two techniques, the literature includes articles that use both for studying organosulfur compounds in onions, often yielding very different results [29]. The comparison of direct and indirect techniques is interesting, as it allows for exploring possible correlations between the analyzed compounds. These investigations can also reveal whether the volatile profile of onions reflects the initial content of sulfur compounds in the bulbs. This is why a comparison between the two techniques is proposed in this work, by studying different varieties of onions. Firstly, for the indirect study of organosulfur compounds in onion, the research group has previously developed a direct thermal desorption–gas chromatography–mass spectrometry (DTD-GC-MS) method to analyze the organosulfur compounds in onion [21]. Regarding the direct study of CSOs and their non-volatile precursors, this work proposes the use of liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) as an alternative technique. Therefore, this work can also be an ideal complement to the previous research, allowing us to carry out, in a non-bibliographic but experimental way, a comparison between DTD-GC-MS and LC-HRMS with chemometric tools to unveil the metabolic profile of different onion samples.

2. Materials and Methods

2.1. Onion Samples

Seven different onion varieties and one shallot, all obtained from local supermarkets in the province of Cadiz (Spain), were used for this study. The characteristics of the samples were as follows: red onion variety (origin Austria, company Tara, 500 g net size, size 50/90 mm), purple onion variety (origin Spain, company la Gramola, 500 g net size, size 50/70 mm), shallot sample (origin France, company Ajos Malsamar S.L., size 250 g net size, size 50/70 mm), Red Label variety (origin Spain, company Linda, 500 g mesh size, 50/70 mm size), yellow onion variety (origin Spain, bulk format, 55/75 mm size), sweet onion variety (origin Spain, company Calidad y origen, bulk format, 70/100 mm size), white onion variety (origin Peru, company Ajos Malsamar S.L., assortment format, size 60/80 mm), and spring onion variety (origin Spain, company La Gramola, 2 kg net format, size 50/90 mm). The whole bulb was used for all the experiments carried out, discarding the outer layers. The bulb was cut into small pieces with a knife, freeze-dried, and ground to obtain a more homogeneous matrix.

2.2. Chemicals and Solvents

Methanol (Fischer Chemical, Loughborough, UK) of HPLC-grade purity and Milli-Q water from a Milli-Q water purification system (manufactured by Millipore, Bedford, MA, USA) was used for UAE extraction. A 1 M HCl solution and a 1 M NaOH solution (Panreac, Barcelona, Spain) were used to adjust the pH levels.

Acetonitrile, methanol, water, and formic acid were used to analyze the organosulfur compounds. All solvents used were of LC-MS purity and were obtained from Honeywell (Guyancourt, France).

For the calibration of the mass spectrum system, a solution of sodium formate (HCOONa) and a solution of leucine enkephalin with a concentration of 100 pg μL^{-1} were utilized. For the preparation of the HCOONa solution, 100 µL of a 0.1 M NaOH solution was mixed with 200 µL of a 10% formic acid solution (Amresco Inc., Fontenay-sous-Bois, France). Finally, 20 mL of 80:20 acetonitrile/water was added. The leucine enkephalin solution was prepared from a 1 mg mL^{-1} concentration stock solution by

1:10 dilution in 0.1:50:50 formic acid/methanol/water. This stock solution was prepared from the commercial reagent leucine enkephalin acetate salt hydrate (Sigma-Aldrich, Saint Louis, MO, USA) ≥ 95% HPLC grade.

Regarding the GC-MS, a standard of C7-C40 saturated alkanes obtained from Supelco (Sigma-Aldrich, Saint Louis, MO, USA) was used for the calculation of the retention indices (RI). The concentration of each compound was 1000 µg/mL in hexane.

2.3. Study of Organosulfur Compounds Using GC-MS

In this case, to analyze the organosulfur compounds present in the onion samples, a TD-20 system (Shimadzu, Kyoto, Japan), coupled with a GCMS-TQ8040 (Shimadzu, Kyoto, Japan) system, was used. Chromatographic separations were conducted using a Suprawax-280 capillary column (Teknokroma, Barcelona, Spain; 60 m length 0.25 mm column I.D. 0.25 m film thickness). The extraction of the organosulfur compounds present in the onion bulb was carried out in a desorption sample tube. Specifically, 10 mg of onion samples were placed in the desorption tube. The thermal desorption process was carried out by heating the cartridge to 205 °C for 906 s with 1 mL min^{-1} He as coolant. The stripped volatiles were trapped in a Tenax GC cold trap (−15 °C), which was subsequently heated at 267 °C for 180 s, allowing a rapid transfer to the GC capillary column. The GC analysis was carried out following the characteristics of the method previously published [21]. For the MS, electron impact ionization was utilized at 70 eV. The device operated in full-scan mode covering a range of 40–400 m/z, with the ion source maintained at a temperature of 200 °C.

The complexity of volatile organic compounds profiles often restricts research to studies on easily identifiable compounds. With the availability of mass spectra libraries and the recent growth of retention index (RI) libraries, volatile organic compounds can be identified using only GC-MS [30]. Specifically, in this work, the compounds were identified by comparing their mass spectra with the Wiley library (Wiley Registry of Mass Spectral Data, 7th edition, 2000), using a similarity criterion of at least 80%. In addition, the retention indices were determined by reference to a homologous series of n-alkanes (C7-C40 Saturated Alkanes Standard, Supelco, Sigma-Aldrich, USA).

2.4. Study of Organosulfur Compounds Using LC-HRMS

2.4.1. Extraction of Organosulfur Compounds

For the extraction of organosulfur compounds before the LC-HRMS analysis, the ultrasound-assisted extraction (UAE) technique (Sonopuls HD 2070.2 processor, BANDELIN electronic GmbH & Co. KG, Heinrichstrabe, Berlin, Germany), previously developed by the research group [31], was employed. The parameters used for the extraction were as follows: 76.8% methanol in water as the extraction solvent at pH 2; 58.5 °C as the extraction temperature; 85% as the UAE amplitude; 0.9 s as the cycle; and 0.2:13 g:mL sample mass/solvent volume as ratio. The extraction time was 2 min. Before mass spectrometric analysis, all samples were filtered using a 0.20 µm nylon syringe filter (Membrane Solutions, Dallas, TX, USA) and diluted 1:10 using the same solvent previously used for extraction.

2.4.2. Analysis of Organosulfur Compounds by LC-HRMS

To analyze the organosulfur compounds present in the onion samples, a Shimadzu LC10-AD (Shimadzu, Noisiel, France) coupled with a SYNAPT XS High-Resolution Mass Spectrometer (Waters Corp., Milford, MA, USA) system was used. Chromatographic separations were conducted using a Sunfire C18 column (2.1 mm × 100 mm. i.d. 3.5 µm) (Waters Corp., Milford, MA, USA) in combination with a C18 guard column cartridge (3.5 µm, Waters Corp., Milford, MA, USA). The analyses were performed in isocratic using a single solvent mixture with the following composition: acetonitrile, methanol, water, and formic acid in a ratio of 12.5:22.5:65:0.1 ($v/v/v/v$). A constant flow rate of 0.1 mL min^{-1} was maintained during all analyses. The injection volume was 20 µL.

All mass measurements were performed in positive ion mode on an electrospray ionization quadrupole-time-of-flight mass spectrometer (ESI-QqToF) instrument. The

temperature of the source was set at 130 °C; nitrogen was used as the desolvation gas at a flow rate of 500 L h^{-1} and a temperature of 250 °C. The electrospray voltages were set at 3.4 kV for the capillary and 0.0 V for the sample cone. During the scan mode, the first and the second analyzers were used with low-mass (LM) and high-mass (HM) resolutions set to 4.9 and 15, respectively. All experiments were acquired during a scan time of 1 s in the range m/z 50–950.

During the CID MS/MS experiments, the first and the second analyzers were used with LM and HM resolutions set to 20 and 15, respectively. Argon was used as a collision gas with a pressure of 1.2×10^{-3} mbar inside the collision cell during the MS/MS experiments (uncorrected Pirani gauge) and a trap collision energy trap. The trap started at 2 eV for all the organosulfur compounds and ended at 15 or 20 eV, depending on the structure of the organosulfur compounds. All MS/MS experiments were acquired over 0.3 min at each excitation voltage, within the m/z range of 10–350.

MassLynx software 4.2 (Waters Corp., Milford, MA, USA) was used for data acquisition and MS and MS/MS processing.

2.4.3. Identification of Organosulfur Compounds by LC-HRMS

Targeted identifications of organosulfur compounds were carried out as follows: (1) literature search; (2) accurate mass measurements; (3) isotopic pattern; (4) CID tandem MS analysis.

(1) Firstly, after a bibliographic search, a list of organosulfur compounds was created, which allows for the first step in the identification of the compounds in the onion samples [22–24,32–35].
(2) Then, from these previously reported compounds, the identification and characterization of compounds in the onion samples involved the evaluation of the mass error between the observed mass and the theoretical mass. To obtain accurate mass measurements, mass spectrometers rely on calibration using ions of known m/z [36]. In this work, the calibration of the instrument was performed externally, before analysis, with a sodium formate solution. In addition, the calibration was validated by acquiring a post-calibration spectrum of the calibration solution itself (NaCOOH) and a known solution of leucine enkephalin. These calibration results showed a relative mass error of about 5 ppm. However, to ensure more accurate identification of the onion samples, candidate structures were considered with relative mass errors of up to $\pm$10 ppm [37].
(3) In addition, the isotopic pattern matching helped determine the chemical formula of the organosulfur compounds. Although the spectral patterns of isotopically generated ions are traditionally used as a secondary means of compound identification, in this work, the careful examination of the theoretical patterns associated with a specific ion is also considered to be a powerful discriminator for uniquely identifying chemical formulae [38].
(4) Finally, tandem mass spectrometry analysis was employed to confirm the structure of the organosulfur compounds previously identified. Compounds for which reference MS/MS data could not be obtained were evaluated at the MS level only [39].

2.5. Statistical Analysis

The data from the organosulfur compounds analyzed by LC-HRMS and GC-MS were organized into two independent matrices (one for each chromatographic technique). The dimensions of the LC-HRMS matrix were 8×15, with 8 corresponding to the number of onion varieties analyzed and 15 to the number of organosulfur compounds quantified with each of the techniques. The dimensions of the GC-MS matrix were 8×18.

For each chromatographic technique, area values were calculated for each organosulfur compound. These area values were used as an estimate of the absolute concentration of each compound, as there are no commercial standards available to obtain the concentration from calibration curves. The multivariate analysis was carried out with the mean values of the area of each organosulfur compound obtained for each variety.

The multivariate analysis was carried out with R software version 4.1.3 [40] and RStudio [41]. Correlations were calculated with the R package "corrplot" [42]. Pearson's chi-square test for 2 × 2 contingency tables was calculated to identify the organosulfur compounds that were significantly correlated (p-value < 0.05). Only the significant correlations were plotted as ellipses. Positive correlation was plotted in blue whereas negative correlation was plotted in red. The darker the color of the ellipse, the more correlated are the variables. In addition to the colors, the ellipses have their eccentricity parametrically scaled to the correlation coefficient. This implies that visually, they will become less prominent (the area of the ellipse will decrease) for higher correlation coefficients.

Principal component analysis (PCA) was carried out with the package "mdatools" [43]. The data were autoscaled before performing the PCA analysis.

3. Results and Discussion

3.1. DTD-GC-MS Analysis and Identification

Using DTD-GC-MS, a total of 18 volatile and semi-volatile organosulfur compounds were tentatively identified according to the Wiley Library, with a match factor of more than 80%. In addition, the RIs obtained were in good agreement with those reported in the NIST Chemistry WebBook under similar analytical conditions [44]. The results obtained for one of the onions studied, the purple variety, are shown in Table 1, and the chromatogram in Figure 3. In addition, the literature RI window [44] used to identify each of the volatile compounds is given in Table 1.

Table 1. DTD-GC-MS-based identification of organosulfur compounds in the purple onion variety.

N°	Retention Time (min)	Compounds	Abbreviation	Chemical Formulae	Measured *m*/*z* Base Peak Ion	Similarity (%)	Experimental RI [2]	Literature RI Window [3]
1	3.718	Methanethiol	Meth	CH_4S	48	98	690	679–690
2	4.157	Dimethyl sulfide	Di-me Su	C_2H_6S	62	98	750	748–757
3	5.118	1-Propanethiol	1-Proth	C_3H_8S	76	95	828	817
4	5.515	Sulfur dioxide	SO2	SO_2	64	96	880	882
5	7.253	Methyl-thiirane	Met-thi	C_3H_6S	74	94	919	915
6	12.315	Dimethyl disulfide	Di-me di-Su	$C_2H_6S_2$	94	95	1063	1069–1085
7	13.058	2-Methyl-thiophene	2-Met-thiph	C_5H_6S	98	93	1080	1093–1095
8	14.203	3-Methyl-thiophene	3-Met-thiph	C_5H_6S	98	96	1106	1120
9	16.013	2,5-Dimethyl-thiophene	2,5-Met-thiph	C_6H_8S	112	91	1145	1157–1202
10	17.543	3,4-Dimethyl-thiophene	3,4-Met-thiph	C_6H_8S	112	94	1178	Not found
11	18.455	1,1′-Thiobis-1-propene	Thi-pro	$C_6H_{10}S$	114	89	1197	Not found
12	18.860	(Z/E)-Allyl(prop-1-en-1-yl)sulfane	Allyl(iso)sulf	$C_6H_{10}S$	114	87	1206	Not found
13	19.238	Propanethial S-oxide	Ox pro	C_3H_6OS	90	96	1216	Not found
14	19.573	Methyl propyl disulfide	Me pro di-Su	$C_4H_{10}S_2$	122	93	1221	1227–1243
15	20.603	2,4-Dimethyl-thiophene	2,4-met-thiph	C_6H_8S	112	95	1243	1250
16	21.112	(Z)-1-Methyl-2-(prop-1-en-1-yl)disulfane [1]	ZMeth(iso)disulf	$C_4H_8S_2$	120	96	1254	Not found
17	22.308	(E)-1-Methyl-2-(prop-1-en-1-yl)disulfane [1]	EMeth(iso)disulf	$C_4H_8S_2$	120	97	1280	Not found
18	26.563	Dimethyl trisulfide	Di-me tri-Su	$C_2H_6S_3$	126	97	1371	1365–1412

[1] The identification of the isomer is tentative. [2] Retention indices were determined concerning a homologous series of n-alkanes on a DB-Wax 60 m length column. [3] The RIs found in the literature can vary depending on the temperature program used, therefore a possible IR window is shown in some cases.

From a qualitative point of view, and as we can see in Table 1, using DTD-GC-MS, all the compounds identified in the samples correspond to the volatile and aromatic organosulfur compounds formed by reactions after the CS-lyase action (the metabolic pathway illustrated in Figure 2). The high temperatures to which the samples are subjected during thermal desorption pre-extraction and subsequent gas chromatographic analysis mean that none of the non-aromatic precursors can be identified in the samples [15]. Other researchers had previously utilized headspace gas chromatography to quantify volatile compounds resulting from reactions catalyzed by CS-lyase, regarding these compounds as indicative of the vegetable's pungency level [26].

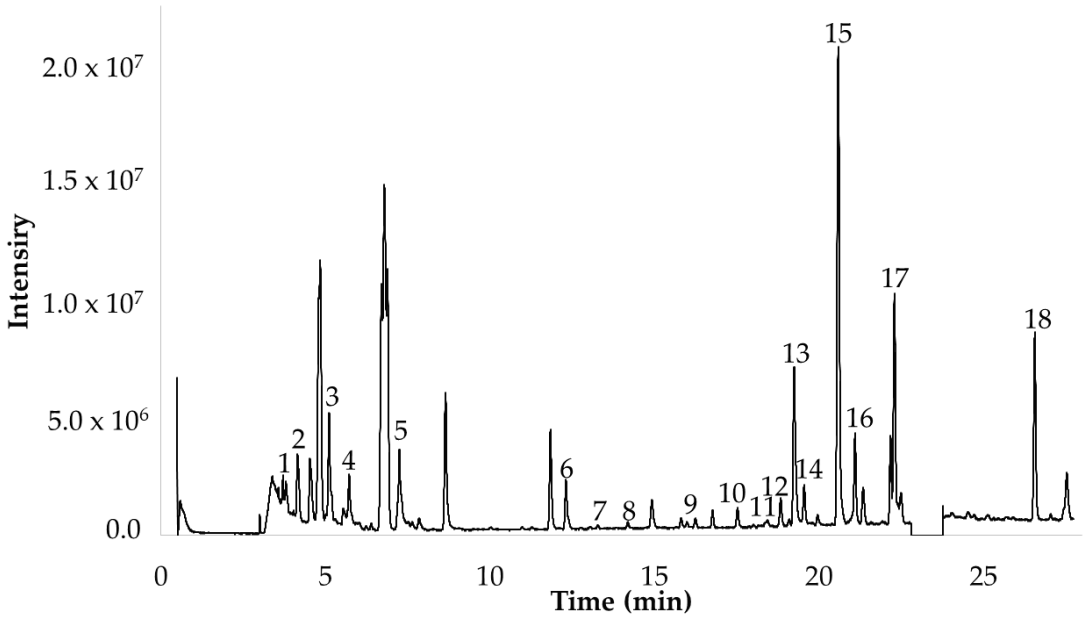

Figure 3. DTD-GC-MS chromatogram of the 18 organosulfur compounds identified in purple onion. The codes for the compounds are shown in Table 1.

3.2. LC-ESI-QqToF Analysis and Identification

Using LC-HRMS, 15 organosulfur compounds were identified in most of the onion samples. The results obtained for one of the onions studied, the purple variety, are shown in Table 2. The mass spectrometry results obtained for the other onion varieties are listed in Tables S1–S7 of the Supplementary Materials. In addition, the extract ion chromatograms obtained for each of the organosulfur compounds identified are given in Figure 4.

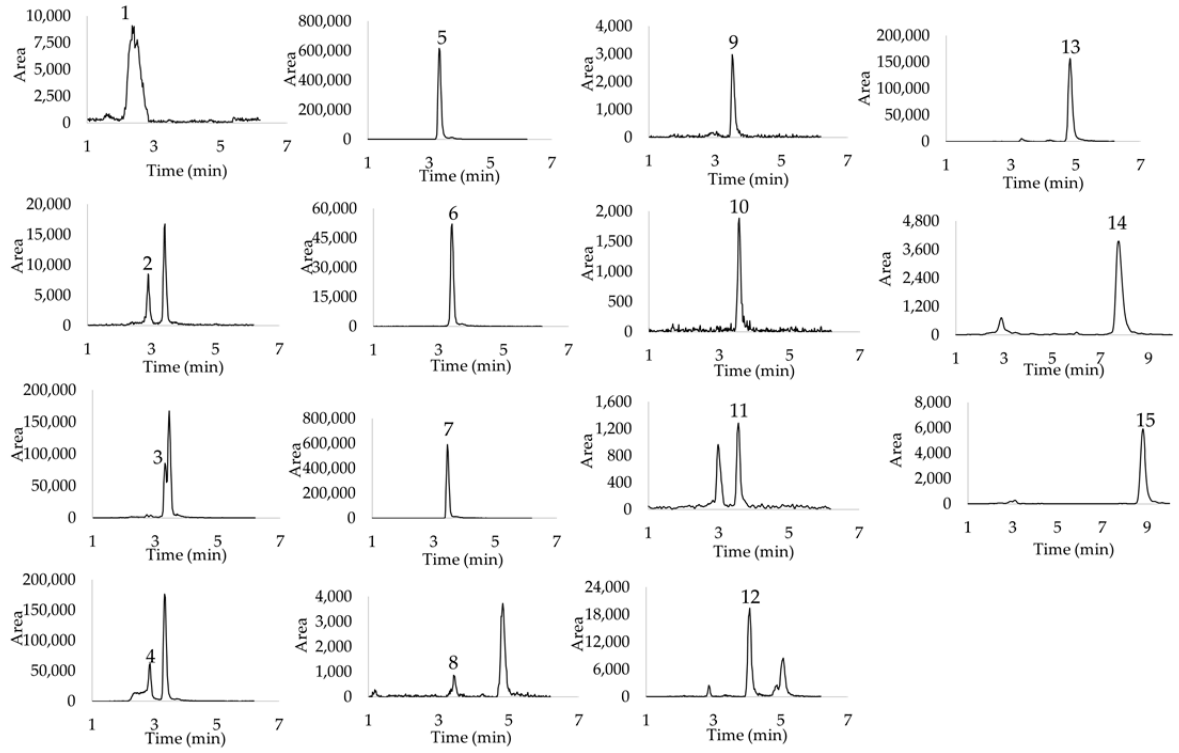

Figure 4. Extract ion chromatograms obtained for each of the organosulfur compounds identified in purple onion. The codes for the compounds are shown in Table 2.

Table 2. LC ESI-QqToF-based identification of organosulfur compounds in the purple onion variety.

N°	Retention Time (min)	Compounds	Abbreviation	Ion Formulae	Measured *m*/*z*	Calculated *m*/*z*	Absolute Error δ (ppm)	Product Ions Formula (Relative Intensity [%], Elemental Composition)
1	2.384	Methiin	Met	$C_4H_{10}NO_3S$	152.0385	152.0381	2.6309	70.0275 (2, $C_3H_4NO^+$), 88.0392 (100, $C_3H_6NO_2^+$), 89.0458 (3, $C_3H_7NO_2^{+\cdot}$), 106.0316 (8, $C_3H_8NOS^+$), 152.0369 (47, $C_4H_{10}NO_3S^+$)
2	2.908	Propiin	Pro	$C_6H_{14}NO_3S$	180.0705	180.0694	6.1088	70.0261 (1, $C_3H_4NO^+$), 88.0376 (100, $C_3H_6NO_2^+$), 116.0290 (3, $C_5H_8OS^+$), 134.0598 (3, $C_5H_{12}NOS^+$), 180.0647 (23, $C_6H_{14}NO_3S^+$)
3	3.331	y-glutamyl-S-methyl-L-cysteine	yGMetC	$C_9H_{17}N_2O_5S$	265.0866	265.0858	3.0179	73.0275 (3, $C_3H_5O_2^+$), 74.9911 (1, $C_2H_3OS^+$), 77.0062 (1, $C_2H_5OS^+$), 84.0451 (2, $C_2H_6NO^+$), 90.0379 (2, $C_3H_8NS^+$), 119.0178 (53, $C_4H_7O_2S^+$), 130.0518 (7, $C_5H_8NO_3S^+$), 136.0414 (41, $C_4H_{10}NO_2S^+$), 202.0557 (5, $C_8H_{12}NO_3S^+$), 230.0497 (7, $C_9H_{12}NO_4S^+$), 248.0576 (13, $C_9H_{14}NO_5S^+$), 265.0860 (100, $C_9H_{17}N_2O_5S^+$)
4	2.874	Isoalliin	Iso	$C_6H_{12}NO_3S$	178.0542	178.0538	2.2465	70.0275 (1, $C_3H_4NO^+$), 73.0117 (1, $C_3H_5S^+$), 88.0229 (2, $C_3H_6NS^+$), 88.0394 (100, $C_3H_6NO_2^+$), 91.0221 (3, $C_3H_7O^+$), 114.0390 (7, $C_5H_8NO_2^+$), 132.0455 (2, $C_5H_{10}NOS^+$), 160.0412 (6, $C_6H_{10}NO_2S$), 178.0536 (32, $C_6H_{12}NO_3S^+$)
5	3.348	γ-Glutamyl-S-(1-propenyl) cysteine sulfoxide	yGIsoCS	$C_{11}H_{19}N_2O_6S$	307.0981	307.0964	5.5357	84.0451 (12, $C_4H_6NO^+$), 88.0394 (19, $C_3H_6NO_2^+$), 130.0518 (46, $C_5H_8NO_3^+$), 154.0496 (5, $C_7H_8NO_3^+$), 178.0536 (10, $C_6H_{12}NO_3S^+$), 200.0552 (5, $C_8H_{10}NO_5^+$), 217.0836 (100, $C_8H_{13}N_2O_5^+$), 307.0952 (29, $C_{11}H_{19}N_2O_6S^+$)
6	3.398	γ-Glutamyl-S-propyl cysteine sulfoxide	yGProCS	$C_{11}H_{21}N_2O_6S$	309.1130	309.1120	3.2351	No MSMS data available
7	3.449	γ–Glutamyl-S-(2-carboxy propyl) cysteine glycine	yG(2-carboxy)CGly	$C_{14}H_{24}N_3O_8S$	394.1303	394.1284	4.8208	No MSMS data available

Table 2. *Cont.*

N°	Retention Time (min)	Compounds	Abbreviation	Ion Formulae	Measured *m*/*z*	Calculated *m*/*z*	Absolute Error δ (ppm)	Product Ions Formula (Relative Intensity [%], Elemental Composition)
8	3.466	S-(1-Propenyl) cysteine	IsoC	$C_6H_{12}NO_2S$	162.0590	162.0589	0.6171	No MSMS data available
9	3.534	γ–Glutamyl-S-(2-carboxypropyl) cysteine glycine hexoside	yG(2-carboxy)CGlyHex	$C_{20}H_{34}N_3O_{13}S$	556.1827	556.1812	2.6970	No MSMS data available
10	3.567	γ–Glutamyl-S-(2-carboxypropyl) cysteine	yG(2-carboxy)C	$C_{12}H_{21}N_2O_7S$	337.1080	337.1069	1.7799	No MSMS data available
11	3.584	S-(2-carboxypropyl) cysteine	(2-carboxy)C	$C_7H_{14}NO_4S$	208.0648	208.0643	2.4031	No MSMS data available
12	4.091	γ-Glutamyl-S-propyl cysteine	yGProC	$C_{11}H_{21}N_2O_5S$	293.1176	293.1171	1.7058	No MSMS data available
13	4.835	γ-Glutamyl-S-(1-propenyl) cysteine	yGIsoC	$C_{11}H_{19}N_2O_5S$	291.1022	291.1015	2.4047	55.018 (1, $C_3H_3O^+$), 58.0654 (1, $C_3H_8N^+$), 73.0117 (8, $C_3H_5S^+$), 84.0451 (3, $C_4H_6NO^+$), 99.0252 (3, $C_5H_7S^+$), 116.0541 (4, $C_5H_{10}NS^+$), 130.0518 (4, $C_5H_8NO_3^+$), 145.0327 (50, $C_6H_9O_2S^+$), 162.0594 (70, $C_6H_{12}NO_2S^+$), 170.0794 (8, $C_8H_{12}NOS^+$), 182.0617 (3, $C_9H_{12}NOS^+$), 228.0688 (4, $C_{10}H_{14}NO_3S^+$), 274.0742 (9, $C_{11}H_{16}NO_5S^+$), 291.0999 (100, $C_9H_{17}N_2O_5S^+$)
14	7.777	γ–Glutamyl-S-(S-1-propenyl)cysteine glycine	yGIsoCGly	$C_{13}H_{22}N_3O_6S_2$	380.0971	380.0950	5.5249	No MSMS data available
15	8.825	γ–Glutamyl-S-(S-propyl)cysteine-glycine	YGProCGly	$C_{13}H_{24}N_3O_6S_2$	382.1113	382.1107	1.5702	No MSMS data available

A comparison of the structures of these compounds with those identified by GC-MS reveals that they are completely different families of organosulfur compounds. The compounds identified by LC-HRMS are associated with the CSOs, accompanied by substantial amounts of their corresponding γ-glutamyl dipeptides precursors. By reviewing Figure 1, it is evident that this methodology facilitates the analysis of the compounds involved in the metabolic pathway of the formation of CSOs in onion. The absence of aromatic compounds in the same onion variety is probably attributed to the relatively mild analytical conditions, where the sample is not exposed to elevated temperatures. Consequently, the reactive processes responsible for forming aromatic compounds do not occur under these conditions.

Regarding the identification, all compounds showed a relative mass error of less than ±10 ppm, in the 6.1–0.6 ppm range. In addition, it was possible to obtain CID MSMS spectra for some of the identified compounds to confirm their structure.

The specific fragmentation patterns obtained for purple onion can be observed in Table 2 and Figure 5. The results reported by Liu P. et al., 2020 and Böttcher C. et al., 2017 [24,31] have been used as a basis for the identification of the products, but not much literature has been found on the fragmentation of the organosulfur compounds characteristic of onion. As can be seen from the MS/MS spectra, in all cases, the precursor ions were $[M + H]^+$. The three main CSOs showed the most intense product ion at m/z 88.039, corresponding to the cleavage of the C-S bond and the consequent loss of the sulfoxide group (methiin $[M + H\text{-}CH_4OS]^+$; propiin $[M + H\text{-}C_3H_8OS]^+$; isoalliin $[M + H\text{-}C_3H_6OS]^+$).

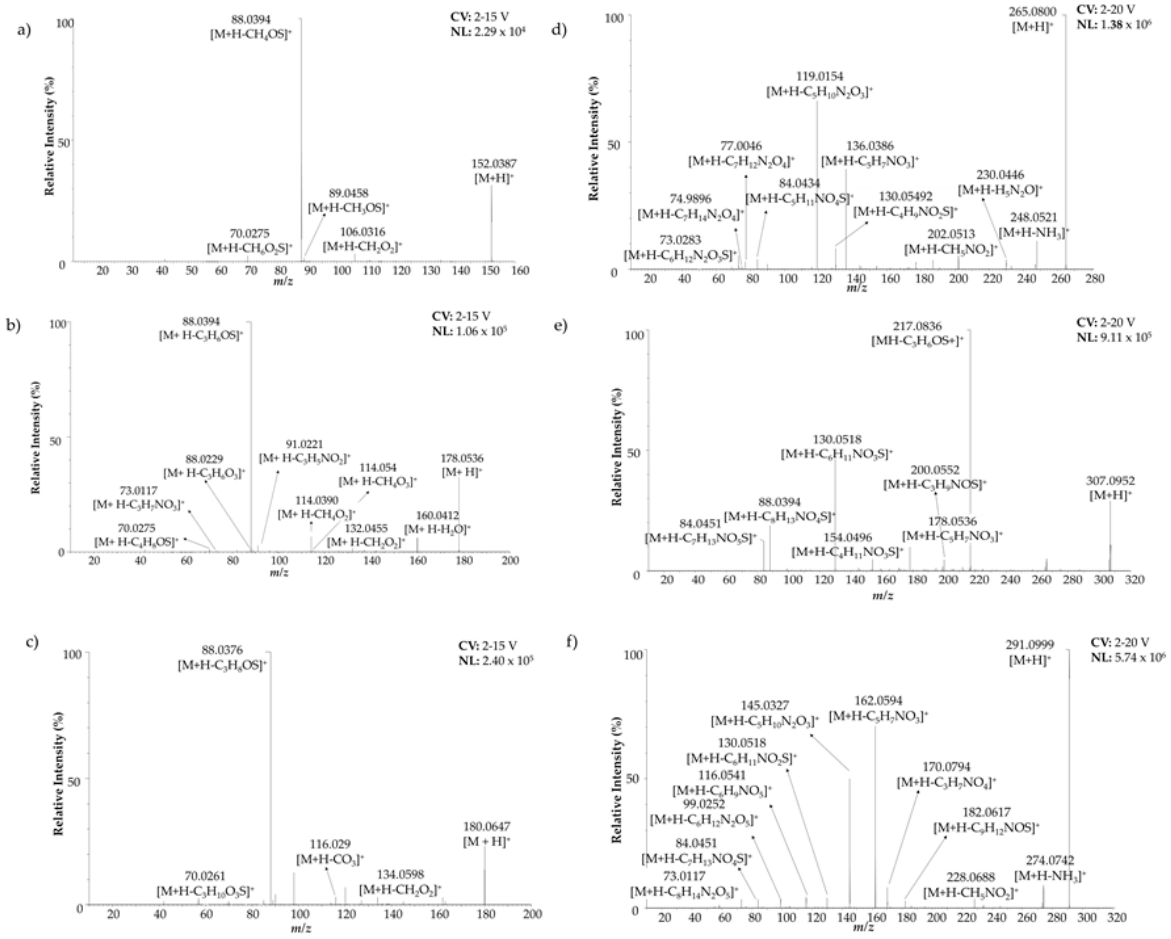

Figure 5. Purple onion sample product ion mass spectra using ESI in positive ion mode. (**a**) Methiin; (**b**) isoalliin; (**c**) propiin; (**d**) γ-glutamyl-S-methyl cysteine; (**e**) γ-Glutamyl-S-(1-propenyl) cysteine sulfoxide; (**f**) γ-Glutamyl-S-(1-propenyl) cysteine.

In cases of unreported MS/MS spectra (Table 2), the problem arises from isobaric contamination affecting the m/z values of the precursor ion. Ions with very close m/z values were reported in the tandem MS spectra. These m/z differences are too small to be discerned by mass spectrometry, even with high-resolution equipment such as the one used in this work. This isobaric contamination complicates MS/MS analysis by making the isolation of the designated precursor ion difficult, resulting in extensive mixtures of fragments.

3.3. Quantification of Organosulfur Compounds

Once the compounds were identified, they were quantified to compare the two analytical techniques. Since no commercial standards were available, the quantification of the different compounds by each of the techniques was performed by area quantification. In the case of GC-MS, the compounds are identified across all varieties. However, when using LC-HRMS, there is significant variation in the organosulfur content among the different varieties. The area values for each sample are given in Table S8 of the Supplementary Materials.

Figure 6 shows the heat map of the chromatographic areas of the eight varieties obtained for each organosulfur compound. In LC, the highest values and variabilities are observed for γ-glutamyl-S-(1-propenyl)-cysteine sulfoxide, γ-glutamyl-S-(2-carboxypropyl)-cysteine glycine, and γ-glutamyl-S-(1-propenyl)-cysteine. Shallots, Red Label, and white onions have very similar organosulfur compositions. Purple onions and spring onions have a very high content of γ-Glutamyl-S-(1-propenyl) cysteine sulfoxide, whereas this organosulfur is not detected in shallots, Red Label, and white onions. In GC, the highest area values and variabilities are observed for 2,4-dimethylthiophene, followed by propanethial S-oxide. The highest concentration of 2,4-dimethylthiophene is observed in shallots, white onions, and spring onions. It can be seen that the variability is higher for LC than for GC. The area values for GC are about 10 times higher than for those for LC.

The box plots in Figure 7 also show that the highest variability in LC among the onion varieties is observed for γ-glutamyl-S-(1-propenyl)-cysteine sulfoxide, γ-glutamyl-S-(2-carboxypropyl)-cysteine glycine, and γ-glutamyl-S-(1-propenyl)-cysteine. These results are in accordance with the heat map for LC (Figure 6a). For these compounds, some onion varieties have very high area values, whereas they are not detected in other varieties. The GC box plots show less variability between onion varieties, as already observed in the heat map (Figure 6b). The highest variability is obtained for 2,4-dimethylthiophene and propanethial S-oxide. The shallot has a much higher amount of 3,4-dimethylthiophene, (Z/E)-allyl(prop-1-en-1-yl)sulfane, dimethyl disulfide, and dimethyl trisulfide than the other varieties. For these compounds, the area values of shallots are marked as black dots in the box plots because they are outside the interval of 1.5 times the interquartile distance.

3.4. Correlations between Organosulfur Compounds

As we have already seen, the use of each analytical technique provides information on different types of organosulfur compounds. When using LC-HRMS, the compounds identified correspond to those represented in Figure 1, whereas when using GC-MS, it is only possible to study the volatile profile of the onion after its reaction with the enzyme allinase (Figure 2). However, in the end, both families of compounds are part of the same biosynthetic pathway of onion aromas. For this reason, it makes sense that the compounds identified by LC ESI-QqToF are the non-aromatic precursors of the aromatic organosulfur compounds analyzed by DTD-GC-MS. In this work, by LC-ESI-QqToF analysis, propiin, methiin, and isoalliin were identified as the main CSOs in onion. Consequently, it is logical that GC analysis reveals mixtures of compounds with propyl (-$CH_2CH_2CH_3$), methyl (-CH_3), and 1-propenyl (-$CHCHCH_3$) functional groups, such as methanethiol, dimethylsulfide, 1-propanethiol, dimethyl disulfide, 1,1′-thiobis-1-propene, methyl propyl disulfide, (*Z*/*E*)-1-methyl-2-(prop-1-en-1-yl) di-sulfane, dimethyl trisulfide, and 1-propanethiol. To study these possible interactions in more detail, Pearson's chi-square test was applied to identify the significant correlations between the organosulfur compounds.

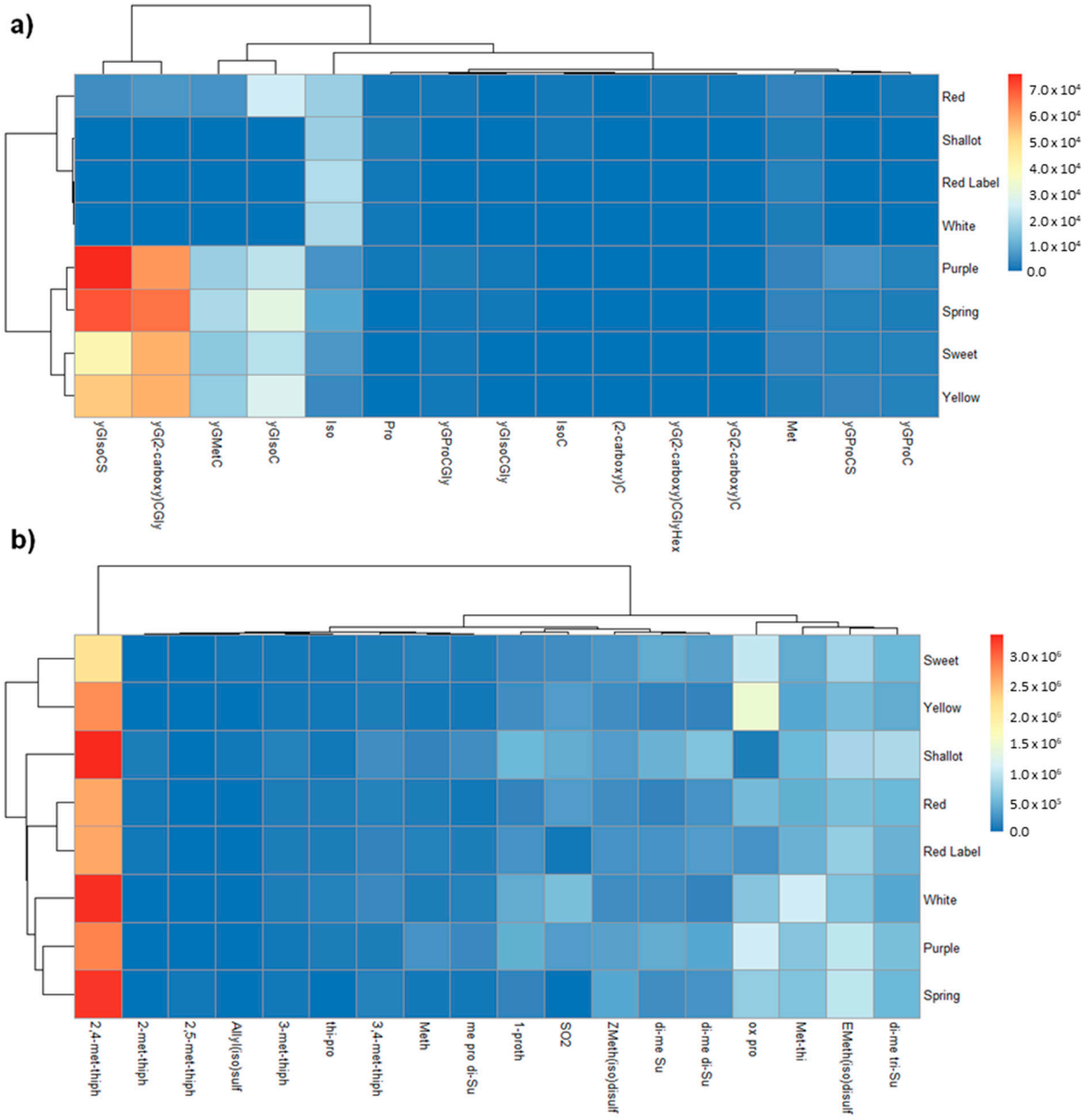

Figure 6. Heat map showing the distribution of organosulfur compounds in different onion varieties: (**a**) identified by LC-HRMS and (**b**) identified by GC-MS.

3.4.1. Correlations between Organosulfur Compounds Identified by LC-ESI-QqToF

First, possible correlations between the compounds identified only by LC-HRMS were studied (Figure 8). We can see that, a priori, there are many correlations between these non-volatile compounds, some of which can be explained by the biosynthetic routes of the formation of CSOs.

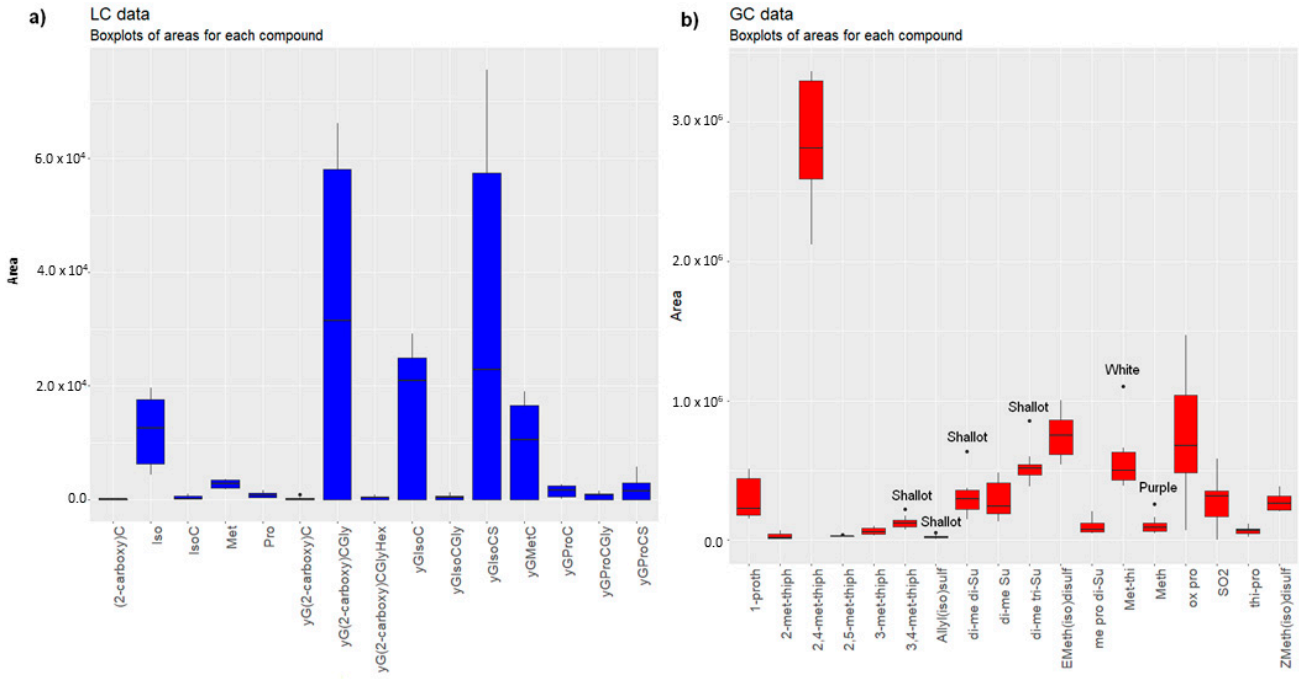

Figure 7. Box plots of the chromatographic areas of the eight onion varieties obtained for each organosulfur compound: (**a**) liquid chromatography (in blue), (**b**) gas chromatography (in red). The black dots correspond to area values that can be considered as outliers (outside the interval of 1.5 times the interquartile distance). Shallot has four outlier observations in the GC box plot.

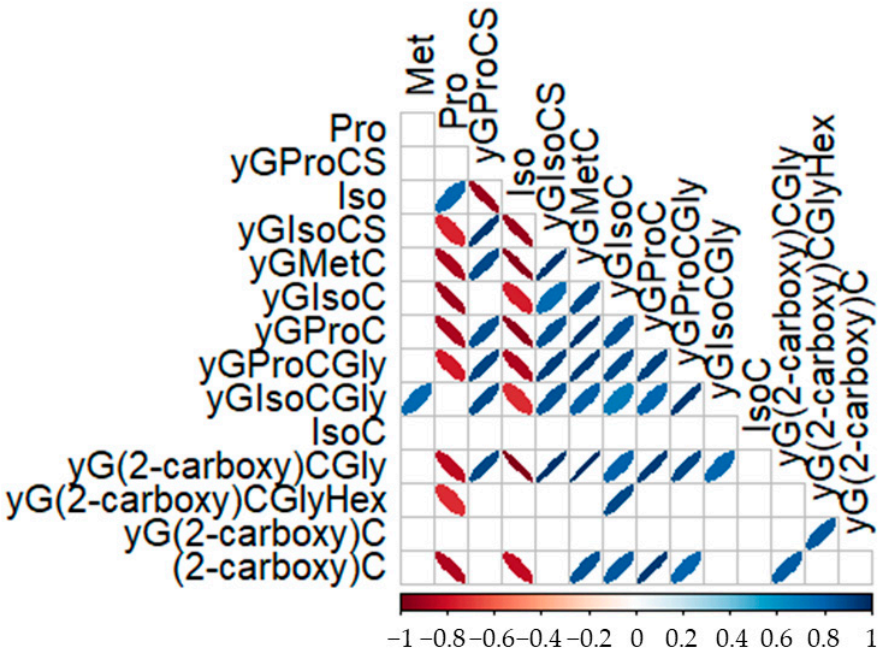

Figure 8. Pearson correlation matrix: Analysis of the relationships between the organosulfur compounds identified by LC-ESI-QqToF. Positive correlations are shown in blue, while negative correlations are displayed in red. The ellipses have their eccentricity parametrically scaled to the correlation coefficient.

From Figure 8, it can be seen that there is a positive relationship between the γ-glutamyl peptide precursors of the CSOs. All these compounds are supposed to be storage compounds of the CSOs during the dormancy stage: when the dormancy stage ends, the γ-glutamyl peptides are converted into the corresponding CSOs [45]. It therefore makes sense

that they are globally positively correlated with each other. More specifically, some correlations exist within the different metabolomic pathways. For example, γ-glutamyl-S-propyl cysteine sulfoxide correlates positively with γ-glutamyl-S-propyl cysteine (r = 0.86) and γ-glutamyl-S-propyl cysteine-glycine (r = 0.91). In the case of y-glutamyl-S-(2-carboxypropyl) cysteine-glycine, which has a more complex metabolic pathway, summarized in Figure S1 of the Supplemental Materials [46], it also correlates positively with y-glutamyl-S-(S-1-propenyl) cysteine-glycine (r = 0.97), γ-glutamyl-S-(1-propenyl) cysteine sulfoxide (r = 0.80), and γ-glutamyl-S-(1-propenyl) cysteine (r = 0.79).

Regarding the CSOs, the isoaliin and propiin are positively correlated. Concerning methiin, this CSO does not show a significant correlation with the others. Montaño, A. et al., 2011 have previously shown that methiin did not correlate with other CSOs in the case of garlic [47]. On the other hand, propiin and isoaliin show a negative correlation with their precursors, according to the metabolic pathways illustrated in Figure 1. As previously mentioned, there is a balance between free cysteine derivatives and their dipeptide forms, with their ratio primarily depending on the growth phase and storage conditions. Authors such as Ichikawa, M. et al., 2006 already showed that during different storage conditions, the increases in isoalliin in garlic almost coincided with the decreases in its precursor, γ-l-glutamyl-S-(trans-1-propenyl)-l-cysteine [48]. This could explain, for example, the negative correlations observed between isoalliin and γ-glutamyl-S-(1-propenyl)-cysteine sulfoxide (r = −0.91) or between propiin and γ-glutamyl-S-propyl-cysteine (r = −0.88).

3.4.2. Correlations between Organosulfur Compounds Identified by DTD-GC-MS

Regarding the results obtained by GC-MS, there is less correlation among the organosulfur compounds analyzed with this technique. Figure 9 shows positive correlations among the thiophenes, which belong to the same family. Simultaneously, these thiophenes exhibit a negative correlation with propanethial S-oxide. This negative correlation is logical when considering the production pathways of these compounds (Figure 2). Both thiophenes and propanethial S-oxide are derived from the same sulfoxide, isoalliin, leading to a competitive process where the sulfoxide can either form thiophenes and other aromatic compounds or produce the small molecule responsible for eye irritation when cutting onions.

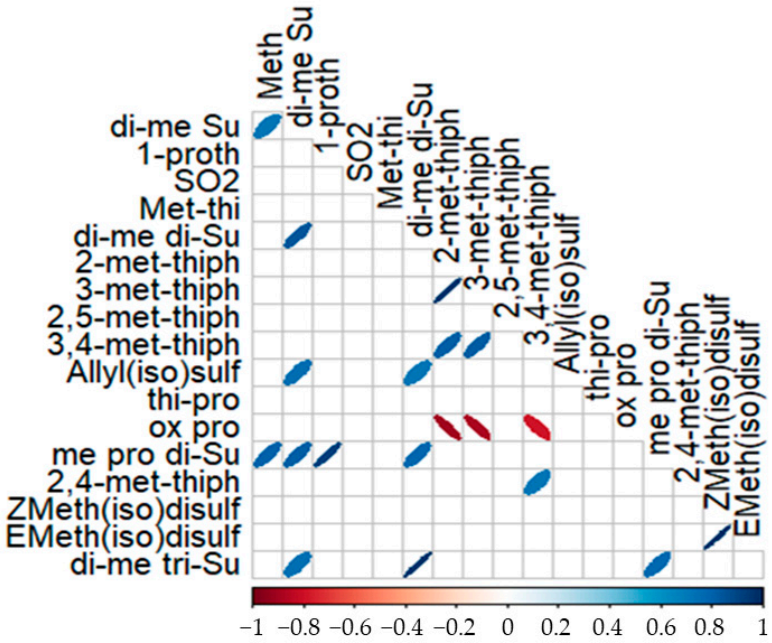

Figure 9. Pearson correlation matrix: Analysis of the relationships between the organosulfur compounds identified by DTD-GC-MS. Positive correlations are shown in blue, while negative correlations are displayed in red. The ellipses have their eccentricity parametrically scaled to the correlation coefficient.

3.4.3. Correlations between Organosulfur Compounds Identified with Both Techniques

Finally, the possible correlations between the compounds analyzed with the two analytical techniques proposed in this work were studied. The results obtained are shown in Figure 10. A priori, it does not seem possible to establish a strong relationship between the content of CSOs, the non-volatile precursors quantified in the samples by LC-HRMS, and the content of aromatic organosulfur compounds analyzed by GC-MS.

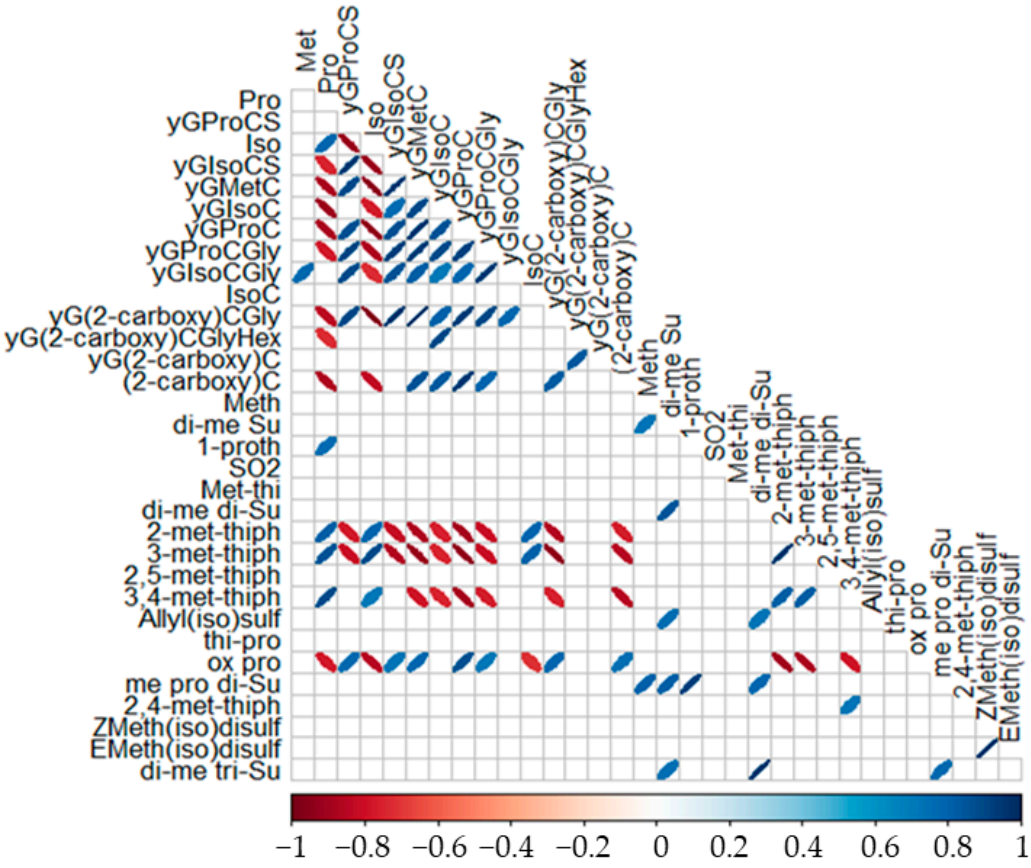

Figure 10. Pearson correlation matrix: Analysis of the relationships between the organosulfur compounds identified with both analytical techniques. Positive correlations are shown in blue, while negative correlations are displayed in red. The ellipses have their eccentricity parametrically scaled to the correlation coefficient.

First, it can be seen that some γ-glutamyl peptide precursors correlate negatively with the volatile compounds analyzed by GC-MS. These correlations do not provide significant information, as these precursors do not directly contribute to flavor, lacking alliinase sensitivity [45].

Regarding the CSOs, positive correlations with some volatile compounds analyzed by GC-MS can be observed. This aligns with CSOs being converted to thiosulfinates by alliinase when tissues are cut or crushed, subsequently producing characteristic flavors [45]. For instance, propiin correlates positively with 1-Propanethiol (r = 0.76), fitting the shared -propiin group between the precursor and the enzymatic product. Conversely, propanethial S-oxide negatively correlates with both propiin and isoalliin. This compound, known as a tear factor because it is responsible for the eye irritation and watering caused by cutting onions, is generated from the main onion precursor, isoalliin. Therefore, this negative correlation appears inconsistent.

Finally, the correlations obtained between the identified compounds were complemented with principal component analysis (PCA) for a better understanding of the trends.

3.5. Principal Component Analysis

Principal component analysis (PCA) was conducted using the area of the organosulfur compounds quantified by the two analytical techniques proposed in this study. All the data were autoscaled. The score plots obtained using the LC-HRMS data matrix, the GC-MS data matrix, and the combined data from both methods are shown in Figure 11.

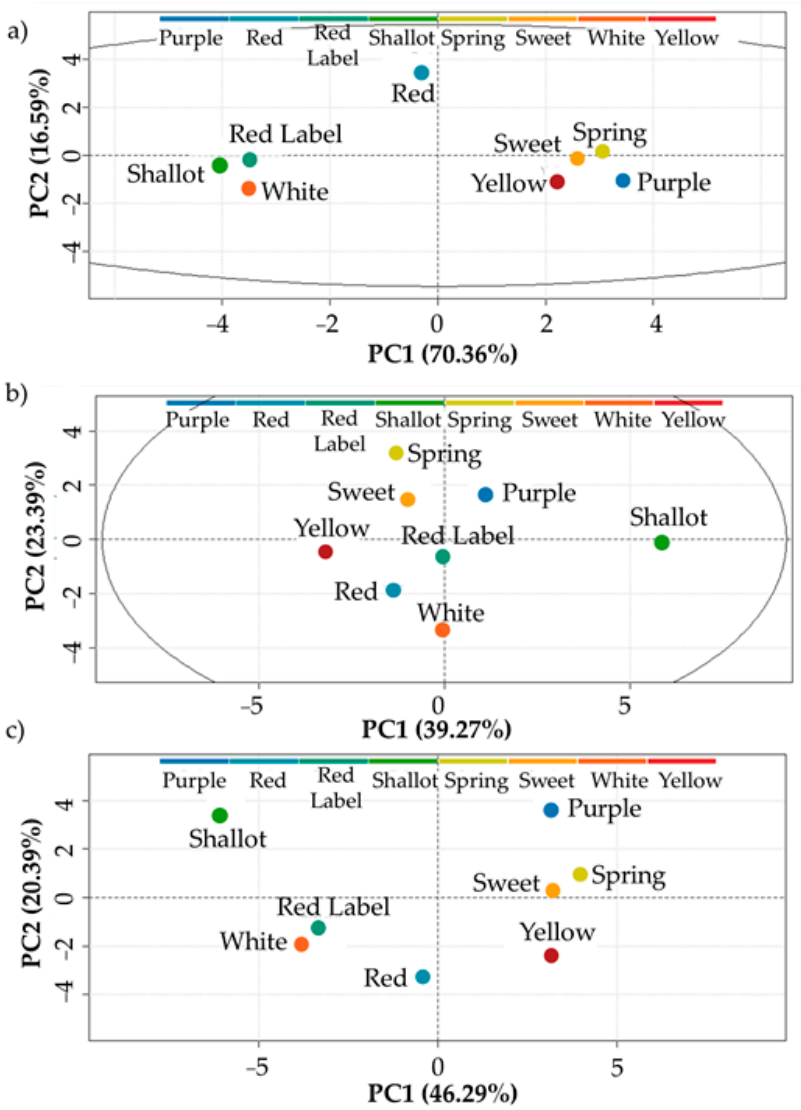

Figure 11. Score plots depicting the results of principal component analysis (PCA) calculated on the dataset of the organosulfur compounds analyzed by: (**a**) LC-HRMS; (**b**) GC-MS; and (**c**) both analytical techniques combined. The ellipse represents the *Hotelling T2*, with a 95% confidence interval.

Figure 11a illustrates the reduction of data from the 15 organosulfur compounds identified by LC-HRMS to two principal components, representing 86.95% of the total explained variance. All the scores were within the *Hotelling T2* ellipse and revealed a tendency to form three distinct clusters among the eight onion varieties studied. The biplot of Figure 12a elucidates which organosulfur compounds influence each discriminant group the most. PC1, representing 70.36% of the explained variance, has high negative loadings for propiin and isoalliin, while exhibiting high positive loadings for the γ-glutamyl peptide precursors of the CSOs. This is consistent with the negative correlation observed between propiin and isoaliin and the γ-glutamyl peptide precursors of the CSOs (Figure 8). Consequently, the samples Red Label, white onions, and shallots, which tend to cluster together in the left part of the PC1, generally display higher levels of propiin and isoaliin. On the other hand, the

other varieties show a higher content of γ-glutamyl peptide precursors and a lower content of propiin and isoalliin (as they have a negative correlation). Other authors have already indicated that the distribution of organosulfur compounds in *Allium* vegetables differed not only among species but also according to the intensity of dormancy, which is presumed to depend on the season and storage conditions [45]. Therefore, based on these results, it could be considered that the Red Label, white, and shallot varieties have a lower content of the γ-glutamyl peptide precursors because they present a different level of dormancy, probably due to different cultivation conditions. Regarding methiin, Ichikawa, M. et al., 2066 showed that no clear changes in the methiin content, or in the total content of GSMC and methiin, were observed under any storage conditions [48]. Finally, the red variety is located more independently in the PCA graph, presenting a more balanced composition between CSOs and γ-glutamyl peptide precursors. In particular, it stands out for its higher content of S-(1-Propenyl) cysteine and γ–Glutam-yl-S-(2-carboxypropyl) cysteine.

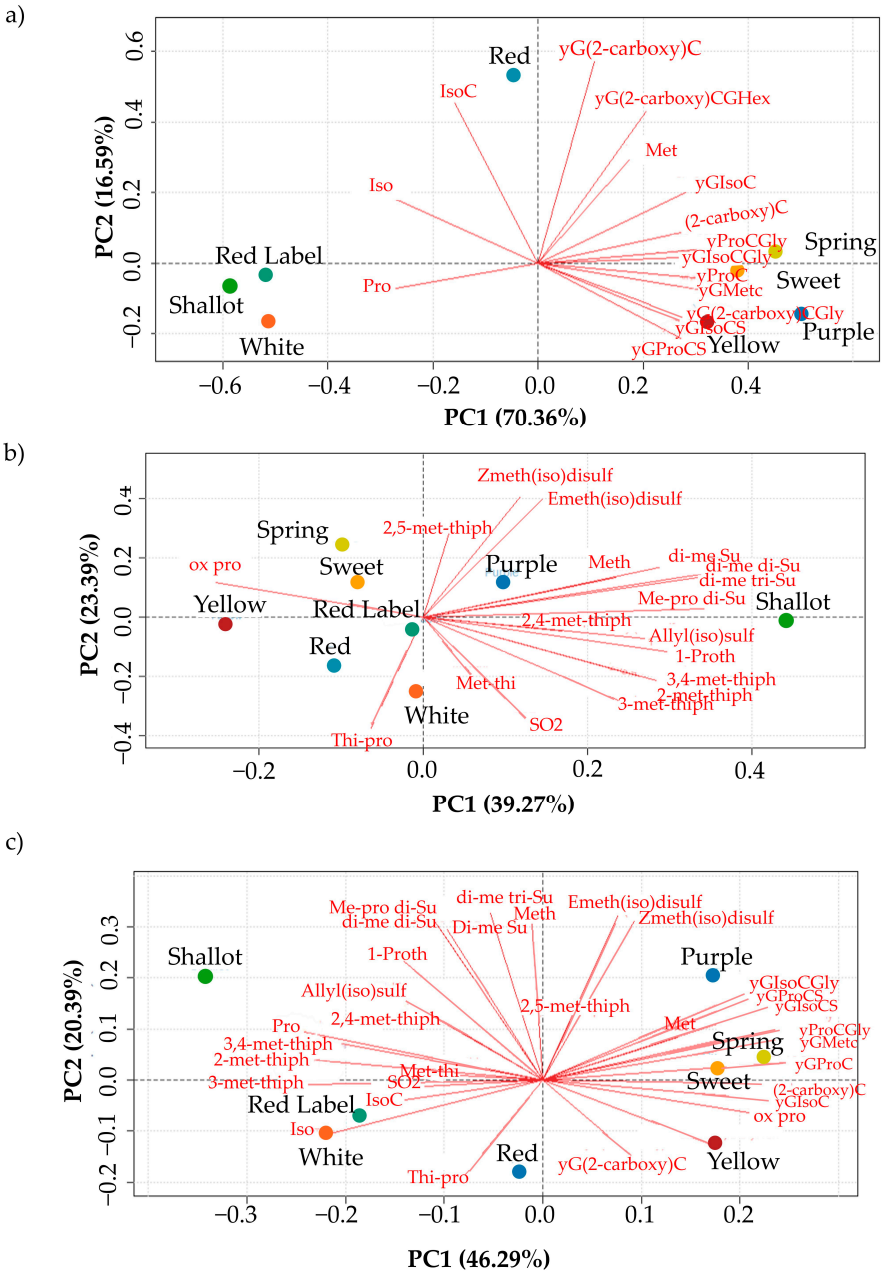

Figure 12. PCA biplots of the organosulfur compounds analyzed by (**a**) LC-HRMS; (**b**) GC-MS; and (**c**) both analytical techniques combined.

Figure 11b shows the score plot of the GC-MS data. In this case, the total explained variance retained by the first two principal components was approximately 62.66%. All the scores of the onion varieties were within the 95% *Hotelling T2* ellipse, showing that there are no outliers. The score plot demonstrates that there is a lower clustering trend between the samples. This could probably be attributed to the harsh analytical conditions, which induce degradation and numerous reactions between the organosulfur compounds. Although clustering is less pronounced, there is a clear distinction in the shallot's profile. This difference is likely because shallots are a different species from onions, resulting in a unique aromatic profile. Looking at the biplot obtained (Figure 12b), the negative loading of propanethial S-oxide has a very important contribution to PC1 and behaves differently from the rest of the organosulfur compounds (which show positive loadings). This opposite behavior can also be explained by the negative correlation already observed between these compounds in Figure 9. The biplot highlights the distinct metabolic pathway that this tear factor has compared to the other volatile compounds. The onion varieties analyzed by GC-MS are then differentiated according to these two metabolic pathways. The 'yellow' onion has a very high concentration of propanethial S-oxide and a low concentration of the remaining volatile organosulfur compounds, while the shallot has the lowest concentration of propanethial S-oxide and the highest concentration of the remaining compounds.

Finally, the PCA was conducted jointly using the results of both techniques (Figure 11c). From the obtained results, it is evident that the classification into three groups is primarily influenced by the findings derived from the LC-HRMS analysis. While GC-MS offers a slightly improved separation of the shallot, the main basis for classification stems from the information gleaned through the LC-HRMS analysis, which is expected, due to the greater variability afforded by this technique.

Regarding the varieties studied and their classification, it is evident that those with a higher content of non-volatile precursors identified by LC-HRMS did not necessarily result in higher concentrations of aromatic compounds detected by GC-MS. Theoretically, authors such as Yamazaki, Y. et al., 2011 [45] assume that varieties with a higher content of CSOs will generate a higher intensity of aromatics. The findings of this study reveal that, while certain varieties, such as sweet onion or spring onion, exhibit more apparent correlations, demonstrating consistent contents across both techniques, there are instances where this correspondence does not apply. For instance, although the shallot onion displayed the lowest levels of organosulfur compounds detected by LC-HRMS, it paradoxically exhibited the highest amounts identified by GC-MS. These disparities could be due to the complexity noted above, where it is difficult to establish an exact stoichiometric relationship between hydrolysates and CSO, as suggested by some authors [49].

3.6. Comparison with Existing Literature on Organosulfur Compound in Onions

In conclusion, the findings from this study suggest that the proposed and compared analytical techniques yield distinct results. Primarily, while GC-MS allows for the analysis of the onion's volatile profile, LC-HRMS provides insights into the initial content of organosulfur compounds before the enzymatic reaction with alliinase. However, neither compound types correlate satisfactorily, indicating no relationship between volatiles and non-volatiles. Therefore, despite the fact that GC is one of the most employed techniques in the literature for studying the sulfur compound content in onions, we believe that for a more comprehensive and accurate depiction of the metabolomic profile in onions, employing LC-HRMS or combining both techniques provides a superior outcome.

To validate the methodology developed in this study, a comparison will be made with results reported in the literature. It is important to consider that the total concentration of flavor precursors varies widely due to factors such as varietal differences, climatic conditions, or maturity stage [50]. Therefore, the comparison will focus on general trends rather than specific varietal differences, as these factors can significantly influence outcomes, as previously noted.

Concerning the results obtained by DTD-GC-MS, a comparison has been made in the previous work published by the authors [21]. Regarding the CSO analyzed by LC-HRMS, it can be observed that in the varieties studied in this work, isoalliin presented a higher concentration, followed by methiin, and lastly, propiin. This agrees with the results shown by other authors, who also point to isoalliin as the highest CSO in onions, with lower amounts of methiin and propiin and with an absence of alliin [14,15,45]. Regarding the γ-glutamyl peptide precursors, y-glutamyl-(1-propenyl)cysteine sulfoxide has been reported by several authors to be the most abundant in onions [51,52].

4. Conclusions

The high popularity of onions, due to their significant content of organosulfur compounds responsible for their sensory attributes and health benefits, has driven the development of various analytical methods to study these compounds. In the literature, GC-MS is one of the most widely used methods for their analysis. However, the high temperatures required in this method lead to the hydrolysis of the initial organosulfur compounds, focusing primarily on the volatile profile of onions. To understand the relationship between this volatile profile and the initial organosulfur compounds in onions, such as CSOs, this study proposes the use of LC-HRMS as an alternative. For this objective, eight onion varieties were analyzed using both techniques. DTD-GC-MS identified 18 volatile organosulfur compounds, such as disulfides, trisulfides, and the compound responsible for eye irritation when cutting onions, while LC-HRMS identified 15 non-volatile organosulfur compounds such as methiin, propiin, isoalliin, and their precursors. Correlating the results from both methods revealed no clear relationships between the volatile and non-volatile compounds identified by each technique. Furthermore, the results showed that LC-HRMS provides more detailed information on the metabolic pathways involved in the formation of onion aromas. As a first preliminary study, it was observed that the LC-HRMS method allows a better identification of similarities between the onion varieties studied, allowing a first classification in space by PCA. On the contrary, the results obtained by GC-MS revealed fewer similarities between the varieties studied, showing a more distinctive aroma profile only for the shallot. Therefore, despite GC-MS being one of the most employed techniques in the literature for studying the sulfur compound content in onions, this work concludes that for a more comprehensive and accurate depiction of the metabolomic profile in onions, employing LC-HRMS or combining both techniques may provide a superior outcome.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors12070130/s1. Figure S1: Metabolic pathways for the synthesis of the isoalliin; Table S1: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the red onion variety; Table S2: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the shallot sample; Table S3: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the Red Label onion variety; Table S4: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the yellow onion variety; Table S5: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the white onion variety; Table S6: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the sweet onion variety; Table S7: LC-ESI-Q-IMS-qToF-based identification of organosulfur compounds in the spring onion variety; Table S8: Absolute mean area values quantified by LC-HRMS and GC-MS analytical techniques.

Author Contributions: Conceptualization, A.M. (Alicia Maroto), G.F.B. and A.M. (Antony Memboeuf); methodology, A.V.G.-d.-P.; software, A.M. (Alicia Maroto) and A.V.G.-d.-P.; validation, A.V.G.-d.-P.; formal analysis, A.V.G.-d.-P. and A.M. (Alicia Maroto); investigation, A.V.G.-d.-P.; resources, G.F.B. and A.M. (Antony Memboeuf); data curation, A.V.G.-d.-P. and A.M. (Alicia Maroto); writing—original draft preparation, A.V.G.-d.-P.; writing—review and editing, A.M. (Alicia Maroto), G.F.B. and A.M. (Antony Memboeuf); visualization, A.M. (Alicia Maroto), G.F.B. and A.M. (Antony Memboeuf); supervision, A.M. (Alicia Maroto), G.F.B. and A.M. (Antony Memboeuf); project administration, G.F.B. and A.M. (Antony Memboeuf); funding acquisition, G.F.B. and A.M. (Alicia Maroto). All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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