# *CHEMICAL AND FUNCTIONAL CHARACTERIZATION OF CHILOE'S GIANT GARLIC (Allium Ampeloprasum L.)*

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## ABSTRACT

The present work reports the chemical and functional profile of Chiloe's giant garlic. Applying AOAC's official methods the proximal content of giant garlic was determined finding a moisture content of 62.34±0.35%, carbohydrates 20.64±0.01%, protein 2.80±0.10%, fat (crude) 0.09±0.00%, ash 0.74±0.02%, and fiber (crude) 13.04±0.01%. Three saccharides [sucrose (5.92±0.02 mg $g^{-1}$), glucose (0.11±0.00 mg $g^{-1}$) and fructose (0.46±0.01 mg $g^{-1}$)], four fatty acids [linoleic acid (57.67±0.00%), palmitic acid (23.46±0.00%), oleic acid (7.20±0.00%), and α-linolenic acid (5.06±0.00%)], and three sulfoxide compounds [alliin (2.66±0.90 mg $g^{-1}$), methiin (9.61±0.33 mg $g^{-1}$) and isoalliin (5.02±1.24 mg $g^{-1}$)] were determined by high-performance thin-layer chromatography (HPTLC), gas chromatography (GC) and HPTLC/mass spectrometry (MS), respectively. Functional profile characterization showed an Oxygen Radical Absorbance Capacity (ORAC) value of 0.250±0.001 mmol TE per 100 $g^{-1}$ and a total (poly)phenols content (TPC) of 40.66±2.08 mg EAG 100 $g^{-1}$. (Poly)phenols profile analyzed by liquid chromatography (LC)/MS showed only the presence of caffeic acid (0.57±0.05 μg $g^{-1}$) and rutin (at traces level). Bioactive molecules with antioxidant (DPPH) and COX-2 inhibition activities were identified through HPTLC (bio)autography and MS analysis, finding the presence of tryptophan (antioxidant) and γ-glutamyl-S-allyl-L-cysteine (GSAC), γ-glutamyl-S-(*trans*-1-propenyl)-L- cysteine (GSPC), alliin and isoalliin with antioxidant and COX-2 inhibitory activity.

**Keywords:** *sulfoxide compounds, (poly)phenols, alliin, COX-2 inhibition, mass spectrometry, antioxidants*.

## 1. INTRODUCTION

Garlic (*Allium sativum* L.) is widely recognized for its medicinal and culinary uses [1]. It contains bioactive molecules with pharmacological properties, including antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic activities. Garlic consumption has been linked to beneficial effects on cardiovascular, digestive, and respiratory systems [2] as well as over metabolic syndrome, by lowering total cholesterol and low-density lipoproteins [3, 4], inhibiting platelet aggregation, and improving glucose metabolism [2, 5]. These effects are primarily due to organosulfur compounds such as S-allyl-cysteine and alliin (S-allyl-L-cysteine sulphoxide) [6]. These kinds of bioactive organosulfur compounds are also present in Chiloe's giant garlic (*Allium ampeloprasum* L.), which is like common garlic but with key differences. At cellular level, it presents a hexaploidy (2n = 6X = 48) resulting in a bigger bulb size and unique bioactive molecules profile. Chiloe's giant garlic is botanically related to the leek and is part of the diverse *Allium ampeloprasum* complex. Commonly referred to as elephant garlic, great-headed garlic, or locally as *"ajo chilote"* because is micro-cultivated on Chiloé Island in Chile. This island has been designated a Globally Important Agricultural Heritage System by the Food and Agriculture Organization (FAO) of the United Nations. The garlic is grown using traditional farming techniques under sustainable conditions, with minimal use of agrochemicals. The plant features a tall flowering stalk (1–2 m) with white to purple flowers that produce either sterile seeds or none at all, requiring propagation through cloves. Its bulb consists of six or more cloves, each measuring 8–10 cm in diameter and weighing 80–100 grams [7]. This descriptive study aims to identify the most representative and significant groups of molecules in Chiloe's giant garlic, including macronutrients, (poly)phenols, and organosulfur compounds. It also seeks to identify molecules with potential pharmacological activities, such as antioxidant properties and enzyme inhibition. To the best of our knowledge, this is the first report providing a chemical and functional characterization of Chiloe's giant garlic.

## 2. MATERIALS AND METHODS

### 2.1 *Reagents, chemicals, and standard solutions*

*trans*-ferulic acid (≥99%), p-coumaric acid (≥98%), gallic acid (>97%), vanillic acid (≥97%), caffeic acid (≥98%), vanillin (≥99%), kaempferol (≥90%), kaempferol-3-glucoside(≥90%), myricetin (≥96%), quercetin hydrate (≥95%), rutin hydrate (≥94%), quercetin-3-β-D-glucoside (≥90%), Cyclooxygenase-2 (COX-2) human (EC 1.14.99.1), arachidonic acid (≥98.5%), Tris–HCl, pyrogallol red, 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 97%), N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD, ≥98.5%), 6-hydroxy-2,5,7,8- tetramethylchromano-2-carboxylic acid (TROLOX, 97%), porcine hematin (≥90%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from Sigma-Aldrich (St. Louis MO, USA). Unless otherwise indicated all solvents were p.a. n-butanol, isopropanol, formic acid (98-100%), water (MS grade), acetonitrile (MS grade), methanol (MS grade), acetic acid (100%), sodium hydrogen carbonate ($NaHCO_3$, ≥99.7%), ammonia (25% v/v), sodium dihydrogen phosphate anhydrous ($NaH_2PO4$, ≥99.9%), ammonium formate (≥99%), disodium hydrogen phosphate ($Na_2HPO_4$ $H_2O$, ≥99%), potassium hydroxide (>85%), and Folin-Ciocalteu phenol reagent (2 M) were purchased from Merck (Darmstadt, Germany). Positive control diclofenac (OPKO, Lot # 220,803) was purchased at a local drugstore. Ultra-pure water (18.2 MΩ cm) was produced using a Purist system from Rephile (Shanghai, China). Filter paper Nº4 and polyvinylidene difluoride (PVDF) 13 mm syringe filters (0.45 μm) were obtained from Whatman (NJ, USA). Individual stock solutions of each (poly)phenol (20 mg $L^{-1}$) were prepared in methanol: water (4:1 v/v). Standard working solutions were prepared by diluting aliquots of the stock solutions. Each saccharide stock solution was prepared in water at a concentration of 0.1 mg $mL^{-1}$. The alliin stock solution (0.1 mg $mL^{-1}$) was prepared using a methanol-water mixture (1:1, v/v).

### 2.2 *Sample preparation*

Chinese (common garlic) and Chiloe's giant garlics were obtained in Concepcion city (36°49′38″S to 73°03′01″W) and Chiloé Island (42º40′36″S to 73º59′36″W) respectively, during the years 2015-2018. Chiloe's giant garlic samples were identified by Professor Dr. Pedro Aqueveque (botanist) and a voucher specimen (LMM-093) was incorporated in the Agroindustrial Department's Herbarium located in Campus Chillan of the University of Concepcion. For organosulfur compounds determination, garlic samples were blanched with hot water (*ca*. 90ºC) for 10 min into top-sealing plastic bags to inactivate the alliinase enzyme responsible for alliin metabolization to allicin. For Total Phenolic Content (TPC), (poly)phenols profile, and Antioxidant Capacity (AC) non-blanched samples were used. Two grams of blanched or non-blanched samples were extracted with 40 mL of methanol-water mixture (4:1 v/v) for 2 min at 9500 rpm using an IKA T25 Ultra-turrax homogenizer

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(Staufen, Germany). Filtered extracts (filter Nº4) were stored refrigerated and protected from light until analysis. Before liquid chromatography (LC) or high-performance thin-layer chromatography (HPTLC) analysis, extracts were filtered again using a 13 mm PVDF syringe filter (0.22 µm).

2.3 *Proximate analysis*

Moisture, ash, protein, crude fat, and crude fiber contents were measured employing the AOAC official methods [8]. The carbohydrate content was calculated as the difference obtained by subtracting all other macronutrients [9, 10]. All samples were analyzed in triplicate and the results were expressed as percentage of fresh weight (FW).

2.4 *Saccharides profile*

Saccharides were evaluated following the method reported by Aranda et al [11] with minor modifications. Briefly, 5-25 µL of extracts or standard (mannose, sucrose, fructose, glucose, xylose, maltose, galactose, arabinose, and raffinose) were applied on 10 x 10 cm HPTLC silica gel 60 plate (pretreated by immersion into 0.1 M $K_2HPO_4$ methanol-water solution (1:1 v/v) and dried at 120 °C for 30 min) using CAMAG (Muttenz, Switzerland) automated TLC sampler (ATS4) with the following settings for 10 tracks per plate: 8.0 mm band length, 8.8 mm track distance, 120 nL $s^{-1}$ application speed and first *x*- and *y*-axis application at 10 mm. Chromatography was carried out by multiple developments (three times) inside a twin trough chamber using 5 mL of acetonitrile + ultrapure water (4+1 v/v) up to a migration distance of 80 mm. Between developments, the plate was dried at 60 °C for 5 minutes using a CAMAG TLC plate heater. Post-chromatographic derivatization was performed *in situ* by plate immersion for 3 s (3 cm $s^{-1}$) using CAMAG Immersion device 3 into aniline–diphenylamine reagent (2.4 g diphenylamine, 4 g aniline, 20 mL $H_3PO_4$ and 200 mL methanol) and heating for 10 min at 110 °C. HPTLC plates were documented under UV/Vis lights employing a CAMAG Reprostar photodocumentation system. Quantification was performed by spectrophotodensitometry at 520 nm using a CAMAG Scanner 3 following previous approaches [12]. Data was acquired and analyzed with CAMAG WinCats 1.4.10 software.

2.5 *Fatty acid profile*

Lipids fraction was extracted applying the Bligh and Dyer method [13]. The methyl ester generation and analysis were performed according to the International Union of Pure and Applied Chemistry (IUPAC) method [14] numbers 2.301 and 2.302 modified by Aranda et al [15]. Briefly, fatty acids methyl esters (FAME) were formed by saponification with KOH/methanol (2 N) and then extracted with n-hexane *via* vortex agitation for 3 min. The supernatant was injected into Agilent (Palo Alto, CA, USA) 6890N gas chromatographic system equipped with 7683B autosampler and flame-ionization detector (FID). FAME separation was carried out on Agilent HP-88 column (100 m x 0.25 mm ID, 0.2 µm) using nitrogen as carrier and make-up gas at flow rates of 1 mL $min^{-1}$ and 60 mL $min^{-1}$, respectively. Analysis was performed using the following operational conditions: injector at 250ºC in split mode (1/50), oven temperature program: 50 °C for 1 min increased 25°C/min up to 175 °C, held 1 min and then 4 °C/min up to 230 °C held for 5 min. FID was set at 280°C with hydrogen and air flows of 40 and 450 mL $min^{-1}$, respectively. Data was acquired and analyzed with Agilent Chem Station Rev. A.10.01 software.

2.6 *Total (poly)phenols content (TPC) and (Poly)phenols profile*

TPC was measured by Folin-Ciocalteu method with slight modifications [16]. Briefly, samples were prepared as indicated in sample preparation section and properly diluted. On a 96-well microplate, 15 µL of sample or standard were mixed with 100 µL of sodium hydrogen carbonate (60 g $L^{-1}$) and 100 µL of Folin–Ciocalteu reagent (previously diluted 10-fold with ultrapure water). After 90 min of continuous agitation, the absorbance was measured at 725 nm into a BioTek (Winooski, VT, USA) Epoch 2 microplate reader. Standard calibration was established with gallic acid (0.02 to 0.30 mg $mL^{-1}$), and results were expressed as mg of gallic acid equivalents (GAE) per 100 g of FW sample. For (poly)phenols profile, samples were analyzed following the method reported by Carrasco-Sandoval et al [16, 17] using a Shimadzu Nexera X2 UHPLC system composed coupled to SPD-M20A diode array detector (DAD) and LCMS-8030 triple quadrupole (TQ) mass spectrometer with electrospray ionization source (ESI). Chromatography was carried out on Phenomenex (Torrance, CA, USA) Kinetex XDB-$C_{18}$ core-shell column (150 mm x 4.6 mm, S-5 µm) connected to Kinetex guard column, both set at 32°C, using a mobile phase composed of water (A) and acetonitrile (B), both MS grade and acidified with 0.1% v/v formic acid. The following gradient program was used at a flow rate of 0.5 mL $min^{-1}$: 0-17 min 20%-45% B; 17-18 min 45%-45% B (isocratic step); 18-20 min 45%-20% B; and 20-30 min 20%-20% B (column conditioning). Separation was completed in 15 min for a total running time of 30 min. UV-detection data was acquired from 200-400 nm and MS analysis was performed using the following settings: ESI (-) voltage of 4.5 kV, nebulizer gas ($N_2$) flow: 3.0 L $min^{-1}$, drying gas flow: 15 L $min^{-1}$, heat block temperature 400 °C and desolvation line temperature 250 °C. MS data were acquired in Full Scan (*m/z* 100-2000) and Selected Ion Monitoring (SIM) modes using the following *m/z* values: gallic acid ($[M-H]^-$, *m/z* 169); vanillin ($[M-H]^-$, *m/z* 151); caffeic acid ($[M-H]^-$, *m/z* 179); *trans*-ferulic acid ($[M-H]^-$, *m/z* 193); vanillic acid ($[M-H]^-$, *m/z* 167); *p*-coumaric acid ($[M-H]^-$, *m/z* 163); quercetin ($[M-H]^-$, *m/z* 301), quercetin 3-ß-D-glucoside ($[M-H]^-$,*m/z* 463); kaempferol ($[M-H]^-$, *m/z* 285); kaempferol 3-glucoside ($[M-H]^-$, *m/z* 447); and myricetin ($[M-H]^-$, *m/z* 317).

2.7 *Antioxidant capacity*

ORAC assay was carried out based on Aravena et al report [18]. Briefly, 15 µL of Trolox standard (50-500 uM) or sample were added into the 96-well microplate, then 20 µL of 64 µM pyrogallol red (PGR) and completed with 194 µL of phosphate buffer (75 mM, pH 7.4). This mixture was incubated at 37°C for 30 min and then 1 µL of AAPH solution (120 mM), previously incubated at 37°C, was added. Absorbance was measured at 540 nm every 30 s for 180 min using a BioTek (Winooski, VT, USA) Epoch 2 microplate reader set up with constant shaking. Absorbance values ($A/A_0$) were presented as a function of incubation time, and the area under the curve (AUC) was evaluated up to reach an $A/A_0$ ratio of 0.2. All experiments were performed in triplicate and reported as Trolox equivalent (TE) per 100 g of FW sample.

2.8 *Organosulfur compounds*

Organosulfur compounds were determined following the CAMAG application note Nº F06A [19]. Briefly, extracts (5-25 µL) and standard (1-10 µL) were sprayed-on 10 x 10 cm HPTLC silica gel 60 plates using ATS4 using the following settings for 10 tracks per plate: band length 8.0 mm, tracks distance 8.8 mm, application speed 120 nL $s^{-1}$ and first *x*- and *y*-axis application at 10.0 mm. Chromatography was performed inside a 10 x 10 cm twin trough chamber using a solvent mixture composed of n-butanol, ultrapure water, acetic acid, and formic acid (28:8:9:2 v/v/v/v) up to a migration distance of 60 mm. *In situ* post-chromatographic derivatization was performed by plate immersion for 3 s (3 cm $s^{-1}$) into a ninhydrin solution (0.6 g of ninhydrin dissolved in 190 mL of isopropanol with 10 mL of acetic acid) using the CAMAG Immersion Device 3. The plate was then heated for 10 min at 120 °C on a TLC plate heater. For analyte identification (selectivity), a non-derivatized plate section was used to elute the bands directly to the MS through the Advion (Ithaca, NY, USA) Plate Express TLC/MS as described in section 2.9. Detection was performed with CAMAG Scanner 3 in Vis-absorption mode at 490 nm with a slit dimension of 6 .0 mm x 0.1 mm and a scanning speed of 20 mm $s^{-1}$. Instruments control and data acquisition and processing were done with winCats 1.4.10 software.

2.9 *HPTLC-Bioassay-MS*

Proper volumes of Chiloe's giant garlic extracts were applied onto HPTLC silica gel 60 $F_{254}$ plates using the ATS4 with the following parameters for 8 tracks per plate: 8.0 mm band length, 8.8 mm track distance, 120 nL $s^{-1}$ application speed and first *x*- and *y*-axis application at 10.0 mm. Chromatographic separation was carried out using a mobile phase composed of isopropanol: n-butanol: water: ammonia (5.5:0.9:1.6:2 v/v/v/v) up to a migration distance of 70 mm. Positive controls were applied post-chromatographic development at 50 mm before the bioassay. The plate was divided in two sections, one for bioassay and another for MS analysis. DPPH assay was carried out using the method reported by Lopez et al [20], using caffeic acid as positive control. One HPTLC plate section was immersed at 3 cm $s^{-1}$ into 0.5 mM (0.1% w/v) DPPH methanol solution for 3 seconds using CAMAG Immersion Device 3. After 30 min incubation at room temperature in darkness, antioxidants were observed as colorless bands on purple background. COX-2 inhibition assay was performed using the method established by Oyarzun et al [21], using diclofenac as positive control. All solutions were applied using CAMAG Derivatizer. After 10 minutes of

incubation in a humidity chamber at 37°C inhibitory bands were observed as colorless bands on blue background. Based on retention factor ($R_F$) all bands of interest were located on the second HPTLC plate section (without bioassay reagents) and marked using a soft pencil inside of Spectroline (Melville, NY, USA) UV-cabinet. Bioactive molecules were identified in two steps, first, bands of interest were directly eluted and analyzed by MS; and second, bands were eluted or scraped off to a micro-vial for liquid chromatography diode array-MS analysis [22, 23]. For first step, bands were eluted by means of Advion Plate Express TLC/MS interface using methanol: 10 mM ammonium formate (19:1% v/v) as elution solvent at a flow rate of 0.1 mL $min^{-1}$ for 2 min to the electrospray ionization (ESI) source of Advion the ESI interface of Advion expression-L Compact MS, and analyzed applying the following conditions: capillary voltage (-2.5; 3.5 kV), nebulizing gas nitrogen ($N_2$) 3 L $min^{-1}$, drying gas flow ($N_2$) 10 L $min^{-1}$, DL temperature 200°C, and block temperature 250°C. Mass spectra were acquired in full scan mode (*m/z* 100-2000) applying positive and negative ionization. Plate background signals were subtracted for each analysis. Data was acquired through Advion Mass Express and processed by Data Express Software. For the second step, bands of interest were scrapped off and extracted with methanol: water (7:3 v/v) solution, filtered (0.22 µm), and injected into a Waters (Milford, MA, USA) Arc LC system composed of a Quaternary Solvent Manager-R pump, Sample Manager FTN-R autosampler, CH-30A column heater, UV detector coupled to Acquity QDa single-MS. Chromatography was carried out using a binary mobile phase composed of acidified (0.1% formic acid v/v) water (A) and acetonitrile (B) on Phenomenex (Torrance, CA, USA) Kinetex 3.5 µm XB-C18 column (150 mm x 4.6 mm, 100 Å) set at 30 °C following the method established by Liu et al [24]. MS analysis was carried out using the following settings: ESI (+) capillary voltage of 0.8 kV, cone voltage of 10 V, probe temperature of 600 °C, and source temperature of 100 °C. Full Scan mode (*m/z* 100–1200) was used for qualitative analysis. MS fragmentation was performed using the same chromatographic parameters using a Waters I-Class LC coupled to Synapt XS HDMS 32K mass spectrometer. Mass acquisition was set using MS$^e$ and continuum modes. Acquisition parameters were set as follows: ESI (+) capillary voltage of 0.8kV, cone voltage of 20V, scan speed 0.3 s on resolution mode. The collision energy was set at 6 V for the low energy and a ramp of 15-45 V for high energy. Lock mass solution of Leucine enkephalin was applied in intervals of 30 s. Before analysis, the mass analyzer and detector were calibrated (*m/z* 50-1200) with sodium formate solution. Data was acquired and processed by means of Waters Masslynx 4.2 software.

## 3. RESULTS AND DISCUSSION

### 3.1 *Proximate analysis*

In Chinese garlic, the values determined for moisture (72.21±0.14%,), protein (5.20±0.10%,); crude fat (0.49±0.14%,), ashes (1.20±0.01%,), crude fiber (12.01±0.35%) and carbohydrates (8.59±0.47%) are in agree with those reported by Odebunmi et al Odebunmi, Oluwaniyi and Bashiru [25] (66.57, 7.87, 0.52 and 1.33%, respectively), except for crude fiber and carbohydrates values (0.73 and 22.98%, respectively). Chiloe's giant garlic showed a moisture (62.34±0.35%), protein (2.80±0.10%,); crude fat (0.09±0.00%,), ashes (0.74±0.02%,), crude fiber (13.04±0.01%) and total carbohydrates (20.64±0.01%) content comparable to those reported by Garcia-Herrera et al García-Herrera, Morales, Fernández-Ruiz, Sánchez-Mata, Cámara, Carvalho, Ferreira, Pardo-de-Santayana, Molina and Tardio [26] for wild populations of *A. ampeloprasum* (78.32%; 1.67%; 0.18%; 0.79%; 4.23% and 16.60%, respectively). Regarding Chiloe's giant and Chinese garlics, all major constituents showed significant differences (Table 1). These differences can be associated with the species, as well as with cultivation areas.

**Table 1.** Comparative values of Chiloe`s giant garlic and Chinese garlic constituents

| **Parameter** | **Giant garlic** | **Chinese garlic** | ***p*** |
|---|---|---|---|
| **Proximate analysis** | (% DW) | (% DW) | |
| Protein | 7.43±0.39 | 18.71±0.39 | <0.0001 |
| Crude fat | 0.24±0.01 | 1.76±0.08 | <0.0001 |
| Ash | 1.96±0.04 | 4.32±0.03 | <0.0001 |
| Fiber | 34.63±0.02 | 43.22±1.26 | 0.0003 |
| Carbohydrate | 54.81±0.33 | 30.91±1.70 | <0.0001 |
| **Saccharides** | (mg $g^{-1}$ FW) | (mg $g^{-1}$ FW) | |
| Sucrose | 5.92±0.20 | 11.96 ±0.01 | <0.0001 |
| Glucose | 0.11±0.00 | 0.49±0.00 | <0.0001 |
| Fructose | 0.46±0.01 | 0.51±0.01 | 0.0036 |
| **Organosulfur compounds** | (mg AE $g^{-1}$ FW) | (mg AE $g^{-1}$ FW) | |
| Alliin | 2.66±0.90 | 10.31±1.24 | 0.0010 |
| Isoalliin | 5.02±1.24 | 8.29±0.74 | 0.0172 |
| Methiin | 9.61±3.33 | 13.61±2.02 | 0.1499 |

Results are expressed as mean ± standard deviation (*n*=3); ND: not detected (<LOD); DW: dry weight; FW: fresh weight; AE: Alliin equivalents

### 3.2. *Saccharides profile*

The main saccharides in both types of garlic were sucrose, glucose, and fructose (Table 1); the presence of mannose, maltose, xylose, arabinose, raffinose, and galactose was discarded. Sucrose was the highest content observed in both types of garlics (5.92±0.20 mg $g^{-1}$ FW in Chiloe's giant garlic and 11.96±0.01 mg $g^{-1}$ FW in Chinese garlic). These values are higher than those reported by Ceccanti, Rocchetti, Lucini, Giuberti, Landi, Biagiotti and Guidi [27] for sucrose (1.1±0.4 and 1.1±0.3 mg $g^{-1}$ dry weight (DW)) in elephant (*A. ampeloprasum* var. *holmense*) and Chinese garlics, respectively. In particular, for Chiloe's giant garlic, glucose (0.11±0.00 mg $g^{-1}$ FW), fructose (0.46±0.01 mg $g^{-1}$ FW) and sucrose (5.92±0.20 mg $g^{-1}$ FW) were lower than the values reported by Loppi, Fedeli, Canali, Guarnieri, Biagiotti and Vannini [28] (8.1±0.3; 15.3±0.1 and 6.5±0.2 mg $g^{-1}$ FW, respectively) for elephant garlic cultivated in Italy. These results could be explained by different varieties and cultivation zones.

### 3.3 *Fatty acid profile*

As described in table 2, the most important fatty acids detected in Chiloe's giant garlic were linoleic acid (57.7%), palmitic acid (23.5%), oleic acid (7.2%) and α-linolenic acid (5.1%). No data are currently available about Chiloe's giant garlic fatty acids profile, however, compared with wild population of wild *A. ampeloprasum,* it was observed the same profile except for the absence of α-linolenic acid in wild types [26]. With regards to Chinese garlic, the reports indicate that the main fatty acids present are palmitic, oleic, linoleic, and linolenic acid [29], which is concordant with the present results.

**Table 2.** Fatty acids profile of Chiloe`s giant garlic

| Cn:m | | Fatty acid | % (RSD<1%) |
|---|---|---|---|
| C13:0 | - | Tridecanoic acid | 0.92 |
| C14:0 | - | Tetradecanoic acid | 0.14 |
| C15:0 | - | Pentadecanoic acid | 0.17 |
| C16:0 | - | Hexadecanoic acid | 23.46 |
| C17:0 | - | Heptadecanoic acid | 0.24 |
| C18:0 | - | Octadecanoic acid | 0.62 |
| C22:0 | - | Docosanoic acid | 0.66 |
| C23:0 | - | Tricosanoic acid | 0.54 |
| C24:0 | - | Tretacosanoic acid | 0.61 |
| C23:0 | - | Tricosanoic acid | 0.54 |
| C15:1 | - | Pentadecaenoic acid | 0.27 |
| C16:1 | - | Hexadecaenoic acid | 0.58 |
| C17:1 | - | Heptadecaenoic acid | 0.21 |
| C18:1 | 9 | Octadecaenoic acid (*cis*) | 7.20 |
| C18:2 | 6 | Octadecadienoic acid (*cis*) | 57.67 |
| C18:3 | 6 | Octadecatrienoic acid | 0.21 |
| C18:3 | 3 | Octadecatrienoic acid | 5.06 |
| C20:1 | 9 | Eicosenoic acid | 0.46 |
| C18:2 | 6 | Octadecadienoic acid (*trans*) | 1.41 |

* Results are expressed as the mean ($n$=3). RSD: relative standard deviation.

### 3.4 *(Poly)phenols profile*

From the thirteen (poly)phenols evaluated, i.e., gallic acid, vanillic acid, caffeic acid, *trans*-ferulic acid, *p*-coumaric acid, myricetin, kaempferol, kaempferol-3-glucoside, quercetin, quercetin-3-β-D-glucoside, rutin, and vanillin, only caffeic acid with an average concentration of 0.57±0.05 μg $g^{-1}$ FW and traces of rutin were found in Chiloe's giant garlic. This profile is different than those reported by Emir, Coban and Emir [30] for elephant garlic from Turkey, who described the presence of several hydroxybenzoic acids like 3-hydroxybenzoic acid and gallic acid and some flavonoids such as flavones (fisetin) and flavonols (quercetin and kaempferol). In Chinese garlic, none of the studied (poly)phenols were observed. Although Beato, Orgaz, Mansilla and Montaño [31], reported the presence of caffeic, vanillic, p-hydroxybenzoic, and *p*-coumaric acids, the inexistence or non-detected levels could be associated with cultivation location/conditions.

### 3.5 *Organosulfur compounds*

Alliin, isoalliin, and methiin were measured by HPTLC/Vis/MS based on compounds' migration distance and mass spectra. In agree with previous reports [32, 33], the observed *m/z* values, i.e., 178.15 (200.15, 216.15, 241.15), 178.15 (200.15, 216.15, 241.15) and 152.20 (174.2), correspond to the protonated molecule $[M+H]^+$ of alliin ($[M+Na]^+$, $[M+K]^+$, $[M+ACN+Na]^+$); isoalliin ($[M+Na]^+$, $[M+K]^+$, $[M+ACN+Na]^+$) and methiin ($[M+Na]^+$), respectively. Chinese garlic showed an average alliin concentration of 10.31±1.24 mg $g^{-1}$ FW, which is in the same range as those reported by other authors, e.g. Siddiqui, Mothana and Alam [34] (24.57 mg $g^{-1}$ DW); Krest and Keusgen [35] (6.20 mg $g^{-1}$ FW); and Kim, Park, Lee, Lee, Ko and Yoo [33] (16-18 mg $g^{-1}$ DW). In Chiloe's giant garlic the alliin, isoalliin, and methiin values (2.66±0.90; 5.02±1.24 and 9.61±3.33 mg $g^{-1}$ FW) were higher than those reported by Kim, Kim, Jin, Park, Yoon, Lee, Kim, Lee, Kim, Lee, Shin, and Yoo [36] (2.61±0.19; 0.18±0.02 and 1.27±0.01 mg $g^{-1}$ DW) and Ferioli, Giambanelli and D'Antuono [37] (2.89; 0.71; and 0.89 mg $g^{-1}$ FW) for elephant garlic.

### 3.6 *Antioxidant Capacity*

Chiloe's giant garlic showed ORAC values (0.250±0.001 mmol TE per 100 $g^{-1}$ FW or 62 μM TE $g^{-1}$ FW) slightly lower than those found in Chinese garlic (0.265±0.004 mmol TE per 100 $g^{-1}$ FW or 66 μM TE $g^{-1}$ FW; $p$=0.0032; F=0.1176). These values are lower but in the same magnitude than those reported by Kim, Kim, Jin, Park, Yoon, Lee, Kim, Lee, Kim, Lee, Shin and Yoo [36] (500 and 400 μM TE $g^{-1}$ DW, respectively), which is concordant with the low TPC values found in Chiloe's giant garlic (see 3.4).

### 3.7 *Total (poly)phenols content*

Chinese garlic showed a higher TPC value (74.56±4.98 mg GAE per 100 g FW) than the one reported by Fei, Tong, Wei and De Yang [38] (84.00±3.14 mg GAE per 100 DW) and Nuutila, Puupponen-Pimiä, Aarni and Oksman-Caldentey [39] (7.5-11.5 mg GAE per 100 g DW); and a much lower level than the ones informed by Ciric, Krajnc, Heath and Ogrinc [40] for Slovenian (1444±3 mg GAE per 100 FW) and Spanish (2644±6 mg GAE per 100 g FW) garlic as well as for several ($n$=43) Chinese cultivars (1716 to 4253 mg GAE per 100 g FW)[41]. Since there are no reports about TPC in Chiloe's giant garlic, the values calculated in the present work were compared with those described for elephant garlic (*A. ampeloprasum* var. *holmense*). TPC value of Chiloe's giant garlic (40.66±2.08 mg GAE per 100 g FW) was lower than the ones reported by Ceccanti, Rocchetti, Lucini, Giuberti, Landi, Biagiotti and Guidi [27] (*ca.* 1400 mg GAE per 100 g FW), and by Loppi, Fedeli, Canali, Guarnieri, Biagiotti and Vannini [28] (300±3 mg GAE/100 g FW). Comparatively, Chinese garlic showed significantly higher TPC values than Chiloe's giant garlic, 74.56±4.98 vs 40.66±2.08 mg GAE per 100 g FW ($p$=0.0004; F=0.2971).

### 3.8 *HPTLC-Bioassay fingerprint*

Chiloe's giant garlic extracts presented three antioxidant bands at $R_F$ (0.47; .0.55; 0.63) and 2 bands with COX-2 inhibition activity at $R_F$ (0.47; 0.55) for a total of 3 bands of interest (Figure 1). First band ($R_F$ 0.47) analyzed by LC-MS showed two compounds with *m/z* of 291 (Figure 2A), which according to the retention time, parent ion and mass fragmentation were identified as γ-glutamyl-S-allyl-L-cysteine (GSAC) and γ-glutamyl-S-(*trans*-1-propenyl)-L- cysteine (GSPC) [21, 24, 42]. The second band ($R_F$ 0.55) corresponds to bioactive sulfoxides compounds alliin and isoalliin, showing the same *m/z* 178 but slight differences in retention time (5.021 and 5.254 min, Figure 2B). These compounds are well-known antioxidants [43] and *in-silico* evidence shows that several compounds of the *Allium* family have COX-2 inhibition capacity where alliin displayed an extraordinary potential with better binding free energy than celecoxib [44]. The last band ($R_F$ 0.63) corresponds to two essentials amino acids, tryptophan (*m/z* 205; Figure 2C) and phenylalanine (*m/z* 166), both showed antioxidant capacity, but the first possess the highest antioxidant capacity amongst the 20 alpha amino acids [45].

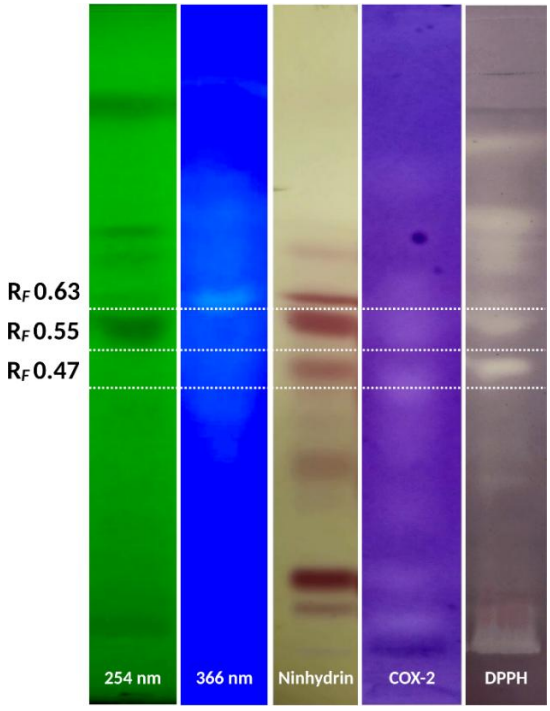


**Figure 1.** HPTLC Chromatogram of Chiloe's giant garlic visualized under UV 254 nm, UV 366 nm, Ninhydrin assay, COX-2 inhibition assay, and DPPH scavenging assay showing the three bands of interest at $R_F$ 0.47, 0.55 and 0.63

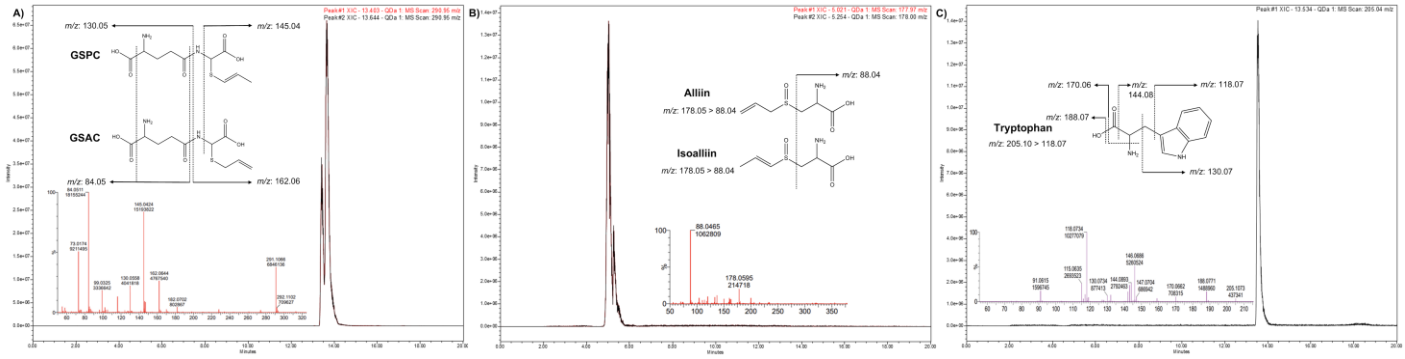


**Figure 2.** Mass chromatogram and mass spectra of bioactive molecules present in Chiloe's giant garlic detected by HPTLC-Bioassay. Band at $R_F$ 0.47 contains two compounds: GSAC and GSPC (A). Band at $R_F$ 0.55 contains alliin and isoalliin (B) and band at $R_F$ 0.63 showed the presence of tryptophan (C).

## CONCLUSION

To the best of our knowledge, for the first time, the chemical and functional profile of Chiloe's giant garlic is reported. Proximate analysis showed significant differences with Chinese garlic in all macro constituents, the same difference was observed for organosulfur compounds. Another interesting finding was the presence of caffeic acid in Chiloe's giant garlic, while in Chinese garlic none of the (poly)phenols studied were detected. This result is contradictory with TPC values because Chinese garlic showed almost 2-fold higher TPC values than Chiloe's giant garlic. Regarding functionality, alliin and isoallin and the precursors, GSAC and GSPC, showed both antioxidant and anti-inflammatory activities. Overall, it can be concluded that Chiloe's giant garlic has very attractive functional properties which demonstrate its classification as functional food.

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