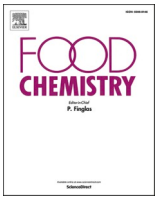
Food Chemistry 439 (2024) 138089

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



White Acqualagna truffle (*Tuber magnatum* Pico): Evaluation of volatile and non-volatile profiles by GC-MS, sensory analyses and elemental composition by ICP-MS

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ARTICLE INFO

Keywords:
Acqualagna *Tuber magnatum*
GC–MS
Sensory analysis
Elemental analysis
ICP-MS

ABSTRACT

The White Truffle is the most expensive edible underground mushroom. In this study the first characterization of the Acqualagna white truffle was delivered, taking into consideration the soil of origin and the human perception. The volatile profile was identified by GC–MS and compared with the descriptors obtained by sensory analysis. The non-volatile characterization was done using elemental composition by ICP-MS analysis, elemental analysis, and spectrophotometric assays. The volatile profile consists mainly of bis(methylthio)methane (78.72%) and other minor constituents, linked to seven odorant descriptors: garlic-like, nutty-like, geosmine-like, floral, mushroom-like, pungent and green/herbal. ICP-MS revealed that truffle has a higher content of K, P, S, Ca and Mg (97% of the elements investigated) and that it assimilates the Rare Earth Elements (REE) from the soil without discriminating them. In conclusion, this project is the first step for the enhancement of local food, linked to the territory conditions in which it is produced.

1. Introduction

The truffle, known by the Babylonians and a source of legends and mystery even in the times of ancient Rome, today is a mushroom of great value. Truffle belongs to the Tuberaceae family and Pezizales order, furthermore, only those of the genus *Tuber* are considered true truffles. The word truffle is believed to have originated from the latin word "tubera", plural of tuber, which means swelling, lump or hump due to the shape of the truffle (Mustafa et al., 2020). Truffles are hypogeous ascomyceteous fungi that grow all over the world but in certain European regions, such as Italy and France, they are particularly important. Truffles are generally globose consisting of an outer protective tissue, the peridium, and an inner mass known as gleba. The fertile part area is rich in spores, used for reproduction (Pacioni et al., 2010). In recent years, the economic value of truffles has grown significantly due to growing demand from consumers for innovative and refined food flavours. The truffle emerges in the food market of many countries for its peculiar properties, it has in fact a wonderful taste and smell resulting from numerous volatile compounds. One of the main reasons for the growth in truffle value is also the local interest in the rural economy therefore it can help to preserve the rural landscape and biodiversity as well as to use the land in a sustainable way. Among the truffles with the highest economic and commercial value, there are four types that occurs also in Europe: the *Tuber melanosporum* Vitt. (Périgord black truffle), the *Tuber aestivum* Vitt. (summer or Burgundy truffle), the *Tuber borchii* Vitt. (bianchetto truffle) and the *Tuber magnatum* Pico (Italian white truffle) (Zambonelli, Iotti & Hall, 2015). It is known that the environment in which the truffle grows influences its characteristics. Several abiotic (rainfall and temperature, mycelial connectivity, soil properties, and microclimatology) and biotic (fungi, yeasts, bacteria, mesofauna, plant host) factors could influence truffle life and the formation of ascocarp (Ceruti, Fontana & Nosenzo, 2003). In particular, the soil has a big impact on truffles and it is studied for their authentication. Due to the growing interest in this fungus, different studies have been performed to characterize different truffles from various areas of the world (Al-Laith, 2010; Hamza et al., 2016). The truffle has a central role in the development of rural communities, in particular because it directly affects the agricultural sector and consequently the development of the territory

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https://doi.org/10.1016/j.foodchem.2023.138089
Received 19 September 2023; Received in revised form 23 November 2023; Accepted 25 November 2023
Available online 6 December 2023

and cities for economic reasons (Zambonelli, Iotti & Hall, 2015). Due to its unique taste, it is an ingredient in numerous delicious dishes all over the world (Mustafa et al., 2020). In recent years some studies have characterized the volatile compounds mainly using the headspace solid-phase microextraction technique (Costa et al., 2015; Gioacchini et al., 2008; Torregiani et al., 2017). However, few articles have undertaken sensory analyses on white truffle samples correlating the human's perception with the molecules identified using analytical techniques (Schmidberger & Schieberle, 2017), and even fewer authors have studied the relationship between the truffle and the soil it comes from (Segelke et al., 2020). In Italy it is particularly known the *T. magnatum*, due to its high gastronomical potential. White Acqualagna Truffle (*T. magnatum*) represents an excellence among all white truffles. In fact, being considered the most valuable variety of truffle, depending on the year and the size, it can also reach the quotation of thousands of euros per kilogram (Eusebi et al., 2015). To date, there are no characterization studies in the literature on the Acqualagna white truffle, and since the truffle is an increasingly used food and consumers are more and more interested in having information on their diet, it is crucial to gather as much knowledge as possible about this food. Our work represents the first characterization of volatile and non-volatile profile of White Acqualagna Truffle using several techniques. All the studies were carried out with the aim of attributing an identity to a truffle with a specific geographical origin, the country of Acqualagna, and to correlate the chemical composition of this truffle with what the consumer perceives. The aroma profile of fresh truffles was performed using gas chromatography-mass spectrometry system (GC–MS) and was compared with the sensorial analysis. The inductively coupled plasma mass spectrometry (ICP-MS) was carried out to obtain an elemental mapping in truffles and also to study the soil where truffles grew. This allows us to evaluate the assimilation and accumulation abilities of this truffle. Elemental analysis was also realized for the quantification of elements (H, C, N, S). Finally, the antioxidant activity was evaluated by measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH), total phenolic content (TPC) and total flavonoid content (TFC). In conclusion, this is a pioneer work realized on these matrices using numerous techniques to give more importance to a local resource with global impact.

2. Materials and methods

2.1. Samples

Intact samples of *T. magnatum* Pico (kingdom: Fungi, division: Ascomycota, class: Pezizomycetes, order: Pezizales, family: Tuberacae, genus: *Tuber*, species: *Tuber magnatum*)(Leonardi et al., 2021) were harvested in the hilly areas of Acqualagna city (43° 37′ 7′' North, 12° 40′ 22′' East), a small city in Central Italy (Fig. S1), with the collaboration of T&C S.r.L. a private company. In particular, to guarantee the species and origin of the samples, the fresh truffles were purchased directly from the harvesting locations selected by an expert truffle trader from T&C S.r.L. The highest possible level of authenticity and the specification of the species was based on the morphology of the carpophore, the shape of the spores, and the structure of the peridium and gleba, with reference to data previously recorded by Mycological Maletti Herbarium (Associazione Micologica Bresadola, Pesaro (PU), Italy). The study was carried out on a total of 27 full-maturity-stage samples, 9 of which were collected every 15 days between October and November 2022, according to Italian rules (Legge n. 752, 16 Dicembre 1985) that provide for legal harvesting and marketing deadlines. Fresh samples, manually cleaned and brushed with a soft bristle to brush away any dirt or debrids, were put in polypropylene (PP) vessels containing an absorbent clean pad. Each vessel contained approximately 10 g of fresh intact truffles.

2.2. Chemicals and reagents

Folin–Ciocalteu (FC), sodium carbonate anhydrous (≥99%, Na_2CO_3, molecular weight 105.99, Cas No 497-19-8), gallic acid (analytical standard, ≥97.5-102.5%, $C_7H_6O_5$, molecular weight 170.12, Cas No 149–91-7), sodium nitrite (≥ 99.0%, $NaNO_2$, molecular weight 69.00, Cas No 7632-00-0), aluminum chloride (≥99%, $AlCl_3$, molecular weight 133.34, Cas No 7446-70-0), sodium hydroxide (≥98%, NaOH, molecular weight 40.00, Cas No 1310–73-2), rutin (analytical standard, ≥95.0% (HPLC), $C_{27}H_{30}O_{16}$, molecular weight 610.52, Cas No 153-18-4), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (≥97.0%, $C_{14}H_{18}O_4$, molecular weight 250.29, Cas No 53188–07-1), 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$, molecular weight 394.32, Cas No 1898–66-4), ethanol (96%,C_2H_6O, molecular weight 46.07, Cas No 64-17-5), ascorbic acid (analytical standard 99%, $C_6H_8O_6$, molecular weight 176.12, Cas No 50-81-7), 3-methylbutanal, (≥95.0%, $C_5H_{10}O$, molecular weight 86.13, Cas No 590–86-3), bis(methylthio)methane, (≥99.0%, $C_3H_8S_2$, molecular weight 108.23, Cas No 1618–26-4), dimethyl sulfide, (≥99.0%, $(CH_3)_2S$, molecular weight 62.13, Cas No 75–18-3), 3-(methylthio)propanal (≥97.0%, C_4H_8OS, molecular weight 104.17, Cas No 3268–49-3), (E,E)-2,4-nonadienal, (≥89.0%, $C_9H_{14}O$, molecular weight 138.21, Cas No 5910–87-2), and 1-octen-3-one (50 wt %, $C_8H_{14}O$, Cas No 4312–99-6)were purchased from Merck (Milan, Italy). Hydrogen peroxide (30–32% Ultrapure) and nitric acid (65–69% Ultrapure) were purchased from Carlo Erba Reagents Srl (Cornaredo, Milan, Italy).

2.3. Aroma characterization by GC–MS

2.3.1. Sample preparation

White truffles (1 g) from Acqualagna were finely ground using a truffle slicer (approximately 0.2 mm in thickness). These small slices of the truffle were put in the vial (20 mL) and closed with a screw cap equipped with a PTFE- silicon septum. The headspace solid phase microextraction gas chromatography/mass spectrometry (HS-SPME-GC–MS) was used to characterize the volatile profile of fresh white truffles using a previous publish method (Torregiani et al., 2017) with some modification.

2.3.2. Headspace solid-phase microextraction (HS-SPME)

Each vial was heated under stirring at around 250 rpm, the temperature was 60 °C, for 15 min in a heating platform. An AgilentChem workstation was used for the GC–MS system. After this time, the fiber DVB/PDMS/CWR/PDMS, (Agilent Technologies, Santa Clara, California, USA), 80 μm (50//30 μm) thickness was exposed to the headspace of the sample for 15 min. Once the extraction time had ended, the fiber was removed from the vial and placed in the injection port of the gas chromatograph for the rapid desorption of the analytes. A desorption time of 15 min, with an injection temperature of 250 °C, was sufficient to desorb most of the analytes from the fiber. After desorption from the fiber the headspace of the sample went in contact with the stationary phase in the column. The fiber was cleaned before each microextraction to prevent contamination using a blank GC–MS run with the same conditions mentioned above.

2.3.3. GC–MS analysis

A gas chromatograph and mass selective detector were used in combination to study the volatile profile of Acqualagna truffles. In particular, it was used an Agilent 8890 gas chromatograph (GC) coupled with a 5977B mass spectrometer (MSD) from Agilent (Santa Clara, California, USA). The system was also constituted of a PAL RTC 120 autosampler (Switzerland). The separation was performed by HP-5 MS capillary column (30 m l. × 0.25 mm i.d., 0.1 μm f.t., Agilent), supplied by Agilent (Santa Clara, California, USA) and coated with 5% phenylmethylpolysiloxane. The carrier gas was He (99.999%) flowing at 3 mL min^{-1} in splitless mode. The oven was thermostatted at 35 °C (5 min) then ramp went to 60 °C, 3 °C min^{-1}, up to 70 °C, 1 °C min^{-1} and 200 °C at 5 °C min^{-1}, hold 2 min, finally the temperature arrives 300 °C at 15 °C min^{-1} and remain 5 min. The mass spectra were acquired in full

scan in the range 40/400 uma and the mass spectrometer used the electron impact (EI) mode with an ionization voltage of 70 eV to produce the spectra of the separated compounds. Peak assignment of the chromatograph was based on the computer matching of the mass spectra with the WILEY275 and NIST 08 using a matching quality of over 60%, published literature, and Kovats retention indices (RI) which were calculated based on *n*-alkane (C_7–C_{30}) series (Sigma Aldrich) under the same chromatographic condition. The relative percentage content of each compound was determined using a peak area normalization procedure based on the total ion flow chromatogram and consequentially expressed as a relative percentage (%) by calculating the ratio of each individual peak area to the sum of the peak areas of all target compounds. Only peaks with area percentage > 0.01 were considered and analyzed by comparing relative peak areas.

2.4. Sensory analysis

To establish how the olfactory characteristics of white truffle from Acqualagna are perceived by the consumer a study of sensory analysis was realized. Identification and selection of descriptors for establishing a sensory profile by quantitative descriptive analysis of white Acqualagna truffle were estimated as the protocols defined in ISO 11035:1994 (Sensory analysis – identification and selection of descriptors for establishing a sensory profile by a multidimensional approach). The room where the sensory analyses were carried out must conform to ISO 8589:2007, in particular for: lighting, temperature, noise and odours. Individual booths should be set up, so that the assessors can work on their own without distraction (modular mobile units can be used). The samples were prepared as follows: about 10 g of each truffle sample was put into the glass goblet (one for each assessor), which is then covered with a plastic lid to preserve the characteristic volatile profile of the truffle. The panel was constructed following ISO 8586:2012 (Sensory analysis – General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors) and consisted of 16 assessors. The panel leader was responsible for coordinating the trials, collecting and processing the results and overseeing the selection, training and monitoring of the performance of all the members of the panel. The panel participated in three sensory sessions of 45 min to train their ability to recognize and describe different aroma attributes in according to ISO 8586:2012. After the training sessions the panelists ultimately came to a final agreement regarding truffle's aroma description. (sulfury or garlic-like, cabbage-like, malty, cooked potato-like, fatty or green and mushroom-like). The following reference compounds were used to prepare the judges: bis(methylthio)methane for sulfury or garlic-like note, dimethyl sulfide for cabbage-like note, 3-methylbutanal for malty note, 3-(methylthio)propanal for cooked potato-like note, (E,E)-2,4-nonadienal for fatty or green note, and finally 1-octen-3-one for mushroom-like note (Feng et al., 2019; Schmidberger & Schieberle, 2017). The single judgment of each panelist was evaluated. Judges were required to generate the maximum number of descriptors defining all the olfactory sensations produced by truffles and to write them down in the appropriate form provided for all the tests. In this phase, no aromatic aspect of the product was overlooked and any distortion due to the influence of an individual in the group was avoided. Secondly, the judges discussed as a group and compare their perceptions under the guidance of the panel leader. This encouraged them to analyze the different components of product perception and to link it to a precise descriptor. All descriptive terms were then collected, including synonyms. In according to ISO 11035:1994, during the group discussions and in the presence of the samples, the panel leader proceeded with the selection of the descriptors. Hedonistic, quantitative, irrelevant words, that can describe the product in their own terms, were eliminated from the discussion. Finally, were identified several descriptors that can be used in the sensory analysis of the product (ISO 11035:1994).

2.5. Sample preparation for non-volatile profile

The samples of truffle used for spectrophotometric tests, elemental analysis and ICP-MS, were obtained in the same way. As described in section 2.1, we took 9 samples every 15 days. Each of these groups was divided into 3 distinct groups of 3 samples. The truffles of each group were carefully sliced (with a thickness of approximately 2 mm) and grounded by using a mortar with liquid nitrogen. For elemental analysis and ICP-MS a part of each blend of truffles obtained was freeze-dried at − 54 °C and 0.05 mbar, through a BUCHI Lyovapor™ L-200 freeze-dryer (Büchi Labortechnik AG, Flawil, Switzerland). The lyophilized samples were ground in a mortar and the obtained powders were maintained at 4 °C until further analyses.

2.6. Phenolic profile and antioxidant activity

2.6.1. Preparation of extracts

Extraction of flavonoids and phenolic compounds were carried out as follows: 1 g of the ground fresh truffles was extracted with 10 mL of ethanol 70% (drug-solvent ratio 1:10), as described by Sezer et al., 2017. Extractions were performed in screw-capped tubes, at room temperature in an ultrasound water bath at 40 kHz (FALC, Treviglio, Italy) for 20 min. The samples were centrifuged at 5000 rpm for 10 min using an IEC CL10 Centrifuge (Thermo Fisher Scientific, Waltham, USA). The supernatant was used for spectrophotometric assays and each trial was performed in triplicate.

2.6.2. Determination of the total phenolic content

The TPC of the extract was determined by the Folin-Ciocalteu method reported by Giusti et al., 2018 with slight variations. Briefly, 0.5 mL of the extract or gallic acid was mixed with 2.5 mL of 0.1 M Folin-Ciocalteu reagent in a polypropylene conical tube and after 5 min of incubation in the dark at room temperature was added 7 mL sodium carbonate (Na_2CO_3) solution 7.5%. The mixture was allowed to stand for 2 h in the dark, and absorbance was measured at 765 nm with an Agilent Technologies (Cary 8454 UV–Vis, Woburn, Massachusetts, USA) spectrophotometer, using the respective solvent as blank. Results were calculated by comparing the absorbance of samples with the standard calibration curve of gallic acid. TPCs were expressed as milligrams of gallic acid equivalents (GAE)/g dry weight (DW).

2.6.3. Determination of the total flavonoid content

Total Flavonoid Content (TFC) was determined following a method described by Laurita et al., 2021. Briefly, 0.5 mL of extract solution, 0.15 mL of $NaNO_2$ (0.5 M), 3.2 mL of methanol (30% v/v) were mixed. After 5 min 0.15 mL of $AlCl_3{\cdot}6H_2O$ (0.3 M) and after other 5 min, 1 mL of NaOH (1 M) were added. The solution was mixed well and was incubated for 30 min in the dark at room temperature. The absorbance was measured, against the blank reagent, at 506 nm. The standard calibration curve for TFC was made using rutin standard solution under the same procedure as described above. TFC was expressed as mg of rutin equivalents (RT)/g dry weight (DW).

2.6.4. Radical scavenging activity assay

The antioxidant activity was determined using the DPPH method. Free radical scavenging activity of the extracts against radical 2,2-diphenyl-1-picrydrazyl (DPPH) was estimated spectrophotometrically as described by Giusti et al., 2017 with some modifications. According to the procedure, 0.5 mL of extract solution or standard (ascorbic acid) or blank (ethanol) was mixed with 4.5 mL of ethanolic solution of DPPH (0.1 mM). DPPH stock solution was prepared by dissolving 3.95 mg DPPH in 100 mL ethanol and kept at 4 °C, protected from light. After 30 min of incubation in the dark at room temperature, the DPPH disappearance was measured spectrophotometrically at 517 nm using (Agilent Technologies, Cary 8454 UV–Vis, Woburn, Massachusetts, USA) spectrophotometer. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-

Table 1
Digestion program used to mineralize the truffle fruits.

Steps	1	2	3
Temperature (°C)	150	190	50
Pressure (Bar)	36	36	0
Power (%)	70	90	0
*Ramp (*min*)*	5	5	1
*Step time(*min*)*	10	20	20

carboxylic acid) was used as the reference antioxidant for the calibration curve and the results were expressed as mg Trolox equivalent (TE)/kg dry weight (DW).

2.7. Elemental analysis

A ThermoFisher Scientific™ FLASH 2000 CHNS/O Analyzers (Thermo Fisher Scientific, Waltham, USA) was used to detect and quantify basic elements, such as C, H, N and S in Acqualagna truffles. The freeze-dried sample was ground, weighed and packed carefully in a tin capsule with an oxidizer (vanadium pentoxide) to be introduced into the instrument in solid form. Calibration samples were prepared by using BBTO (2,5-Bis(5-*tert*-butyl-benzoxazol-2-yl)thiophene). The truffle is heated and combusted in a furnace at 950 °C with a constant flow of helium stream 140 mL/min in a temporarily enriched oxygen atmosphere (flow oxigen 250 mL/min) occurred the combustion and were generated four reduced components: N_2, CO_2, H_2O and SO_2. These was separated in a chromatographic column and detected by a detector. Data were processed with specific software.

2.8. Quantitative elemental analysis by ICP-MS

The freeze-dried truffle was previously mineralized, by acid digestion, using a Berghof speedwave 4 microwave mineralizer (Berghof, Eningen, Germany). The acid digestion was performed in Teflon vessels using 4 mL 30%–32% H_2O_2, 1 mL 65% −69% HNO_3, 0.05 g of freeze-dried truffles powder and 50 μL of a solution containing 2 mg/l of Au, Be and Ru added as recovery standard. The latter solution was appropriately prepared from the single-element standard solutions (1 g/L, ICP-MS grade, Fluka Analytical, Merck, Darmstadt, Germania). The digestion program follows three steps indicated in the Table 1. The mineralized sample was then transferred to a plastic tube and then diluted 1:10 with ultrapure water (resistivity 18.2 MΩ cm) produced by Millipore Milli-Q system (Millipore, Molsheim, France). In addition, the soil, where the truffles have been collected, was dried in oven at 100 °C for 24 h, diluted in a 1:5 ratios with ultrapure water, stirred for 24 h, and finally allowed to settle for 2 h. The obtained supernatant (release water) was filtered by PTFE-0.2 μm filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany). Both, the mineralized truffle solutions and the release water samples were characterized by an ICP-MS 7500cx series (Agilent Technologies, Santa Clara, CA, USA). The ICP-MS instrumentation operated under the following conditions: power 1550 W, carrier gas 0.9 L/min, make-up gas 0.00 L/min, sample depth 7 mm, nebulizer pump 0.1 r.p.s. and spray chamber temperature 2 °C. The 7500cx series can operate in NoGas/He mode, in order to overcome most of the polyatomic interference by the collision cell. A solution containing ^{45}Sc, ^{115}In, ^{140}Ce and ^{209}Bi (10 mg/l) was properly prepared from the single-element standard solutions (1 g/L, ICP-MS grade, Fluka Analytical, Merck, Darmstadt, Germania) and used as the internal standard for ICP-MS measurements. Standard solutions of the investigated elements were prepared by dilution with 1.0% HNO_3, conveniently prepared from stock solution (Fluka Analytical, Merck, Darmstadt, Germania). The calibration line for the micro-elements (Li, Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ru, Pd, Ag, Cd, Sn, Sb, Cs, Ba, Pb, U) was performed using the following solutions: 0.01 ppb; 0.10 ppb; 1.00 ppb; 5.00 ppb; 10.0 ppb; 50.0 ppb; 100.0 ppb and 500.0 ppb. For the macro-elements (Na, Mg, P, S, K, Ca), the calibrations line was performed by using the following standard solutions: 0.50 ppm; 1.00 ppm; 2.50 ppm; 5.00 ppm; 10.0 ppm; 25.0 ppm and 50.0 ppm. The calibration of Hg element was operated as follow: 0.1 ppb; 0.5 ppb; 1.0 ppb; 5.0 ppb; 10.0 ppb. For the rare earth elements (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Th), the calibration line was performed by using the following standard solutions: 0.001 ppb, 0.01 ppb; 0.10 ppb; 1.00 ppb; 5.00 ppb; 10.0 ppb; 50.0 ppb; 100.0 ppb and 500.0 ppb, in this case as internal standard was used only ^{115}In and ^{209}Bi. All the calibration standards solutions for the ICP-MS analysis were prepared using ICP-MS calibration standards (10 mg/L, Agilent Technologies, Santa Clara, CA, USA).

3. Results and discussion

3.1. Aroma characterization by GC–MS

Truffles represent a promising food among chefs and food lovers thanks to their desirable organoleptic properties and rarity due to their flavour and unique aroma. Consequently, today truffles have become increasingly popular in the alimentary industry and are considered one of the world's most highly prized foods. In recent years natural truffles are in fact used for different food applications as ingredients in many sophisticated culinary creations: in sauces, soups, and stews, pasta dishes and they can also be used to season meats and vegetables so their importance cannot be underestimated. Aroma truffle is very complex and it is widely accepted that not all volatile molecules but only a small fraction is responsible for what humans perceive as truffle smell, these molecules are defined as odorants (Vahdatzadeh, Deveau & Splivallo, 2015). Generally, in fact, the volatile constituents contain alcohols, esters, aldehydes, ketones, acids, amines, aromatic ethers, and sulfur compounds but the distinctive and intense aroma perceived from human derives mainly from bis(methylthio)methane or 2,4-dithiapentane. The key odorous notes of truffles are different from one species to another or with respect to its geographical distribution giving rise to their own unique aroma. In fact, it has been reported that the aroma of a truffle is profoundly influenced not only by genetic factors but also by cultivation environment, stage of maturation, and storage conditions. (Strojnik, Grebenc & Ogrinc, 2020). The SPME-GC–MS method allowed to obtain the separation of a high number of compounds: 50 molecules were tentatively identified by mass spectral library matching with a good level of acceptance (Table 2). Volatile compounds derived from white truffle *T. magnatum* are known as almost one component: bis(methylthio)methane or 2,4-dithiapentane, accompanied by the presence of other minor constituents. Also in this study, in all samples analyzed its chemical composition resulted largely dominated by bis(methylthio) methane (medium content: 78.72%, sulfury, garlic-like), followed by a substance which is very common in nature: dimethyl sulfide (medium content: 9.74%, cabbage, sulphur), presents in 25 of 27 samples and this result is in agreement with earlier reports (Piloni et al., 2005). For instance, the chemical profile herein was comparable with that of Costa et al., 2015, where the volatile profile of truffle also showed bis(methylthio)methane as the main constituent followed by dimethyl sulfide and other minor compounds. In detail, from a semiquantitative point of view, other minor constituents were also identified in the aroma of white truffle including 2-methyl-1-butanol (2.45%, malty), 2-acetyl-5-methylfuran (2.31%, nutty, caramel), phenylethyl alcohol (1.57%, floral, rose), 1-methoxy-3-methylbenzene (1.82%, narcissus), toluene (1.21%, sweet, fruity), 3-methyl-1-butanol (0.90%, malty, roasted), 1-hexanol (0.72%, alcoholic, pungent, green). Furthermore, hexanal (0.63%, leafy, fruity, sweaty), disulfide, bis((methylthio)methyl) (0.47%, cabbage, garlic-like), 2-heptanone (0.09%, fruity) were present in low percentages. However, 2-acetyl-5-methylfuran and 1-methoxy-3-methylbenzene were identified in 23 samples of 27, followed by 1-hexanol presents in 20 samples. 3-methyl-1-butanol was instead detected in 18 samples, followed by 2-methyl-1-butanol and disulfide, bis((methylthio)methyl)

Table 2
Compounds detected in white Acqualagna truffle samples by GC–MS. Data was expressed in relative percentages of area values (%).

No.	Component	RI exp.	RI lit.	Samples of fresh white Acqualagna truffle								
				1	2	3	4	5	6	7	8	9
1	Ethanol	–	–	–	0.12 ± 0.2	–	–	–	–	–	–	–
2	Dimethyl sulfide	–	–	10.30 ± 1	14.09 ± 1.47	12.51 ± 2	–	16.17 ± 2	16.16 ± 3	19.27 ± 3.4	6.42 ± 1.2	29.60 ± 2.87
3	3-Methylbutanal	710	697	0.98 ± 0.19	–	–	–	–	–	–	–	–
4	3-Methyl-1-butanol	748	741	–	1.97 ± 0.32	0.65 ± 0.12	–	0,43 ± 0.08	–	–	–	0.15 ± 0.02
5	2-Methyl-1-butanol	750	743	1.10 ± 0.15	3.70 ± 0.55	5.04 ± 0.4	–	0.29 ± 0.02	–	–	–	4.45 ± 0.74
6	1-Pentanol	753	762	–	–	–	–	–	–	–	–	0.21 ± 0.03
7	Dimethyl disulfide	753	746	–	–	–	–	0.42 ± 0.08	1.18 ± 0.2	–	–	–
8	Toluene	769	759	–	–	5.37 ± 0.9	2.03 ± 0.22	2.28 ± 0.03	–	0.61 ± 0.1	1.73 ± 0.32	0.20 ± 0.04
9	Octane	800	800	–	–	–	–	0.12 ± 0.02	–	4.36 ± 0.85	0.65 ± 0.06	0.24 ± 0.04
10	Hexanal	801	801	0.69 ± 0.14	0.63 ± 0.01	2.31 ± 0.42	2.23 ± 0.43	0.35 ± 0.07	–	–	–	0.71 ± 0.13
11	3-Methylbutanoic acid	855	845	–	0.08 ± 0	–	–	–	–	–	–	–
12	1-Hexanol	870	870	–	0.40 ± 0.08	1.59 ± 0.3	0.06 ± 0	2.97 ± 0.6	–	–	–	0.76 ± 0,13
13	Bis(methylthio)methane	889	889	70.55 ± 3,51	74.20 ± 0,81	37.46 ± 1.32	67.07 ± 2	63.89 ± 5	71.32 ± 3.7	60.73 ± 4.8	85.79 ± 1.8	56.30 ± 2.9
14	2-Heptanone	900	891	–	0.04 ± 0.01	0.18 ± 0.03	–	–	0.19 ± 0.03	0.27 ± 0.05	–	0.13 ± 0.02
15	Heptanal	902	903	1.12 ± 0.04	–	–	–	–	–	–	–	–
16	Dimethyl sulfone	905	914	–	–	–	0.28 ± 0.05	–	–	0.88 ± 0.07	0.18 ± 0.01	–
17	2(5H)-Furanone	907	915	0.08 ± 0.01	–	–	–	–	–	–	–	–
18	2-Heptenal	941	954	–	–	0.28 ± 0.02	–	–	–	–	–	–
19	Benzaldehyde	953	961	0.15 ± 0.1	0.04 ± 0.01	1.27 ± 0.25	1.22 ± 0.23	–	–	0.99 ± 0.15	–	0.20 ± 0.04
20	3-Octanone	985	988	2.25 ± 0.03	–	–	–	–	–	–	–	–
21	2-Pentylfuran	989	993	–	–	–	2.16 ± 0.42	–	0.24 ± 0.05	0.92 ± 0.18	–	–
22	2-Acetyl-5-methylfuran	994	1004	1.41 ± 0.13	4.09 ± 0.67	18.64 ± 3.7	2.71 ± 0.52	9.09 ± 2	0.26 ± 0.03	1.25 ± 0.23	0.65 ± 0.05	1.87 ± 0.27
23	3-Octanol	994	995	–	–	0.31 ± 0.05	–	–	–	–	–	–
24	Decane	999	1000	–	–	–	–	–	2.60 ± 0.09	5.38 ± 0.44	3.02 ± 0.19	1.91 ± 0.34
25	Phenol	1007	994	7.67 ± 1.52	–	–	–	–	–	–	–	–
26	Anisole (Methoxybenzene)	1014	1006	0.46 ± 0.06	–	–	–	–	–	–	–	–
27	1-Methoxy-3-methylbenzene	1014	1008	–	–	4.87 ± 0.21	–	3.08 ± 0.5	4.61 ± 0.6	–	0.33 ± 0.02	0.77 ± 0.13
28	5-Ethylcyclopent-1-enecarboxaldehyde	1024	1026	–	–	0.60 ± 0.02	0.57 ± 0.02	–	–	–	–	0.15 ± 0.03
29	Benzeneacetaldehyde	1036	1046	–	–	0.38 ± 0.07	–	–	–	–	–	–
30	Acetophenone	1058	1061	–	–	0.41 ± 0.02	–	–	–	–	–	–
31	2-Nonanone	1081	1090	–	–	–	–	–	–	–	0.39 ± 0.06	1.47 ± 0.19
32	Phenylethyl alcohol	1108	1108	–	0.62 ± 0.11	7.25 ± 0.48	–	–	–	–	–	–
33	Disulfide, bis((methylthio) methyl)	1125	1134	–	–	0.40 ± 0.07	1.36 ± 0.1	0.42 ± 0.08	1.09 ± 0.03	1.76 ± 0.34	0.38 ± 0.06	–
34	Naphtalene	1173	1176	–	–	0.19 ± 0.03	12.28 ± 2	–	0.15 ± 0.02	–	–	–
35	Dodecane	1196	1200	–	–	–	–	–	2.20 ± 0.3	2.85 ± 0.33	0.49 ± 0.08	0.60 ± 0.11
36	2-Decanone	1200	1194	–	–	–	–	–	–	0.73 ± 0.14	–	–

(*continued on next page*)

Table 2 (*continued*)

No.	Component	RI exp.	RI lit.	Samples of fresh white Acqualagna truffle								
				1	2	3	4	5	6	7	8	9
37	3-Phenyl-2-butanone	1238	1240	0.88 ± 0.14	–	–	–	–	–	–	–	–
38	2-Undecanone	1288	1291	2.35 ± 0.09	–	0.28 ± 0.04	0.77 ± 0.08	0.48 ± 0.01	–	–	–	0.29 ± 0.03
39	Caryophyllene	1409	1411	–	–	–	7.25 ± 0.86	–	–	–	–	–

No.	Component	RI exp.	RI lit.	Samples of fresh white Acqualagna truffle								
				10	11	12	13	14	15	16	17	18
1	Dimethyl sulfide	–	–	11.54 ± 1.21	6.44 ± 1	6.75 ± 1.1	10.19 ± 1.7	5.82 ± 0.9	7.35 ± 1.23	12.73 ± 1	4.46 ± 0.6	4.95 ± 0.2
2	2-Heptane	693	700	–	0.03 ± 0	–	–	0.02 ± 0	–	–	–	–
3	3-Penten-2-one	731	731	–	–	–	–	–	–	0.20 ± 0.03	–	–
4	3-Methyl-1-butanol	748	741	0.21 ± 0.03	0.14 ± 0.01	0.14 ± 0.03	–	–	–	–	0.61 ± 0.07	6.12 ± 1
5	2-Methyl-1-butanol	750	743	–	–	–	–	1.17 ± 0.13	0.85 ± 0.12	–	3.61 ± 0.6	–
6	1-Pentanol	753	762	–	–	–	–	–	0.07 ± 0	–	–	–
7	Dimethyl disulfide	753	746	0.40 ± 0.06	0.89 ± 0.16	0.36 ± 0.06	0.32 ± 0.06	0.50 ± 0.03	–	–	–	0.89 ± 0.15
8	Toluene	769	759	0.14 ± 0.02	0.29 ± 0.04	0.62 ± 0.06	–	–	–	–	–	0.02 ± 0
9	Octane	800	800	0.23 ± 0.03	–	–	–	–	–	–	–	–
10	Hexanal	801	801	–	0.33 ± 0.05	0.12 ± 0.02	0.09 ± 0.01	0.23 ± 0.01	0.16 ± 0.03	–	0.24 ± 0.03	–
11	3-Methylbutanoic acid	855	845	–	–	–	–	–	0.23 ± 0	–	0.90 ± 0.08	–
12	1-Hexanol	870	870	0.26 ± 0.04	0.23 ± 0.03	0.24 ± 0.03	0.71 ± 0.12	0.06 ± 0.01	0.29 ± 0.05	0.24 ± 0.03	4.35 ± 0.2	–
13	Bis(methylthio)methane	889	889	72.78 ± 4.2	84.14 ± 2.13	88.04 ± 1.5	86.52 ± 2.1	87.37 ± 0.04	83.32 ± 0.15	84.75 ± 1	80.38 ± 0.09	86.45 ± 1
14	2-Heptanone	900	891	–	–	–	0.03 ± 0	–	–	0.02 ± 0	–	0.06 ± 0
15	Dimethyl trisulfide	958	969	–	0.06 ± 0.01	–	0.04 ± 0.01	–	0.02 ± 0	–	–	–
16	3-(methylthio)propanol	977	983	–	–	–	–	–	0.23 ± 0.03	–	–	–
17	3-Octanone	985	988	–	–	0.76 ± 0.02	–	–	–	–	–	–
18	2-Acetyl-5-methylfuran	994	1004	–	4.65 ± 0.73	–	0.76 ± 0.03	0.22 ± 0.02	2.20 ± 0.42	0.47 ± 0.02	0.49 ± 0.2	0.19 ± 0.02
19	3-Octanol	994	995	–	–	–	0.04 ± 0	–	0.05 ± 0	–	0.03 ± 0	–
20	Decane	999	1000	0.63 ± 0.07	–	–	–	–	–	–	–	–
21	1-Methoxy-3-methylbenzene	1014	1008	11.31 ± 2.3	2.37 ± 0.38	2.51 ± 0.46	1.21 ± 0.23	3.86 ± 0.7	1.57 ± 0.16	0.73 ± 0.09	1.18 ± 0.08	0.36 ± 0.04
22	Benzeneacetaldehyde	1036	1046	–	–	–	–	0.21 ± 0.04	0.13 ± 0.02	0.19 ± 0.05	–	–
23	2-Octenal	1053	1056	–	–	–	–	0.47 ± 0.09	0.36 ± 0.06	0.48 ± 0.01	–	–
24	2-Nonanone	1081	1090	0.68 ± 0.11	–	–	–	–	–	–	–	0.78 ± 0.12
25	Nonanal	1102	1103	–	–	–	–	0.06 ± 0.01	0.05 ± 0	0.07 ± 0	–	–
26	Phenylethyl alcohol	1108	1108	–	0.14 ± 0.02	0.14 ± 0.02	–	–	2.98 ± 0.61	–	4.57 ± 0.7	–
27	Disulfide, bis((methylthio) methyl)	1125	1134	1.41 ± 0.23	0.30 ± 0.03	0.25 ± 0.04	0.09 ± 0.02	0.04 ± 0	0.08 ± 0	0.11 ± 0	0.12 ± 0	0.10 ± 0.01
28	2-Decanone	1200	1194	0.41 ± 0.06	–	–	–	–	–	–	–	–
29	2-Undecanone	1288	1291	–	–	0.07 ± 0.01	–	–	0.05 ± 0	–	0.08 ± 0	0.09 ± 0.01

No.	Component	RI exp.	RI lit.	Samples of fresh white Acqualagna truffle								
				19	20	21	22	23	24	25	26	27
1	Ethanol	–	–	–	0.38 ± 0.08	–	0.04 ± 0	0.05 ± 0	–	–	–	0.03 ± 0.02
2	Dimethyl sulfide	–	–	–	1.54 ± 0.14	3.20 ± 0.63	4.45 ± 0.28	13.53 ± 2.5	9.78 ± 1.24	3.84 ± 0.33	5.85 ± 0.83	6.62 ± 0.88
3	2-Propanone	–	–	–	–	–	–	0.05 ± 0	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

No.	Component	RI exp.	RI lit.	Samples of fresh white Acqualagna truffle								
				19	20	21	22	23	24	25	26	27
4	Acetic acid	671	662	–	–	–	–	0.61 ± 0.06	–	–	1.80 ± 0.2	–
5	3-Methyl-1-butanol	748	741	0.77 ± 0.08	0.71 ± 0.09	0.43 ± 0.07	0.45 ± 0.05	0.32 ± 0.05	0.72 ± 0.09	0.88 ± 0.19	0.71 ± 0.05	0.82 ± 0.16
6	2-Methyl-1-butanol	750	743	2.28 ± 0.04	2.97 ± 0.64	2.89 ± 0.5	3.26 ± 0.29	1.40 ± 0.15	2.13 ± 0.37	2.09 ± 0.37	2.27 ± 0.25	2.11 ± 0.35
7	1-Pentanol	753	762	0.20 ± 0.02	–	–	–	–	0.06 ± 0	–	–	–
8	Dimethyl disulfide	753	746	–	–	2.06 ± 0.18	–	–	–	–	–	–
9	Toluene	769	759	–	–	–	–	0.03 ± 0.01	–	–	–	–
10	Octane	800	800	–	–	–	–	–	0.08 ± 0.02	–	–	–
11	Hexanal	801	801	0.51 ± 0.05	–	–	–	–	0.25 ± 0.02	–	–	–
12	3-Methylbutanoic acid	855	845	0.65 ± 0.02	0.11 ± 0	–	0.47 ± 0.07	–	–	0.32 ± 0.06	–	0.32 ± 0.06
13	2-Methylbutanoic acid	862	860	–	–	–	0.15 ± 0	–	–	0.04 ± 0	–	0.02 ± 0
14	1-Hexanol	870	870	0.30 ± 0.05	0.42 ± 0.08	0.06 ± 0.01	0.72 ± 0.10	–	0.23 ± 0.01	0.13 ± 0.01	0.07 ± 0.01	–
15	Bis(methylthio)methane	889	889	93.56 ± 0.26	92.40 ± 0.78	90.12 ± 1.46	74.34 ± 0.32	83.30 ± 2.74	85.56 ± 0.9	89.5 ± 0.76	87.44 ± 0.46	88.26 ± 0.08
16	2-Heptanone	900	891	–	0.09 ± 0.01	0.05 ± 0	–	–	–	0.03 ± 0	0.03 ± 0	0.04 ± 0
17	Benzaldehyde	953	961	–	–	–	–	–	0.06 ± 0	–	–	–
18	Dimethyl trisulfide	958	969	–	–	0.03 ± 0	–	0.23 ± 0.03	–	–	–	–
19	3-Octanone	985	988	–	–	–	–	–	–	–	0.14 ± 0	–
20	2-Pentylfuran	989	993	–	–	–	–	–	–	–	–	–
21	2-Octanone	990	992	–	–	–	–	–	–	0.38 ± 0.05	–	–
22	2-Acetyl-5-methylfuran	994	1004	0.22 ± 0.01	–	0.11 ± 0	2.29 ± 0.36	0.05 ± 0	0.19 ± 0	1.02 ± 0.21	–	0.41 ± 0.01
23	3-Octanol	994	995	–	–	–	–	–	–	–	–	0.01 ± 0
24	1-Methoxy-3-methylbenzene	1014	1008	0.19 ± 0.03	0.55 ± 0.05	0.51 ± 0.07	0.57 ± 0.05	0.10 ± 0	0.10 ± 0.01	0.33 ± 0.06	0.37 ± 0.05	0.34 ± 0.02
25	5-Ethylcyclopent-1-enecarboxaldehyde	1024	1026	–	–	–	–	0.02 ± 0	0.05 ± 0	–	–	–
26	2-Nonanone	1081	1090	0.74 ± 0.13	0.42 ± 0.08	–	–	0.22 ± 0.03	0.48 ± 0.07	–	–	–
27	Phenylethyl alcohol	1108	1108	0.22 ± 0	0.32 ± 0.05	0.45 ± 0.08	3.55 ± 0.64	0.08 ± 0	0.32 ± 0.02	1.36 ± 0.15	0.50 ± 0.09	1.01 ± 0.17
28	Disulfide, bis((methylthio) methyl)	1125	1134	–	–	0.09 ± 0	–	0.03 ± 0	–	–	–	–
29	2-Undecanone	1288	1291	0.36 ± 0.06	0.09 ± 0.01	–	–	–	–	–	–	–

- indicates not detected. Compounds are listed in order of their calculated RI.
RI lit represent the retention index present in literature; RI exp represent the calculated retention index.

present in 17 samples. Phenylethyl alcohol was detected in 15, hexanal in 14, 2-heptanone in 13 and finally toluene in 11 samples of 27 truffles. In addition, small traces of other compounds have been detected but still contribute to the aroma of the truffle (Table S2). In our sample of *T. magnatum*, the level of bis(methylthio)methane is similar to *T. magnatum* reported by Mustafa et al., 2020. Our level of bis(methylthio)methane is higher than those reported in the literature for *T. melanosporum,* but the molecule is not detected in *T. aestivum* (Torregiani et al., 2017). Regarding dimethyl sulfide our results are perfectly in line with *T. magnatum* in Piloni et al., 2005, but higher than *T. melanosporum* (Torregiani et al., 2017) and *T. aestivum* of Díaz et al., 2003. In this study the level of 2-methyl-1-butanol is lower than those reported for *T. magnatum* in Piloni et al., 2005, but similar to the amount reported for *T. melanosporum* and higher than the value of *T. aestivum* (Díaz et al., 2003). On the other hand, 2-acetyl-5-methylfuran level is similar to those reported for *T. magnatum* (Piloni et al., 2005) and this molecule is not detected in *T. melanosporum* and *T. aestivum* (Mustafa et al., 2020). For the lower constituents of the volatile profile these results are linear with data reported in the literature in fact they are present in lots of articles as a minor part of all the volatile profiles. Despite Gioacchini et al., 2008 detected terpenoid compounds such as caryophyllene in *T. magnatum*, conversely, our results are in contrast with that in fact only caryophyllene was identified in only one of 27 samples. The changes found for the volatile compounds present in truffle might be linked to several factors, such as the region where the truffle was harvested, and the environmental conditions in which it grew. Climatical, seasonal, geographical, and geological changes could influence the truffle molecules and consequently their aroma.

3.2. Sensory analysis

Sensory analysis is a crucial tool in understanding and evaluating the quality of truffles, as it allows us to assess their aroma, flavour, and texture. The aroma of truffles is made up of a complex mix of odorants, which are chemical compounds that can be detected by the human nose. Our data explore the correspondence between odorants and molecules of truffle, and how they work together to create the unique aroma of truffles. Several odorants have been identified in the white Acqualagna

Table 3
Total phenolic, flavonoid content and radical scavenging ability of nine blend sample of white Acqualagna truffles.

	First collecting			Second collecting			Third collecting		
	blend 1	*blend 2*	*blend 3*	*blend 1*	*blend 2*	*blend 3*	*blend 1*	*blend 2*	*blend 3*
Phenolics(mg GAE/g of dw)	2.05 ± 0.06	2.41 ± 0.12	1.96 ± 0.22	2.47 ± 0.6	2.06 ± 0.08	2.49 ± 0.44	2.54 ± 0.50	2.08 ± 0.11	2.03 ± 0.05
Flavonoids(mg RT/g of dw)	26.60 ± 0.09	24.17 ± 1.17	25.25 ± 0.35	23.25 ± 1.06	24.00 ± 1.41	23.32 ± 0.02	24.75 ± 0.35	21.98 ± 0.44	25.07 ± 1.08
DPPH (mg TE/kg of dw)	540.10 ± 13.92	578.64 ± 30.23	563.87 ± 8.71	574.89 ± 34.32	571.92 ± 29.85	551.28 ± 1.78	518.88 ± 2.47	513.99 ± 23.00	570.85 ± 27.12

Values are means ± SD of three measurement.

truffle: garlic-like, nutty-like, geosmine-like, floral, mushroom-like, pungent and green or herbal. The identified truffle odorant sulfury or garlic-like is the key aroma compound of white truffle which corresponds to sulphur compounds such as bis(methylthio)methane and dimethyl sulfide present in our sample. This odorant is found in our sample and is responsible for the strong and sulfuric aroma of white truffles. Two of the most important odorants in truffle are also nutty-like and geosmin-like. The first one corresponds to rich, slightly sweet, and green nutty and derives from molecules: 2-acetyl-5-methylfuran, benzaldehyde. Instead, the second one derives from: 2-octanone, 3-octanol, and gives the idea of the earthy aroma that is often associated with soil. Other important odorants in truffles are mushroom-like and pungent, which respectively correspond to molecules 3-octanone, 3-octanol and 1-hexanol, acetic acid. The mushroom-like notes in truffles are often described as being earthy, fresh mushrooms and gently nut-like, with hints of decay. Instead, when the aroma of the truffle is described as having a "pungent" quality, it refers to a complex mix of sensory characteristics capable of evoking a powerful, strong and intense smell. The pungent notes in truffles are often described as being similar to delicate fatty-fruity, fermented fruit, vegetable and alcoholic flavors. Overall, the smell is overwhelming, with a sharp, almost bitter quality. Another descriptor identified is floral, an odorant that can evoke the fragrance of various types of flowers so it is delicate, sweet, and slightly perfumed, with hints of fruitiness and earthiness. This derives from benzeneacetaldehyde, phenylethyl alcohol and 1-methoxy-3-methylbenzene. Finally, the complex mix of odorants found in truffles, including green or herbal. This one is expressed for the presence of 3-methyl-1-butanol, 2-heptanal, 1-hexanol, heptanal and 2-pentylfuran. The green or herbal notes in truffles are often described as being fresh, slightly vegetal, and with a hint of bitterness. This odorant derives from the fact that different judges recognize in truffles the scent of various types of plants and vegetation. Among all the odorants found in white Acqualagna truffles in our study, sulfury or garlic-like, mushroom-like and green, are also detected in the white Alba truffle studied by Schmidberger & Schieberle, 2017. Differently from us, they have found cooked potato-like, amine-like, sperm-like and malty but their judges did not testify to the presence of floral, pungent, nutty-like and geosmin-like, detected by our panel. These differences may arise from a combination of environmental or genetic factors that could influence the chemical compounds present in truffles and consequently the aroma. Soil composition, temperature, humidity, rainfall and sunlight may affect the volatile compound of truffle samples (Díaz et al., 2003; Mustafa et al., 2020; Strojnik, Grebenc & Ogrinc, 2020). Overall, the state of maturation and above all the species of truffle are important for attributing a specific smell to a truffle. The results identified for the first time the natural mixture of key odorants responsible for the distinct aroma profiles of white Acqualagna truffles and show the correspondence between odorants and molecules of the truffle. The unique aroma and flavour of truffles derive in fact from the correspondence between odorants and molecules. This makes truffles such an important ingredient in the culinary world and consequently a highly prized food because it can create a sensory experience that is difficult to replicate with other ingredients.

3.3. Phenolic profile and antioxidant activity

In truffles there are various antioxidant compounds such as phenols, flavonoids, and carotenoids, responsible for their antioxidant activity. Today there are little data on the antioxidant power of *T. magnatum* and none on the white truffle of Acqualagna. Generally, it is reported that black truffles have higher antioxidant activity and phenolic content than white ones (Beara et al., 2014). Table 3 shows the total phenolic, flavonoid content and radical scavenging ability of the white Acqualagna truffle extract. The percentage of inhibition of DPPH, calculated according to the formula: % Inhibition = [(A0-A1)/A0] * 100 where A0 is the absorbance value of the DPPH blank solution and A1 is the absorbance value of the sample solution, goes from 53 to 63%. This antiradical activity was in good correlation with total phenolics content (TPC) and total flavonoid content (TFC). Although our results indicate that the antioxidant capacity of white truffles is higher than in other reports, the TPC is lower than the same one (Beara et al., 2014). Our TPC results varied, from 1.96 to 2.54 mg GAE/g of dw and are similar to those reported by Tejedor-Calvo et al., 2021. Otherwise, a good level of flavonoids are found in the truffle range from 21.98 to 26.60 mg RT/g of dw. The differences between our results and the data presented in the literature may be explained at first by the various species of truffle but also by the environmental conditions. The growing conditions, the different harvesting period, and different stages of maturation and the soil in which the samples were collected could in fact change the number of active compounds in truffle. The estimation of phenols and flavonoids may also vary depending on the method of analysis. It is important to take in to consideration that with a spectrophotometric method it could be possible to obtain an overestimated polyphenolic content due to different non-phenolic materials present in the extracts that could interfere with the spectrophotometric analysis.

3.4. Elemental analysis

The estimation of different element contents which are present in truffle material is useful for those who consume them, so it is one of the key aspects in the study of truffles. Carbon, hydrogen, sulphur and nitrogen are very important for plant growth and by estimating the amount of these elements it is possible to know the nutritional value and metabolic pathways of different plants. Carbon is generally the most abundant element in truffles followed by nitrogen. In the present study, the values of carbon, hydrogen, nitrogen and sulphur were analyzed and there are no differences between all samples. As we expected carbon is the major compound with a percentage from 38.46 to 39.99%. Hydrogen, nitrogen and sulphur are respectively 5.77, 5.48 and 0.66% (Table S1). Therefore, in the tested sample carbon to nitrogen ratio is higher than 6:1. A higher content of carbon than nitrogen in organic matter suggests a higher content of carbohydrates and proteins (Mandal et al., 2017). On the other hand, despite sulfur and hydrogen are present in smaller amounts they still play important roles in the chemistry of truffles (García-Montero et al., 2007).

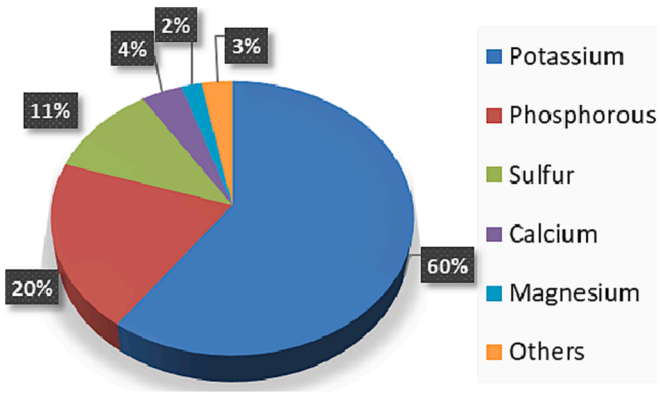

Fig. 1. Distribution of the main elements present in the Acqualagna white truffle.

3.5. Quantitative elemental composition by ICP-MS analysis

The elemental composition of white Acqualagna truffles is characterized by five main elements K, P, S, Ca and Mg, that give the 97% of the total mass while the other elements investigated represent only the 3%; this result is according to previous analysis on truffles mushroom (Kruzselyi & Vetter, 2014) (Fig. 1). From ICP-MS analysis, all the elements investigated are detected except Tl, Hg, Pt and Be, Au, Ru, that resulted not present in the truffles fruit samples; for these reasons the last three elements are then used as recovery standards. For the elements Pd and Sb, the concentrations are lower than the LOD values. The element profile concentration (mg/Kg) of the nine blend of truffles is reported in Fig. 2 while the elemental concentrations for each of the analysed elements are summarized in Table 4. Comparing the results between the fruits and the released water of soils (Table S3), interesting is the pattern of the higher mass elements. In particular, the Rare Earth Elements (REE), that identify the geographical area and predominantly influenced by the soil, are assimilated without discrimination (Oddone et al., 2009; Segelke et al., 2020). Essential elements for the human metabolism such as Zn and Cu appear to be increased in the fruits, in a lesser extent also Se shows this behaviour. Specifically, Zn is concentrated 222 times, Cu 50 times and Se 23 times. On the other hand, also Cd concentration increased in the truffles (3480 times) and in a lesser extent Cr increase its concentration (20 times), as reported for other fungi (García et al., 2013; Kalač et al., 2004); in addition, even Ag (40 times) increased its concentration on the truffle fruits.

4. Conclusions

Today there are no other studies in the literature on Acqualagna truffle, so this is the first study to obtain its characterization with different approaches: GC–MS, panelists analyses, ICP-MS, elemental

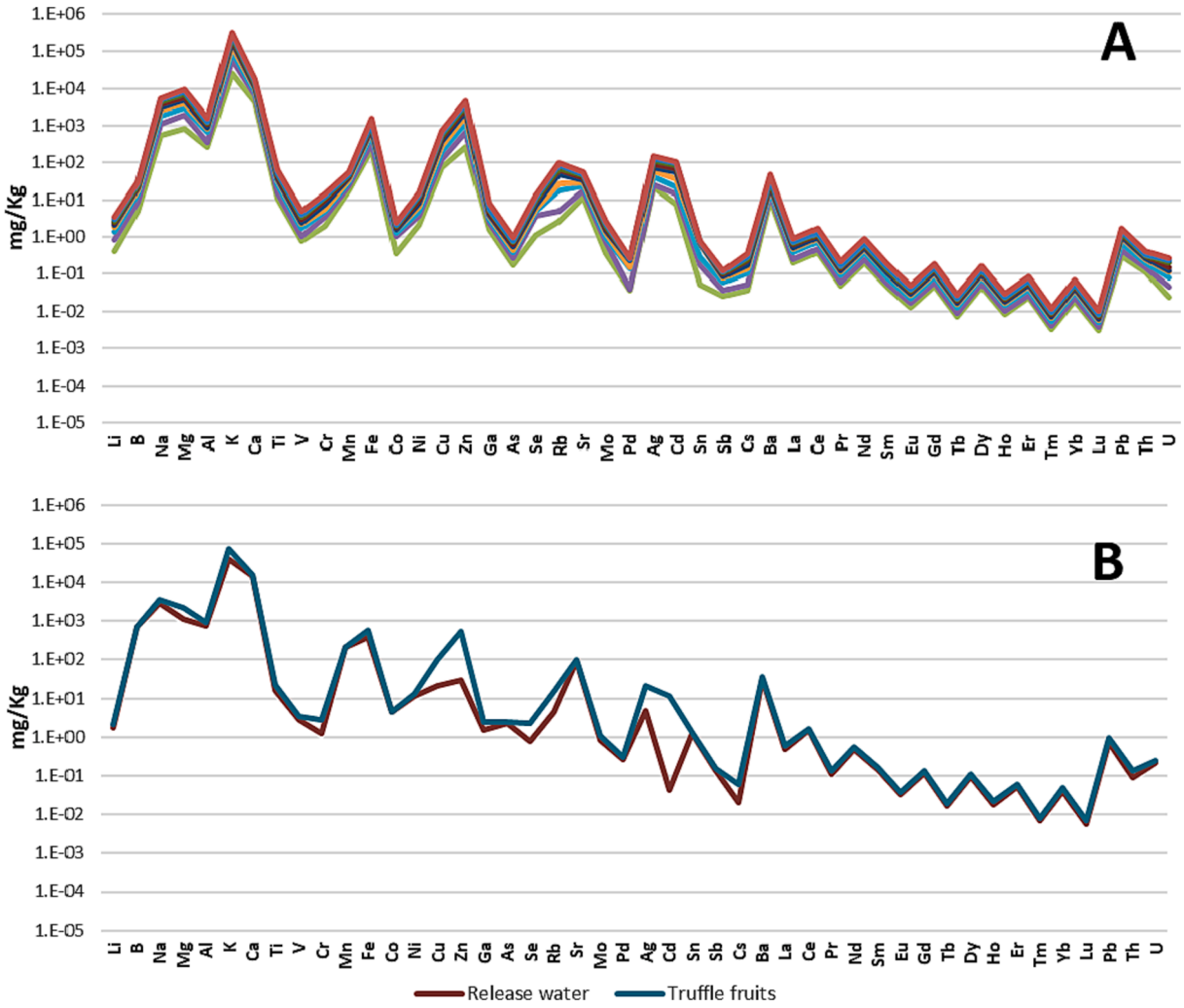

Fig. 2. Element pattern of nine blend white Acqualagna truffles by using ICP-MS (A). Element pattern comparison between truffles fruits (average) samples and release water (average) by ICP-MS (B). Data expressed as mg/Kg on a logarithmic scale.

Table 4
Elements concentration in nine freeze-dried blend sample of white Acqualagna truffles by ICP-MS analysis, data expressed as mg/Kg.

Element	First collecting			Second collecting			Third collecting			Mean
	blend 1	*blend 2*	*blend 3*	*blend 1*	*blend 2*	*blend 3*	*blend 1*	*blend 2*	*blend 3*	
Li / 7	0.41 ± 1.07	0.42 ± 1.15	0.56 ± 2.14	0.49 ± 3.54	0.19 ± 3.68	0.35 ± 3.56	0.27 ± 4.18	0.25 ± 4.45	0.500 ± 2.88	*0.38* ± 2.96
B / 11	5.08 ± 3.23	3.82 ± 2.39	5.56 ± 4.56	4.09 ± 4.01	2.10 ± 5.12	2.06 ± 4.46	1.60 ± 4.92	2.47 ± 6.51	2.05 ± 4.22	*3.21* ± 4.38
Na / 23	533.20 ± 5.44	605.20 ± 7.52	702.40 ± 4.88	832.80 ± 8.12	405.00 ± 5.51	647.80 ± 3.50	608.20 ± 6.62	564.20 ± 4.22	529.80 ± 4.26	*603.18* ± 5.79
Mg / 24	860.00 ± 4.20	1123.60 ± 7.56	980.80 ± 5.32	1014.40 ± 5.71	885.00 ± 4.32	999.60 ± 3.18	939.80 ± 6.40	1074.80 ± 2.84	1233.20 ± 2.08	*1012.36* ± 4.63
Al / 27	262.92 ± 11.32	92.72 ± 17.24	295.56 ± 5.44	208.52 ± 5.81	57.48 ± 5.46	172.22 ± 1.98	83.38 ± 4.82	109.90 ± 3.82	355.60 ± 2.78	*182.03* ± 6.52
P / 31	8208.23 ± 0.96	12244.24 ± 2.13	10784.89 ± 1.01	13084.78 ± 1.50	12268.24 ± 1.71	12286.85 ± 1.16	11616.44 ± 2.45	12978.48 ± 1.52	11596.25 ± 1.26	*11673.78* ± 1.52
S / 34	5628.01 ± 2.93	4816.56 ± 6.62	5548.45 ± 2.73	6492.36 ± 4.48	9112.22 ± 1.92	5916.53 ± 3.06	6534.62 ± 4.25	8280.35 ± 2.71	5078.75 ± 2.58	*6378.22* ± 3.47
K / 39	26048.00 ± 3.88	35184.00 ± 10.24	38172.00 ± 5.24	40960.00 ± 6.81	40680.00 ± 3.62	36960.00 ± 3.14	35280.00 ± 4.40	37100.00 ± 3.61	37340.00 ± 3.12	*36413.78* ± 2.15
Ca / 44	4408.00 ± 7.44	2124.00 ± 10.72	1557.60 ± 4.32	1711.20 ± 11.96	1014.20 ± 6.46	1585.40 ± 2.10	1284.40 ± 5.14	1792.00 ± 2.86	1924.40 ± 4.20	*1933.47* ± 6.13
Ti / 47	10.58 ± 7.23	5.03 ± 5.76	10.09 ± 4.32	7.69 ± 7.65	3.94 ± 2.32	6.27 ± 2.40	3.80 ± 1.86	5.72 ± 7.13	11.28 ± 4.98	*7.164* ± 4.85
V / 51	0.78 ± 1.26	0.25 ± 2.10	0.64 ± 1.68	0.58 ± 2.91	0.28 ± 1.19	0.55 ± 0.98	0.49 ± 2.03	0.48 ± 1.43	0.84 ± 1.56	*0.54* ± 1.68
Cr / 52	1.94 ± 0.98	1.45 ± 0.96	1.45 ± 1.47	1.08 ± 1.15	1.86 ± 1.56	2.07 ± 1.48	1.66 ± 3.02	1.25 ± 4.05	1.61 ± 5.41	*1.60* ± 2.23
Mn / 55	17.4 ± 5.16	6.43 ± 2.16	6.17 ± 1.21	4.81 ± 1.47	3.42 ± 1.26	4.31 ± 2.45	3.15 ± 2.56	3.53 ± 3.51	8.12 ± 4.01	*6.37* ± 3.12
Fe / 56	227.28 ± 4.48	101.32 ± 18.46	259.60 ± 5.48	173.28 ± 7.16	68.40 ± 3.62	163.06 ± 2.94	87.84 ± 5.11	128.70 ± 2.90	334.60 ± 4.32	*171.56* ± 6.05
Co / 59	0.35 ± 1.12	0.69 ± 1.43	0.26 ± 5.34	0.24 ± 2.84	0.10 ± 1.69	0.17 ± 1.09	0.20 ± 1.26	0.18 ± 2.10	0.25 ± 1.23	*0.27* ± 2.01
Ni / 60	2.08 ± 1.54	1.58 ± 2.24	1.29 ± 3.13	1.47 ± 1.31	1.63 ± 0.88	2.21 ± 1.45	1.98 ± 0.80	1.54 ± 1.25	1.56 ± 2.56	*1.71* ± 1.68
Cu / 63	74.40 ± 5.12	47.36 ± 5.12	77.28 ± 4.92	90.96 ± 5.21	90.82 ± 3.86	85.98 ± 3.41	76.76 ± 5.38	76.50 ± 3.72	79.56 ± 2.68	*77.74* ± 4.38
Zn / 66	273.68 ± 5.36	399.80 ± 5.36	546.80 ± 6.04	602.00 ± 6.04	552.80 ± 3.64	635.40 ± 3.01	501.80 ± 5.20	581.00 ± 3.62	439.80 ± 2.86	*503.68* ± 4.57
Ga / 69	1.65 ± 2.63	0.78 ± 2.54	0.64 ± 3.49	0.65 ± 2.45	0.37 ± 1.79	0.63 ± 1.55	0.45 ± 1.74	0.68 ± 1.12	2.23 ± 3.02	*0.90* ± 2.26
As / 75	0.17 ± 3.35	0.09 ± 4.3	0.13 ± 3.56	0.05 ± 5.51	0.10 ± 5.94	0.10 ± 6.52	0.06 ± 4.56	0.09 ± 5.62	0.13 ± 5.68	*0.10* ± 5.00
Se / 77	1.16 ± 2.39	2.51 ± 3.21	1.19 ± 2.56	0.84 ± 1.30	1.52 ± 2.89	2.21 ± 4.78	0.93 ± 5.24	1.27 ± 5.52	1.75 ± 6.25	*1.49* ± 3.79
Rb / 85	2.60 ± 3.72	2.52 ± 4.51	13.04 ± 4.82	10.17 ± 2.92	19.26 ± 2.41	15.97 ± 1.54	6.98 ± 1.58	13.31 ± 1.96	15.58 ± 1.32	*11.05* ± 2.75
Sr / 88	11.50 ± 6.22	6.98 ± 4.23	5.46 ± 6.12	6.63 ± 5.21	2.98 ± 3.56	5.50 ± 3.31	4.91 ± 2.56	6.07 ± 4.01	5.23 ± 3.05	*6.14* ± 4.25
Mo / 95	0.37 ± 1.27	0.37 ± 1.38	0.24 ± 2.35	0.32 ± 1.52	0.18 ± 2.48	0.23 ± 5.12	0.30 ± 6.28	0.26 ± 7.51	0.37 ± 6.04	*0.30* ± 3.77
Pd / 105	0.04 ± 2.15	0.001 ± 3.65	0.10 ± 9.61	0.01 ± 5.01	0.08 ± 4.40	0.02 ± 2.40	0.01 ± 4.79	0.01 ± 10.52	0.01 ± 3.56	*0.03* ± 5.12
Ag / 107	22.58 ± 6.53	3.18 ± 1.81	16.01 ± 6.31	20.15 ± 4.28	14.48 ± 2.91	15.29 ± 1.04	20.24 ± 1.81	21.84 ± 2.16	15.47 ± 1.84	*16.58* ± 6.52
Cd / 111	7.49 ± 3.13	6.98 ± 3.15	7.58 ± 4.61	13.84 ± 3.88	21.40 ± 1.82	12.40 ± 2.22	14.42 ± 1.46	15.86 ± 2.78	5.16 ± 1.90	*11.68* ± 2.77
Sn / 118	0.05 ± 3.85	0.12 ± 2.25	0.12 ± 3.88	0.37 ± 1.64	0.03 ± 1.43	0.02 ± 1.57	0.02 ± 3.45	0.01 ± 4.49	0.02 ± 2.59	*0.09* ± 2.79
Sb / 121	0.03 ± 4.34	0.01 ± 3.56	0.02 ± 3.43	0.02 ± 3.89	0.01 ± 2.94	0.01 ± 4.02	0.01 ± 2.86	0.01 ± 3.72	0.01 ± 4.88	*0.01* ± 3.74
Cs / 133	0.04 ± 2.35	0.01 ± 3.02	0.06 ± 2.28	0.04 ± 2.14	0.03 ± 3.51	0.06 ± 3.76	0.03 ± 5.48	0.04 ± 7.94	0.07 ± 5.48	*0.04* ± 4.00
Ba / 137	10.32 ± 3.08	4.38 ± 7.44	3.67 ± 3.88	3.95 ± 6.61	2.26 ± 2.04	3.86 ± 2.20	2.94 ± 2.84	4.38 ± 2.62	14.30 ± 2.14	*5.56* ± 3.65
La / 139[a]	197.00 ± 88.21	50.76 ± 102.2	130.48 ± 108.83	110.84 ± 192.56	34.54 ± 194.23	94.88 ± 105.23	48.62 ± 105.26	63.34 ± 112.25	192.62 ± 165.12	*102.56* ± 130.43
Ce / 140[a]	378.56 ± 100.56	96.16 ± 105.23	244.12 ± 150.35	200.16 ± 96.25	54.10 ± 103.25	165.24 ± 201.23	73.86 ± 202.53	112.18 ± 202.35	372.20 ± 182.42	*188.51* ± 149.35
Pr / 141[a]	47.52 ± 101.10	11.90 ± 201.26	30.90 ± 161.25	25.64 ± 52.31	6.71 ± 223.12	21.30 ± 105.32	10.06 ± 101.32	14.01 ± 202.45	46.12 ± 184.52	*23.79* ± 147.07
Nd / 146[a]	201.16 ± 144.21	49.40 ± 121.31	127.16 ± 156.21	102.72 ± 365.21	28.02 ± 121.02	86.10 ± 130.26	39.10 ± 108.21	56.84 ± 136.21	185.48 ± 148.05	*97.33* ± 158.97
Sm / 147[a]	46.40 ± 103.21	10.27 ± 202.5	26.83 ± 125.12	21.24 ± 156.12	5.86 ± 223.21	18.54 ± 195.52	8.03 ± 201.25	12.03 ± 156.23	39.60 ± 96.25	*20.98* ± 162.16
Eu / 153[a]	13.03 ± 160.81	3.43 ± 121.03	6.336 ± 101.32	4.944 ± 158.59	1.545 ± 251.23	4.55 ± 263.25	2.48 ± 262.35	3.29 ± 201.23	9.01 ± 123.25	*5.40* ± 182.56
Gd / 157[a]	49.64 ± 121.2	11.42 ± 125.3	27.360 ± 105.23	20.684 ± 90.23	5.562 ± 226.35	18.28 ± 196.54	9.60 ± 165.23	11.69 ± 196.25	38.58 ± 105.21	*21.43* ± 136.84
Tb / 159[a]	7.36 ± 221.56	1.741 ± 150.32	3.862 ± 205.23	2.768 ± 163.47	0.820 ± 275.45	2.42 ± 253.26	1.29 ± 251.23	1.66 ± 268.51	5.56 ± 131.25	*3.05* ± 213.36
Dy / 163[a]	46.04 ± 131.23	9.604 ± 220.12	22.376 ± 121.23	15.612 ± 154.24	4.964 ± 201.32	14.00 ± 201.54	8.23 ± 263.21	8.72 ± 201.52	31.84 ± 152.25	*17.93* ± 182.69
Ho / 165[a]	8.40 ± 223.21	1.812 ± 250.32	4.328 ± 205.23	2.742 ± 263.25	0.924 ± 295.65	2.73 ± 362.25	1.44 ± 295.24	1.64 ± 231.24	5.95 ± 201.54	*3.33* ± 258.66
Er / 166[a]	23.40 ± 153.21	4.98 ± 172.53	11.65 ± 156.23	8.47 ± 142.32	2.47 ± 241.23	7.67 ± 295.64	4.56 ± 240.51	4.76 ± 195.26	17.54 ± 186.25	*9.50* ± 198.13
Tm / 169[a]	3.39 ± 252.31	0.65 ± 253.21	1.48 ± 263.24	1.09 ± 201.22	0.35 ± 285.62	1.07 ± 235.62	0.60 ± 301.25	0.59 ± 301.25	2.21 ± 262.25	*1.27* ± 261.89

(*continued on next page*)

Table 4 (*continued*)

Element	First collecting			Second collecting			Third collecting			Mean
	blend 1	*blend 2*	*blend 3*	*blend 1*	*blend 2*	*blend 3*	*blend 1*	*blend 2*	*blend 3*	
Yb / 172 [a]	19.86 ± 184.56	3.68 ± 186.24	9.74 ± 152.35	7.19 ± 194.52	2.11 ± 201.56	6.34 ± 298.35	3.70 ± 296.25	3.93 ± 201.23	14.25 ± 152.32	*7.87* ± 207.49
Lu / 175 [a]	3.06 ± 152.32	0.68 ± 201.23	1.31 ± 256.21	0.94 ± 218.23	0.38 ± 223.56	0.91 ± 325.5	0.58 ± 321.15	0.54 ± 250.12	2.14 ± 258.36	*1.17* ± 245.19
Pb / 208	0.30 ± 3.84	0.15 ± 4.52	0.24 ± 3.52	0.27 ± 4.31	0.07 ± 4.10	0.16 ± 0.3.02	0.11 ± 4.02	0.14 ± 2.14	0.24 ± 1.92	0.19 ± 3.49
Th / 232	0.11 ± 0.86	0.03 ± 1.55	0.06 ± 0.59	0.04 ± 0.44	0.02 ± 0.76	0.03 ± 0.12	0.02 ± 1.02	0.02 ± 0.64	0.08 ± 0.25	0.08 ± 0.69
U / 238	0.02 ± 2.06	0.02 ± 2.80	0.03 ± 1.86	0.04 ± 1.57	0.01 ± 1.02	0.03 ± 1.01	0.04 ± 1.26	0.04 ± 1.03	0.04 ± 1.01	0.03 ± 1.51

[a] μg/Kg.

analysis and spectrophotometric assays. Sensory panelists' analysis reported a good correlation with our instrumental data obtained with GC–MS. The key aroma compounds of our truffle was sulfury or garlic-like which corresponds to sulphur compounds such as bis(methylthio) methane and dimethyl sulfide. The Acqualagna *T. magnatum* was studied by ICP-MS and 48 elements were detected and quantified. Our white truffle was characterized by five main elements K, P, S, Ca and Mg, which give 97% of the total mass while the other elements investigated represent only 3%. CHNS elemental analysis showed in the tested sample that carbon to nitrogen ratio was higher than 6:1, despite sulfur and hydrogen being present in smaller amounts they still play important roles in the chemistry of truffles. Furthermore, this truffle exhibited good antioxidant activity so it could be valued by consumers for its positive impact on human health. The strong association between the elements identified in soil and in *T. magnatum* testifies to the fundamental role of the soil environment for the elementary composition of truffles.

In conclusion, this pioneering study on Acqualagna white truffles and it is could be a starting point considering that these fungi are still an unvalued source of compounds with high economic value.

CRediT authorship contribution statement

Diletta Piatti: Conceptualization, Formal analysis, Methodology, Writing – original draft. **Riccardo Marconi:** Data curation, Formal analysis. **Giovanni Caprioli:** Resources, Supervision, Writing – review & editing. **Marco Zannotti:** Conceptualization, Data curation, Supervision. **Rita Giovannetti:** Funding acquisition, Supervision. **Gianni Sagratini:** Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors thank the company T&C S.r.l. (Via Pole 26/A, 61041 Acqualagna, Pesaro-Urbino, Italy) for supplying the samples of Acqualagna white truffles.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.138089.

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