Compositional characterization of commercial sparkling wines from cv. Ribolla Gialla produced in Friuli Venezia Giulia

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Keywords:	Ribolla Gialla; sparkling wine; volatile compounds; tryptophan metabolites; lipids

Characterization of the metabolomic profile of Ribolla Gialla commercial sparkling wines

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Abstract

Ribolla Gialla (RG) is a white grape variety used in the production of high-quality sparkling wines. It is widespread in North-East Italy, particularly Friuli Venezia Giulia (FVG), as well as Slovenia. Because of its limited area of cultivation, however, there is little information about the composition and chemical characteristics of the sparkling wines produced. This work used different analytical approaches to characterize thirty-three commercial sparkling RG wines from different areas of FVG. The characteristics included the overall volatile profile and content of terpenes, C_{13}-norisoprenoids, lipids, and tryptophan metabolites. The aroma profile of RG wines was mainly characterized by fermentative esters and β-damascenone, whereas other norisoprenoids and varietal aromas were below the odor threshold. Appreciable amounts of certain fatty acids were found (e.g., palmitic acid), which could be potentially correlated with greater foam stability. However, high concentrations of tryptophan metabolites highlighted a higher risk of developing atypical ageing defects.

KEYWORDS: Ribolla Gialla; sparkling wine; volatile compounds; tryptophan metabolites; lipids

Introduction

Ribolla Gialla is a promising white grape variety that has recently been used for the production of premium sparkling wines, which have received good appreciation in international wine markets. Ribolla has been cultivated since ancient times in North-Eastern Italy (in the Friuli Venezia Giulia region), as well as Slovenia and the Ionian Islands (Kefalonia), where it is known as Rebula and Robola, respectively [1]. A certain number of studies have been carried out on the genetic identity and characterization of Ribolla grapes [1–4], but very few studies have dealt with the metabolomic profile of the wines [5, 6]. In addition, none of them have focused on the aromatic characteristics of sparkling wines obtained from Ribolla grapes, including their volatile organic compound (VOC) content, lipids, polyphenols, and tryptophan metabolites. All of these compounds are important because they may be linked with positive and negative aspects of wine quality.

VOCs are fundamental for determining the sensory characteristics of wine and its profile. These compounds are classified into four groups [7]: i) primary grape VOCs, which are present in the cells of grapes; ii) secondary grape aroma, which are formed during the pre-fermentation phases (crushing, pressing, and skin contact) or by thermal, chemical, and enzymatic reactions in must; iii) fermentation bouquet, which includes aroma compounds that form during alcoholic fermentation; iv) and maturation bouquet, which refers to the aroma compounds that develop when ageing the wine. Primary aroma is particularly important for the production of certain wine typologies, such as white sparkling wines. Furthermore, different aroma compounds may be produced after fermentation and ageing that further affect the aroma profile of the final wine, which are mainly due to pre-fermentative processes and yeast metabolism (e.g., C6-compounds, norisoprenoids, fatty acids, alcohols, esters, carbonyl compounds) [8–11].

Moreover, yeast autolysis takes place during the ageing of sparkling wine, which leads to the release of substances in the medium. Lipids represent a wide class of compounds among these substances [8]. Fatty acids may have a significant impact on the sensory properties of the wine.

A large part of the fatty acids originates from the firm tissues of the grapes, but the greatest amount is formed during alcoholic fermentation. Therefore, fatty acids may be present in wine in free or bound forms as ethyl esters. Both forms directly contribute to the flavor of the wine, while unsaturated fatty acids such as oleic, linolenic, and linoleic acids act as precursors of C6-aldehydes and alcohols with herbaceous notes, and are critical for yeast growth during fermentation [12]. Another part of the sensory assessment of sparkling wine is the relationship between the foaming properties and lipid compounds. Therefore, it has been reported that medium-chain free fatty acids C8, C10, and C12 are negatively correlated with foamability, while the ethyl esters of hexanoic, octanoic, and decanoic acids have a positive effect on the formation and stability of the foam [13].

The essential amino acid tryptophan (TRP) and its metabolites, especially indole-3-acetic acid (IAA), are considered to be potential precursors of 2-aminoacetophenone (2-AAP), which is an aroma compound that causes an atypical ageing off-flavor (ATA) in *Vitis vinifera* wines. The off-flavor is described with aroma descriptors such as “acacia blossom,” “furniture polish,” “wet wool,” “mothballs,” or “fusel alcohol,” which are combined with a loss of the typical bouquet of the grape variety [14]. Depending on the wine matrix, the detection threshold of 2-AAP varies from 0.5 to 1.5 µg L^{-1}. Strongly aromatic wines are able to integrate more than 1.5 µg L^{-1} of 2-AAP, while meager wines might be rejected as tainted by ATA with less than 0.5 µg L^{-1}. It is generally accepted that the ultimate cause of ATA development in white wines is a stress reaction in the vineyard triggered by drought, nutritional deficiency, and other viticultural factors, such as the time of harvest and leaf removal [14, 15].

Most papers on sparkling wines focus on the characterization of internationally known varieties with a worldwide distribution [16–19], but there are few studies on local or less relevant cultivars. Therefore, the aim of this work was to investigate the compositional profile of different Ribolla Gialla sparkling wines produced in different DOC (*Denominazione di Origine Controllata*) districts in the Friuli Venezia Giulia region in North-East Italy. The wines were

characterized for their chemical composition (pH, titratable acidity, residual sugars, alcohol, and sulfur dioxide), volatile profile (overall profile of aroma compounds, free and bound terpenes, and norisoprenoids), and content of lipids and tryptophan metabolites. The results are critically discussed with the aim of defining the metabolomic profile of Ribolla Gialla sparkling wines.

Materials and Methods

Reagents and materials

Hydrogen peroxide (30% w/w), ethanol (96% v/v), ACS grade hydrochloric acid (37%), anhydrous sodium sulfate and citric acid were purchased from Carlo Erba Reagents (Milan, Italy). Sodium chloride (99.5%) was obtained from Honeywell Fluka (Morris Plains, New Jersey). HPLC-grade solvents dichloromethane, *n*-pentane, and methanol, LC-MS grade methanol, acetonitrile, 2-propanol, chloroform, formic acid, ammonium formate, ethyl heptanoate, 1-heptanol, 2-octanol, ethyl hexanoate-d_{11}, 3-(2-hydroxy ethyl)-indole, kynurenic acid, D-tryptophan methyl ester, L-tyrosine ethyl ester, *N*-acetyl tyrosine ethyl ester, and 3,5-di-*tert*-4-butylhydroxytoluene (BHT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). C_7-C_{30} *n*-alkane solution in *n*-hexane was purchased from Supelco (Bellefonte, PA, USA), while cholesterol-d_7 and octadecanoic acid-d_3 were obtained from CDN Isotopes (Quebec, Canada). Finally, the glycosidase preparation (Rapidase Revelation Aroma) used for the determination of bound monoterpenes was obtained from Oenobrands (Montellier, France).

Wine samples

Local wineries located in the North-East Italian region of Friuli Venezia Giulia provided thirthy-three commercial Ribolla Gialla sparkling wines. The locations of the wineries are shown in Figure 1.

Basic analysis of wine samples

The basic quality control parameters (reducing sugars, alcoholic strength, total acidity, volatile acidity, pH, malic, lactic and tartaric acid) were determined by FTIR spectroscopy with a Winescan™ FT-120 instrument (FOSS, Hillerød, Denmark). All of the samples were analyzed two times, and the mean value of the two measurements was considered for the data analysis.

Determination of free and bound terpenes and norisoprenoids by SPE-GC-MS

Terpenes and C_{13}-norisoprenoids in free and bound forms were extracted on Isolute® 500-mg, 6-mL, C_{18} SPE cartridges (Biotage, Uppsala, Sweden) according to the method reported by Comuzzo et al. [20]. A GCMS-QP-2010 system (Shimadzu, Kyoto, Japan) was used for GC-MS analyses. Volatile compounds were separated on a J&W DB-Wax capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) provided by Agilent Technologies Inc. (Santa Clara, CA, USA) using the operating conditions described by Loira et al. [21].

Electron impact mass spectra were recorded at 70 eV and volatile compounds were tentatively identified by comparison of their mass spectra and retention times with those of standard compounds or by comparison of the mass spectrum with those reported in the Wiley 6, NIST 21, and NIST 107 mass spectrum libraries (provided by the manufacturer). Linear retention indices were calculated according to the retention times of *n*-alkanes and compared with those reported in literature. The semi-quantitative analysis was based on the internal standard method using 1-heptanol (312 mg L^{-1} in 96 % v/v ethanol) as the internal standard while considering the response factor to be equal to 1.00.

Determination of volatile compounds

The volatile compounds in the commercial wines were determined using two analytical approaches: liquid-liquid extraction (LLE) and solid-phase microextraction (SPME). Samples

were degassed before analysis by placing them in an ultrasonic bath (Falc Labsonic, Treviglio, Italy) for 2 min.

Liquid-liquid extraction (LLE-GC-MS)

Aroma compounds were determined as reported by Loira et al. [21] by mixing 5 mL of wine with 5 mL of a 30% (w/v) sodium chloride solution and 200 µL of internal standard (ethyl heptanoate, 422 mg L^{-1} in 96% v/v ethanol). The mixture was subjected to three extractions using 2.5 mL of pentane:dichloromethane (2:1 v/v) each. The organic phase was collected in a Pyrex tube, dehydrated with anhydrous sodium sulfate, and concentrated under nitrogen flow to a final volume of about 1 mL. The samples obtained were subjected to GC-MS analysis as reported previously[21].

SPME-GC-MS/MS

Degassed wine (1 mL) was spiked with 50 µL of 2-octanol at 2.13 mg L^{-1} (IS) in ethanol and placed in a 20-mL headspace vial containing 1.5 g of sodium chloride. Two technical replicates were prepared for each sample, along with a blank sample containing only sodium chloride. GC analysis was performed using a Trace GC Ultra gas chromatograph coupled with a TSQ Quantum Tandem mass spectrometer, which was upgraded to the XLS configuration and equipped with a Triplus autosampler (Thermo Fisher Scientific, Waltham, MA, USA). The method used was adopted by Carlin et al. [22]. Samples were incubated for 5 min at 35°C,s and VOCs were extracted for 20 min with a 2-cm-long 50/30-µm coated divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Supelco, Sigma Aldrich, Milan, Italy). After solid-phase microextraction, the fiber was desorbed for 3 min at 250°C in the GC system with the injector set in splitless mode. The fiber was reconditioned between each sample at 270°C for 7 min. Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1}.

The GC oven was equipped with a 30-m × 0.25-mm VF-WAXms column (Agilent Technologies Inc., Santa Clara, CA, USA) with a film thickness of 0.25 µm. The oven temperature was held for 2 min at 40°C after the injection, ramped at 6°C min^{-1} up to 250°C, and held for 5 min. The ion source was set at 230°C, and electron impact mass spectra were recorded at 70 eV. Data acquisition and analyses were performed using the Xcalibur Workstation software supplied by the manufacturer.

Determination of tryptophan metabolites by UHPLC-MS/MS

Sparkling wine samples were filtered at 0.22 µm by a Millex-GV filtration unit (Merc, Darmstadt, Germany) and directly collected in 2-mL HPLC amber vials. The analyses were performed on an Acquity UHPLC system provided with an autosampler and coupled with a XEVO TQMS mass spectrometer equipped with an electrospray source (Waters Corporation, Milford, MA, USA). Tryptophan metabolites were separated at a flow rate of 0.4 mL min^{-1} on a 1.8-µm 150 × 2.1-mm Waters Acquity HSS T3 column (Waters Corporation), which was conditioned at 40°C. A linear gradient of water containing 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) was used according to the conditions reported by Arapitsas et al.[23]. The injection volume was 10 µL, and the wine samples were stored in the autosampler at 6°C during analyses.

The MS conditions included a capillary voltage of 3.5 kV in positive mode and –2.7 kV in negative mode. The ion source temperature was set at 150°C, while the desolvation temperature was 500°C. Nitrogen was used as the cone gas with a flow rate of 50 L h^{-1}. The data were processed using Waters MassLynx (version 4.1) and TargetLynx software (Milford, MA, USA).

Analysis of lipid molecules by UHPLC-MS/MS

Lipid analysis was performed as described by Della Corte et al. [24] with slight modifications. An aliquot (3 mL) of degassed wine was introduced in a 50-mL Falcon tube and spiked with 30 µL of a methanolic solution containing cholesterol-d_7 and octadecanoic acid-d_3 (IS) at concentrations of 1.01 mg mL^{-1} and 1.002 mg mL^{-1}, respectively. Lipids were extracted two times in 21 mL of a chloroform–methanol solution (2:1 v/v) containing BHT (10 mg L^{-1}). The total lower lipid-rich layer was collected in 100-mL flasks, and the solvent was evaporated to dryness using a rotary evaporator. The samples were reconstituted in 300 µL of acetonitrile/2-propanol/water (65:30:5 v/v/v) and filtered (0.22 µm) into 2-mL HPLC amber vials for UHPLC-MS/MS analysis.

UHPLC separation was performed on a Dionex 3000 chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an autosampler and coupled with an API 5500 triple-quadrupole mass spectrometer (Sciex, Concord, Vaughan, ON, Canada), which was provided with an electrospray ion source (ESI). Lipids were separated on a 2.7-µm 150 × 2.1-mm RP Ascentis Express column (Sigma-Aldrich, Milan, Italy) set at 55°C. The injection volume was 5 µL, and the samples were stored in an autosampler at 10°C during the analyses. The mobile phase and chromatographic conditions are those described by Della Corte et al. [24], and the flow rate was 0.260 mL min^{-1}.

The spray voltage of the ESI source was set at 5500 V for positive mode and –4500 V for negative mode, and the source temperature was 250°C. The nebulizer (Gas 1) and heater gas (Gas 2) pressures were set at 40 and 20 psi, respectively. Ultra-high-purity nitrogen (99.999%) was used as both a curtain gas and collision gas (CAD) at 20 and 9 psi, respectively. Instrument control and data acquisition were performed by Analyst software (Applera Corporation, Norwalk, CT, USA), and the data were processed using MultiQuant, version 2.1 (Sciex, Concord, Vaughan, ON, Canada).

Statistical analysis

Statistica for Windows Version 8 (Statsoft, Tulsa, OK, USA) was used to calculate the means, standard deviations (SDs), coefficients of variation (CVs), minimum values (MIN), and maximum values (MAX) of the different analytical parameters determined for wine samples.

Results and Discussion

Basic quality parameters

Table 1 shows the results related to the basic chemical composition of the wines. The mean values, SDs, and CVs show that the samples are very homogeneous concerning alcoholic strength, titratable acidity, tartaric acid, pH, and even volatile acidity, which shows quite low values despite a slightly higher CV. This relatively homogeneous composition is probably related to the fact that the sparkling wines analyzed are all DOC productions and were manufactured in fulfillment of the standards reported in the DOC production guidelines.

However, some differences that are more relevant from a practical point of view were observed for residual sugars (min-max range: 6-19 g L^{-1}), malic acid (0.01-3.79 g L^{-1}), and lactic acid (0.00-2.75 g L^{-1}). These differences could be related to the application of different winemaking protocols by the producers, leading to different styles of wine for Ribolla Gialla DOC, even if they are in agreement to the DOC production guidelines. In regard to sugars, most of the wines analyzed showed a residual sugar content of less than 12 g L^{-1} or between 12 and 17 g L^{-1}. According to the European Commission Regulation (EC) n. 607/2009 [25], these wines are classified as *brut* and *extra dry*, respectively. Only one sample had higher sugar content (about 19 g L^{-1}) and was categorized as *sec* (or *dry*).

The variations of malic and lactic acid highlight the different ways of managing malolactic fermentation (MLF), which are probably connected with the different storage times of the wines on yeast lees after refermentation. Most of the wines did not contain lactic acid in appreciable

amounts, which was probably due to the lack of malolactic fermentation. MLF generally occurs during ageing on the lees because of the positive effect that they have on promoting the growth of lactic acid bacteria [26]. For this reason, most winemakers would consider Ribolla Gialla sparkling wine as a fresh and young wine according to the data.

Ribolla Gialla is generally refermented via the Martinotti/Charmat short method, which involves refermentation in stainless steel autoclaves without contact with the lees at the end of refermentation. However, six wine samples had a higher content of lactic acid. This accounted for the increase of the CV calculated for lactic acid in Table 1 and was combined with the decrease of malic acid concentration. This evidence may be related to the manufacture of certain Ribolla Gialla wines, with some products being kept in contact with the lees after refermentation in an autoclave. Such *sur-lies* aging may be prolonged for up to several months (2-4 months on average). In addition, certain winemakers also produce Ribolla Gialla by the traditional refermentation method in bottles.

Wine aroma profile

Table 2 shows the results of the qualitative and quantitative determination of the aroma compounds detected in wine samples by the different analytical techniques. Concerning non-varietal aromas, a total number of fifty-eight volatiles were tentatively identified in the wines, including acids, alcohols, esters, C6 compounds, diols, and carbonyls. Fatty acids are some of the most representative volatiles in the wines analyzed, and generally, they are described as having cheese, rancid, and fatty notes. If their amounts are higher than the odor detection threshold (ODT), they may negatively affect the organoleptic characteristics of the wines.

In the samples analyzed, the average concentration of fatty acid was often close and sometimes higher than their ODT. Nevertheless, their odor activity value (OAV), which is the ratio between the concentration and ODT, was in the range of 1-4 for many of the compounds analyzed. The exception was octanoic acid, which is the most representative fatty acid in the

volatile composition of Ribolla wines (OAV ≈ 30). These results suggest that fatty acids might have minor relevance in the aromatic characteristics of Ribolla Gialla. Acetic acid (which is responsible for the typical vinegar off-flavor) was found to be lower than ODT in all samples analyzed.

Interestingly, the variability among the samples (i.e., the CVs) for fatty acids and volatile compounds in general (Table 2) was higher than that detected for the basic parameters in Table 1. This highlights a certain level of differentiation among Ribolla Gialla DOC wines, which is probably related to a number of variables, such as those typically connected with the development of wine aromas. Concerning fatty acids, for instance, the differences among the samples might have resulted from the different origin of the grapes used, the amount of lipid substances in the musts, the diverse winemaking conditions, and the yeast strains used for fermentations [27].

Higher alcohols (HA) are also important volatiles among the compounds listed in Table 2. They are produced by yeasts during alcoholic fermentation as products of amino acid metabolism [11]. Their contributions to wine aroma vary from honey, rose, and floral characters (2-phenylethyl alcohol and benzyl alcohol) to pungent and solvent-like smells (1-propanol, 1-butanol, 2- and 3-methyl-1-butanol) [28], and the effects depend on their concentration [9]. The average amounts of HA observed in the wines analyzed were generally found to be lower than their ODT except for 2- and 3-methyl-1-butanol and 2-phenylethanol (ODT: 40 and 10 mg L^{-1}, respectively). This composition may be linked with the yeast strains used, as well as the winemaking conditions adopted and the amino acidic composition of the grapes. The presence of significant amounts of 2-phenylethanol is very interesting for its fresh rose-like odor [29], even if its OAV range was about 1-6.

Esters are the most representative class of volatile compounds found in Ribolla Gialla sparkling wines. Isoamyl acetate (3-methyl-1-butanol acetate), ethyl hexanoate, ethyl lactate, ethyl octanoate, diethyl succinate, and 2-phenylethyl acetate showed the highest concentrations. The

compounds eluted at higher retention times (e.g., diethyl malate) presumably had a minor impact on the aroma profile because of their low volatility. Some esters, such as ethyl butanoate and ethyl hexanoate, were close to or higher than their ODT (20 µg and 65 µg) in all the samples analyzed. Others such as isoamyl acetate, 2-phenylethyl acetate, and hexyl acetate were detected in only certain wines with concentrations higher than their ODT. Compounds such as ethyl hexanoate and isoamyl acetate were found to have significant OAVs (e.g., up to 18 for ethyl hexanoate, 85 for hexyl acetate, and approximately 200 for isoamyl acetate). This observation is very interesting from a sensory point of view because esters generally confer floral, fresh, and fruity notes (rose, banana, pear, green apple) to the wines [28, 30, 31], which are generally recognized as typical in Ribolla wines. For this reason, esters appeared to be an important component of the volatile profile of Ribolla Gialla.

Among esters, ethyl lactate and diethyl succinate are considered "ageing esters" (AE), and their content in wines generally increases during ageing and after malolactic fermentation [29, 32]. The same consideration is true for diethyl malate, but this compound is normally detected less frequently in wines. The concentration of AE was generally found to be lower than the ODT in most of the wines analyzed. However, in some samples, ethyl lactate had higher concentrations than its odor threshold (60 mg/L) in accordance with the amount of malic and lactic acid. In fact, the highest concentration of these esters was observed in the same products in which malolactic fermentation was hypothesized to have taken place (i.e., the wines where malic acid was not detected), as shown in Table 1. This may confirm that certain Ribolla Gialla sparkling wines are produced with a more or less prolonged period of *sur-lies* ageing or by refermenting them in bottle by the traditional method.

Carbonyls, diols, and C6 compounds appeared not to have an important contribution to the aromatic characterization of the samples, and most of the cases, they were detected at levels below their ODT. Hexanal was found to overcome its ODT in some samples, but at the low concentrations detected, it may or may not contribute to the fresh vegetal notes of Ribolla wines.

Table 3 reports the detected varietal aromas (terpenes and C_{13}-norisoprenoids) in free and bound form in the samples. Terpenes are well known to confer floral odors to wines [29]. In Ribolla, they were especially found in the free form, but in most cases, their average concentrations were below their ODT with a few exceptions for linalool and geraniol. Although it was present below its ODT, α-terpineol was the most abundant free terpenic alcohol in the wines. Interestingly, even if Ribolla Gialla cannot be considered as an aromatic variety, the concentrations detected for α-terpineol and linalool were higher than those normally observed in certain international white varieties, such as Pinot blanc, Pinot Gris, Chardonnay, and Sauvignon blanc [19]. Also β-citronellol, was found at lower levels with respect to its ODT (100 μg L^{-1}) [33], it showed similar average values to those of some Riesling wines [19].

Considering the glycosylated forms, only geraniol was significantly detected as both free and bound terpenol with a prevalence of the latter (combined) form of the molecule. According to other experiments, geraniol (with minor traces of nerol) was the only terpenic alcohol normally found in Ribolla Gialla grapes [34]. For this reason, in Ribolla Gialla, linalool, α-terpineol, and β-citronellol may be formed from such terpenic alcohols during grape processing. Citronellol, for instance, is reported to be produced by *S. cerevisiae* from geraniol and nerol during alcoholic fermentation, and other formation pathways have also been suggested for linalool (from geraniol) and α-terpineol (by cyclization of nerol) [35]. For both free and bound geraniol, the average values were generally lower than the ODT reported for the alcohol, but some of the samples showed significant levels of total geraniol (Table 3). Considering that the bound form generally prevails for this terpenol, one interesting way to improve the volatile profile of these wines might be the application of techniques to increase the release of free geraniol from its glycosides in the production process of Ribolla Gialla sparkling wines.

In contrast to terpenes, which did not appear particularly characteristic for the aroma profile of the wines, some C_{13}-norisoprenoids were detected in appreciable concentrations (Table 3). TDN and β-damascenone, for instance, overcame their sensory thresholds in different samples.

Norisoprenoids are generally not connected to specific aromatic grapevine varieties and generally have a low odor threshold. The ODT of β-damascenone is 0.04-0.06 μg L^{-1} in a model dilute alcohol solution [29], while a relatively wide range of ODTs was found in wine: Sefton et al. [36] reported 0.14 μg L^{-1} in deodorized white wine, 0.85 – 2.10 μg L^{-1} in deodorized red wine, and 7.00 μg L^{-1} in red wine. The presence of β-damascenone may be particularly interesting for Ribolla wines for not only the relatively high concentrations found but also because it is reported to modify the sensory perception of some esters. Escudero et al.[37] found that the addition of low levels of β-damascenone (0.85 μg L^{-1}) to a solution of esters increased the fruity notes of the mixture, while higher levels (3.5 μg L^{-1}) accounted for the development of strong raisin/dry-plum odors.

The amount of norisoprenoids is also dependent on the winemaking conditions. In general, β-damascenone is observed at higher concentrations in young wines [36], whereas for 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), a positive correlation between its occurrence in wine and ageing was reported.[38] Their odor descriptors range from floral (e.g., vitispiranes) and fruity-honey scents (β-damascenone) to notes of kerosene and petrol (for TDN) [39]. The presence of TDN among the compounds listed in Table 3 may represent further evidence about the ageing of certain Ribolla Gialla sparkling wines after refermentation.

Another interesting aspect related to norisoprenoids is the high concentration observed for some of them in bound form, particularly 3-oxo-α-ionol and 3-hydroxy-β-damascone, which are the most representative compounds. As for terpenes, Ribolla Gialla appeared to have an unexpressed aromatic potential for norisoprenoids. Thus, from a technical point of view, the optimization of winemaking process to maximize the release of this bound aromatic potential might be an interesting way to improve the quality of Ribolla Gialla sparkling wines.

Metabolomic fingerprint of tryptophan metabolites and indoles

The second group of metabolites analyzed was the amino acid tryptophan and its catabolites, as shown in Table 4. There were twenty-eight compounds detected, among which the sulfonated derivates of indole 3-lactic acid, tryptophol, indole 3-lactic acid glucoside, and indole 3-acetic acid are presented. Sulfonation is the addition of a sulfonic acid group ($-SO_3H$) to an organic compound and is a widespread industrial process used in a diverse range of products. It also has a major function in modulating biological activities that are known to occur in wine, which involve several metabolites such as polyphenols and indoles [23].

Sparkling wines are well known to have much lower amounts of tryptophan than other wines, which is probably due to the second fermentation [23]. Tryptophan values for the commercial samples ranged from 0.01 to 0.39 mg L^{-1} with a mean value of 0.13 mg L^{-1}, and which similarly occurred for its ethyl ester (0.44 mg L^{-1} mean value). TOL-SO_3H had higher amounts among all the other tryptophan metabolites with a mean value of 3.73 mg L^{-1}. The unsulfonated form of tryptophol varied from 0.01 to 1.08 mg L^{-1} in all samples. This could mean that the sulfonated/unsulfonated TOL ratio that favored TOL-SO_3H may also give rise to further products similar to 2-AAP.

The ILA (0.03 mg L^{-1}) and ILA-SO_3H (8.53 mg L^{-1}) pairing showed similar behavior to the previously described TOL and TOL-SO_3H pairing. Arapitsas et al. [23], found that young red wines and rosé wines had a much higher concentration of ILA and especially ILA-GLU, which is synthesized by plants [40]. This was probably due to maceration with the skins during winemaking. In contrast, white and especially sparkling wines had the lowest concentrations due to soft pressing and secondary fermentation, which necessitate further nitrogen consumption.

Sulfonated indole 3-acetic acid was detected at concentrations between 0.13 and 0.39 mg L^{-1}, while its parent compounds were detectable at very low concentrations. Hoenicke et al. [41] showed that the sulfonation of indoles in a model wine solution could be responsible for their

degradation and the formation of aromatic aminobenzenes such as 2-aminoacetophenone, which are responsible for some of the heavy aromatic characteristics of white wines. On other hand, the structural similarity of TOL to IAA could also cause the detection of 2-AAP or other similar aromatic compounds in wine. Because of the lack of anthocyanins and the low flavanol content in white wines, the sulfonation of indoles can increase the risk of developing an atypical off-flavor. Thus, the sparkling wines produced from the Ribolla Gialla variety might have a higher tendency to develop atypical ageing defects, which could also be promoted by inappropriate storage temperature.

Metabolomic fingerprint of lipids

Table 5 shows the twenty-nine lipid compounds found in the samples of commercial sparkling wines according to UHPLC-MS-MS. Most of the compounds found are saturated long-chain fatty acids (LCFAs, more than 12 carbon atoms), although the mid-chain fatty acids (MCFAs, 4–12 carbons) and their esters have a major influence on the organoleptic properties of wine. In addition to the fact that lipids are an integral part of solid grape tissues, they are also an important building block in wine yeasts, where the majority of LCFAs are esterified with glycerol or glycerophosphate to form mono-, di-, and tri-acylglycerides or glycerophospholipids, respectively [42].

The two most abundant LCFAs detected in the samples were palmitic acid (C16:0), which ranged from 5.21 to 11.49 mg L^{-1} with a mean value of 9.24 mg L^{-1}, and stearic acid (C18:0), which ranged from 4.43 to 8.40 mg L^{-1} (mean value: 6.49 mg L^{-1}). Previous studies showed that after the beginning of second fermentation, which is after the inoculation of the medium with yeast, the concentration of saturated fatty acids C16:0 slightly increased, while the proportion of C18:0 remained constant. However, after the end of the growth phase of the yeasts, the distributions of both saturated fatty acids decreased exponentially [43].

Unlike saturated fatty acids (SFA), the values of unsaturated fatty acids (UFA) were lower in the wine samples (*cis*-11-eicosanoic acid 0.11 mg L^{-1}, myristoleic acid 0.05 mg L^{-1}, linoleic acid 0.04 mg L^{-1}, palmitoleic acid 0.02 mg L^{-1}, and linolenic acid 0.01 mg L^{-1}). UFAs also had a much smaller proportion than SFAs. Because of their membrane fluidity, wine yeasts are able to modify the UFA/SFA ratio in their membrane composition in response to stress conditions, such as the presence of ethanol or cooler fermentation conditions under which fermentation takes place, especially for white wines. Normally, the UFA/SFA ratio is close to 1:1, but a deficiency of UFA leads to impaired biosynthesis of phospholipids and consequently stuck fermentations [12].

Similarly, Pueyo et al. [44] studied the effect of the total contents of linolenic acid and palmitic acid as compounds that best define foam stability in wines and foam height in the cavas, respectively. They found a positive correlation between these compounds and the measured properties, meaning that the wines with a greater amount of these fatty acids in the lipidic fraction could form more foam with high stability than those with lower concentrations. Since the analyzed samples in the present study contained a greater concentration of palmitic acid compared to the linolenic acid, it can be expected that foam formation of the Ribolla Gialla sparkling wines will be increased, which is a key parameter for determining the quality of sparkling wines.

In conclusion, Ribolla Gialla sparkling wines showed homogeneous characteristics concerning basic parameters (e.g., sugar content, alcoholic strength, pH, and titratable acidity) as well as their aroma composition. Sparkling Ribolla wines are generally characterized by low levels of free terpenols, and their aroma seems to be mostly characterized by the development of volatile esters and β-damascenone during fermentations, storage, and processing. The presence of these compounds is probably connected with the fresh and fruity notes that normally characterize the sparkling wines produced from Ribolla grapes.

A minor amount of malolactic fermentation was found, which reflects that two distinct wine styles coexist for Ribolla sparkling wines. The first characterizes young wines normally produced by refermentation in stainless-steel autoclaves (Martinotti/Charmat short refermentation method), and the second is based on a more or less prolonged ageing period on refermentation lees in an autoclave or bottles (according to the traditional refermentation method). The analysis of lipid molecules, tryptophan, and its metabolites highlighted other interesting features of Ribolla sparkling wines from a practical point of view. Ribolla Gialla shows a high ratio between saturated (e.g., palmitic acid) and unsaturated fatty acids (e.g., linolenic acid), which may lead to higher foam height in the sparkling wines produced and represents one of the key quality features of sparkling wines in general. In contrast, due to the high amounts of certain tryptophan metabolites, Ribolla Gialla could be prone to the formation of atypical ageing aromas. Considering that this specific Italian product has only recently been appearing in wine markets, further investigations should carefully consider these results to properly address the production practices and techniques towards producing high quality products, thus increasing the local and international competitiveness of Ribolla Gialla sparkling wines.

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Figure Captions

Figure 1. Geographical position of Friuli Venezia Giulia region. Grey areas mark the municipalities in which the wineries that supplied wine samples are located. The number of samples/wineries for each municipality are also reported.

Table 1. Chemical composition of Ribolla Gialla commercial wines. SD: standard deviation; MIN: minimum value; MAX: maximum value; CV: coefficient of variation.

Parameter	Mean ± SD	MIN	MAX	CV
alcoholic strength (% v/v)	11.68 ± 0.64	10.40	12.70	0.05
reducing sugars (g L^{-1})	10.64 ± 2.91	6.00	18.87	0.27
titratable acidity (g L^{-1})	5.94 ± 0.48	5.00	7.25	0.08
volatile acidity (g L^{-1})	0.31 ± 0.06	0.20	0.48	0.21
pH	3.22 ± 0.12	3.03	3.51	0.04
malic acid (g L^{-1})	2.08 ± 0.93	n.d. [a]	3.79	0.45
lactic acid (g L^{-1})	0.26 ± 0.63	n.d.	2.75	2.45
tartaric acid (g L^{-1})	2.83 ± 0.36	2.30	3.93	0.13

[a] n.d.: not detected

Table 2. Non-varietal aroma compounds detected in Ribolla Gialla commercial sparkling wines by different analytical approaches. Concentrations are expressed in µg L^{-1}.

Compounds	AM [a]	IM [b]	Mean ± SD [c]	MIN [d]	MAX [e]	CV [f]	ODT (µg L^{-1}) [g]	
Acids								
acetic acid	LLE	MS RI S	2624 ± 1206	1000	6388	0.46	200000	[33]
2-methylpropanoic acid	LLE	MS RI S	376 ± 168	n.d. [h]	877	0.45	230	[28]
butanoic acid	LLE	MS RI S	423 ± 126	218	812	0.30	10000	[33]
3-methylbutanoic acid	LLE	MS RI S	228 ± 242	n.d.	1103	1.06	250	[28]
hexanoic acid	LLE	MS RI S	4196 ± 1298	2111	7817	0.31	3000	[33]
heptanoic acid	LLE	MS RI	56 ± 70	n.d.	224	1.24	-	
octanoic acid	LLE	MS RI S	6923 ± 2390	2878	15070	0.35	500	[45]
nonanoic acid	SPME	MS S	454 ± 96	239	708	0.21	-	
decanoic acid	LLE	MS RI S	872 ± 736	n.d.	4043	0.84	1000	[45]
benzoic acid	SPME	MS S	5 ± 3	2	17	0.61	-	
dodecanoic acid	SPME	MS S	3 ± 2	1	9	0.67	-	
hexadecanoic acid	LLE	MS RI	2065 ± 2072	n.d.	7714	1.00	-	
Alcohols								
2-methyl-1-propanol	LLE	MS RI	8784 ± 2825	5569	18165	0.32	40000	[33]
1-butanol	LLE	MS RI S	210 ± 103	n.d.	515	0.49	40000	[46]
2- and 3-methyl-1-butanol	LLE	MS RI S	125666 ± 24151	85297	190584	0.19	40000	[28]
3-ethoxy-1-propanol	LLE	MS RI	172 ± 349	n.d.	1651	2.03	-	
2-phenylethanol	LLE	MS RI S	23645 ± 12214	9954	63961	0.52	10000	[33]
C6 compounds								
1-hexanol	LLE	MS RI S	957 ± 231	603	1443	0.24	2500	[28]

trans-3-hexen-1-ol	SPME	MS S	3 ± 1	2	9	0.41	1000	[28]
trans-2-hexen-1-ol	SPME	MS S	7 ± 6	n.d.	35	0.84	-	
cis-3-hexen-1-ol	LLE	MS RI S	39 ± 118	n.d.	646	3.01	400	[28]
hexanal	SPME	MS S	2 ± 7	n.d.	42	3.19	5	[47]
trans-2-hexenal	SPME	MS S	4 ± 1	1	8	0.36	82	[48]
Diols								
2,3-butanediol	LLE	MS RI	2987 ± 1079	1497	5601	0.36	-	
1-2 propandiol	LLE	MS RI	674 ± 250	258	1216	0.37	-	
Esters								
2-methyl-1-propanol acetate	SPME	MS S	0 ± 0	n.d.	1	1.07	-	
ethyl butanoate	SPME	MS S	44 ± 12	18	64	0.26	20	[33]
3-methyl-1-butanol acetate	LLE	MS RI	1182 ± 1168	n.d.	5908	0.99	30	[33]
methyl hexanoate	SPME	MS S	1 ± 0	n.d.	2	0.38	-	
ethyl hexanoate	LLE	MS RI S	554 ± 268	132	1165	0.48	65	[45]
hexyl acetate	LLE	MS RI	8 ± 32	n.d.	171	3.94	2	[47]
ethyl lactate	LLE	MS RI	20976 ± 33732	1589	141330	1.61	60000	[28]
methyl octanoate	SPME	MS S	3 ± 1	1	6	0.39	200	[47]
ethyl octanoate	LLE	MS RI S	433 ± 326	n.d.	982	0.75	580	[45]
3-methylbutyl lactate	SPME	MS S	1 ± 0	n.d.	1	0.30	-	
ethyl-2-hydroxy-4-methylpentanoate	SPME	MS S	6 ± 3	1	14	0.46	-	
methyl decanoate	SPME	MS S	0 ± 0	n.d.	1	0.54	4	[48]
ethyl 3-hydroxybutanoate	LLE	MS RI	42 ± 87	n.d.	295	2.04	-	
ethyl decanoate	LLE	MS RI S	36 ± 80	n.d.	307	2.25	200	[45]
methyl ethyl succinate	SPME	MS S	96 ± 54	25	283	0.56	-	
3-methylbutyl octanoate	SPME	MS S	5 ± 2	2	9	0.35	125	[49]
diethyl succinate	LLE	MS RI	2555 ± 1849	263	8391	0.72	100000	[28]
ethyl 9-decenoate	SPME	MS S	9 ± 11	1	50	1.15	-	
methyl salicylate	SPME	MS S	5 ± 6	1	29	1.31	40	[47]

2-phenylethyl acetate	LLE	MS RI S	136 ± 341	n.d.	1707	2.50	250	[33]
ethyl dodecanoate	LLE	MS RI	57 ± 125	n.d.	456	2.20	-	
diethyl malate	LLE	MS RI	5544 ± 4199	679	18639	0.76	760000	[28]
ethyl tetradecanoate	LLE	MS RI	57 ± 140	n.d.	504	2.48	-	
diethyle 2-hydroxypentanedioate	LLE	MS RI	605 ± 406	n.d.	1472	0.67	-	
ethyl hexadecanoate	LLE	MS RI S	1069 ± 1764	n.d.	6185	1.65	-	
ethyl hydrogen succinate	SPME	MS S	57 ± 30	19	125	0.52	1000000	[28]
Carbonyl compounds								
3-hydroxy-2-butanone (acetoin)	LLE	MS RI S	599 ± 311	162	1230	0.52	150000	[49]
furfural	SPME	MS S	29 ± 25	7	138	0.86	770	[28]
benzaldehyde	SPME	MS S	14 ± 28	4	164	2.05	350	[47]
3,4-dimethyl benzaldehyde	SPME	MS S	1 ± 0	n.d.	2	0.34	-	
Others								
dihydro-2-methyl-3(2*H*)-thiophenone	SPME	MS S	4 ± 3	1	11	0.59	-	
dihydro-2(3*H*)-furanone (γ-butyrolactone)	LLE	MS RI S	691 ± 204	316	1237	0.30	1000	[28]
methionol	LLE	MS RI	232 ± 145	n.d.	623	0.62	1000	[49]

[a] AM: analytical method; [b] IM: identification method (S comparison of mass spectra and retention time with those of standard compounds; RI comparison of order of elution with those reported in literature; MS comparison of mass spectra with those reported in mass spectrum libraries); [c] SD: standard deviation; [d] MIN: minimum value; [e] MAX: maximum value; [f] CV: coefficient of variation; [g] ODT: odor detection threshold; [h] n.d.: not detected

Table 3. Free and bound terpenes and norisoprenoids detected in Ribolla Gialla commercial sparkling wines by different analytical approaches. Concentrations are expressed in µg L^{-1}.

Compounds	AM [a]	IM [b]	Mean ± SD [c]	MIN [d]	MAX [e]	CV [f]	ODT (µg L^{-1}) [g]	
Free terpenes								
β-myrcene	SPME	MS S	0 ± 1	n.d. [h]	7	3.56	-	
limonene	SPME	MS S	2 ± 1	1	4	0.33	200	[47]
cis-linalool oxide (furanic)	SPE	MS RI S	4 ± 4	n.d.	12	0.88	6000	[28]
trans-linalool oxide (furanic)	SPE	MS RI S	1 ± 2	n.d.	9	1.61	6000	[28]
linalool	SPE	MS RI S	10 ± 15	n.d.	80	1.51	50	[28]
terpinen-4-ol	SPME	MS S	1 ± 0	1	2	0.34	340	[47]
α-terpineol	SPE	MS RI S	23 ± 34	n.d.	194	1.49	250	[49]
β-citronellol	SPE	MS RI S	2 ± 4	n.d.	18	1.81	100	[33]
nerol	SPME	MS S	1 ± 1	n.d.	6	0.80	60	[50]
geraniol	SPE	MS RI S	4 ± 10	n.d.	39	2.87	30	[49]
geranic acid	SPME	MS S	7 ± 4	2	23	0.65	-	
Free C_{13}-norisoprenoids								
β-damascenone	SPE	MS RI	5 ± 6	n.d.	28	1.27	7	[36]
3-oxo-α-ionol	SPE	MS RI	10 ± 11	n.d.	42	1.11	-	
riesling acetal	SPME	MS S	4 ± 2	n.d.	7	0.49	-	
vitispirane (isomer 1)	SPME	MS S	4 ± 3	2	14	0.68	800	[38]
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	SPME	MS S	5 ± 6	1	28	1.19	20	[38]
vitispirane (isomer 2)	SPME	MS S	14 ± 9	3	37	0.61	800	[38]
Bound terpenes								
nerol	SPE	MS RI S	3 ± 4	n.d.	20	1.54		

geraniol	SPE	MS RI S	13 ± 13	n.d.	51	1.03
1-hydroxylinalool	SPE	MS RI	2 ± 4	n.d.	15	1.99
geranic acid	SPE	MS RI	8 ± 9	n.d.	36	1.13
Bound C_{13}-norisoprenoids						
3-hydroxy-β-damascone	SPE	MS RI	6 ± 10	n.d.	50	1.83
trans,trans-2,6-dimethyl-2,6-octadiene-1,8-diol (Z8-hydroxygeraniol)	SPE	MS RI	11 ± 13	n.d.	56	1.15
3-oxo-α-ionol	SPE	MS RI	40 ± 32	n.d.	135	0.81
3-oxo-7,8-dihydro-α-ionol (blumenol C)	SPE	MS RI	16 ± 22	n.d.	103	1.38
3-hydroxy-7,8-dihydro-β-ionol	SPE	MS RI	1 ± 2	n.d.	7	2.02

[a] AM: analytical method; [b] IM: identification method (S comparison of mass spectra and retention time with those of standard compounds; RI comparison of order of elution with those reported in literature; MS comparison of mass spectra with those reported in mass spectrum libraries); [c] SD: standard deviation; [d] MIN: minimum value; [e] MAX: maximum value; [f] CV: coefficient of variation; [g] ODT: odor detection threshold; [h] n.d.: not detected

Table 4. Tryptophan metabolites detected in Ribolla Gialla commercial sparkling wines. Concentrations are expressed in mg L^{-1}. SD: standard deviation; MIN: minimum value; MAX: maximum value; CV: coefficient of variation.

Compound [a]	Mean	±	SD	MIN	MAX	CV
ILA-SO_3H	8.53	±	10.31	0.66	54.20	1.21
TOL-SO_3H	3.73	±	2.55	0.23	12.49	0.68
TYL	2.55	±	0.97	0.93	4.79	0.38
TYR	1.13	±	0.49	0.08	1.98	0.43
ILA-GLU-SO_3H	0.53	±	0.37	0.12	1.79	0.70
PHE	0.51	±	0.26	0.03	0.99	0.51
TRP-EE	0.44	±	0.21	0.11	0.89	0.48
TOL	0.29	±	0.27	0.01	1.08	0.93
ABA	0.22	±	0.11	0.02	0.46	0.50
IAA-SO_3H	0.20	±	0.07	0.13	0.39	0.35
ABA-GLU	0.13	±	0.05	0.04	0.26	0.38
TRP	0.13	±	0.10	0.01	0.39	0.77
ILA-GLU	0.10	±	0.05	0.01	0.25	0.50
TYR-EE	0.08	±	0.03	0.02	0.13	0.38
ILA	0.03	±	0.03	n.d.[b]	0.12	1.00
KYNA	0.02	±	0.01	n.d.	0.04	0.50
AA	0.01	±	0.06	n.d.	0.33	6.00
IAA-ASP	4.41	±	2.87	n.d.	12.35	0.65
N-TYR-EE	3.86	±	2.11	n.d.	9.63	0.55
KYN	2.42	±	1.98	n.d.	9.24	0.82
IAA	1.75	±	1.63	n.d.	8.84	0.93
N-TRP-EE	1.08	±	0.81	n.d.	3.47	0.75

[a] ILA-SO_3H: indole-lactic acid-2-sulfonate; TOL-SO_3H: tryptophol-2-sulfonate; TYL: tryptophol; TYR: tyrosine; ILA-GLU-SO_3H: sulfonated indole-3-lactic acid; PHE: phenylalanine; TRP-EE: tryptophan-ethyl ester; TOL: tryptophol; ABA: abscisic acid; IAA-SO_3H: sulfonated indole 3-acetic acid; ABA-GLU: glucoside of abscisic acid; TRP: tryptophan; ILA-GLU: indole 3-lactic acid glucoside; TYR-EE: tyrosine-ethyl ester; ILA: indole 3-lactic acid; KYNA: kynurenic acid; AA: anthranilic acid; IAA-ASP: indole 3-acetic acid conjugate with aspartic acid; N-TYR-EE: N-acetyl-tyrosine-ethyl ester; KYN: kynurenine; IAA: indole 3-acetic acid; N-TRP-EE: N-acetyl-tryptophan-ethyl ester.

[b] n.d.: not detected

Table 5. Lipid compounds detected in Ribolla Gialla commercial sparkling wines. Concentrations are expressed in mg L^{-1}. SD: standard deviation; MIN: minimum value; MAX: maximum value; CV: coefficient of variation.

Compounds	Mean	±	SD	MIN	MAX	CV
Fatty acids						
palmitic acid	9.24	±	13.52	5.21	11.49	1.46
stearic acid	6.49	±	9.32	4.43	8.40	1.44
myristic acid	0.31	±	0.84	0.11	0.50	2.68
oleic acid + *cis*-vaccenic acid	0.19	±	0.32	0.12	0.26	1.72
arachidic acid	0.15	±	0.40	0.08	0.26	2.70
cis-11-eicosanoic acid	0.11	±	0.03	0.10	0.12	0.28
heptadecanoic acid	0.06	±	0.14	0.04	0.10	2.19
myristoleic acid	0.05	±	0.04	0.05	0.06	0.78
linoleic acid	0.04	±	0.08	0.02	0.06	2.29
lignoceric acid	0.03	±	0.16	n.d. [a]	0.09	5.52
behenic acid	0.02	±	0.08	0.01	0.05	4.21
palmitoleic acid	0.02	±	0.36	n.d.	0.14	20.00
linolenic acid	0.01	±	0.04	0.00	0.03	8.00
Sterols						
ergosterol	0.18	±	0.97	0.03	0.35	5.54
lupeol	0.10	±	1.10	0.01	0.48	10.89
Glycerolipids						
1-linoleoyl-rac-glycerol	0.02	±	0.02	0.01	0.02	1.18
1-oleoyl-rac-glycerol	0.01	±	0.02	0.01	0.02	1.43
glyceryl tripalmitoleate	0.01	±	0.09	n.d.	0.04	6.43
Fatty acid esters						
ethyl stearate	0.14	±	0.18	0.10	0.17	1.31
ethyl palmitate	0.13	±	0.22	0.07	0.17	1.64
ethyl oleate	0.03	±	0.05	0.02	0.05	1.47
ethyl linoleate	0.01	±	0.01	0.00	0.01	2.00
methyl palmitate	0.08	±	1.20	n.d.	0.50	15.19
methyl stearate	0.04	±	0.36	n.d.	0.15	9.73
methyl oleate	0.01	±	0.06	n.d.	0.03	6.67
Triacylglycerols						
tripentadecanoin	0.01	±	0.04	n.d.	0.02	5.00

[a] n.d.: not detected

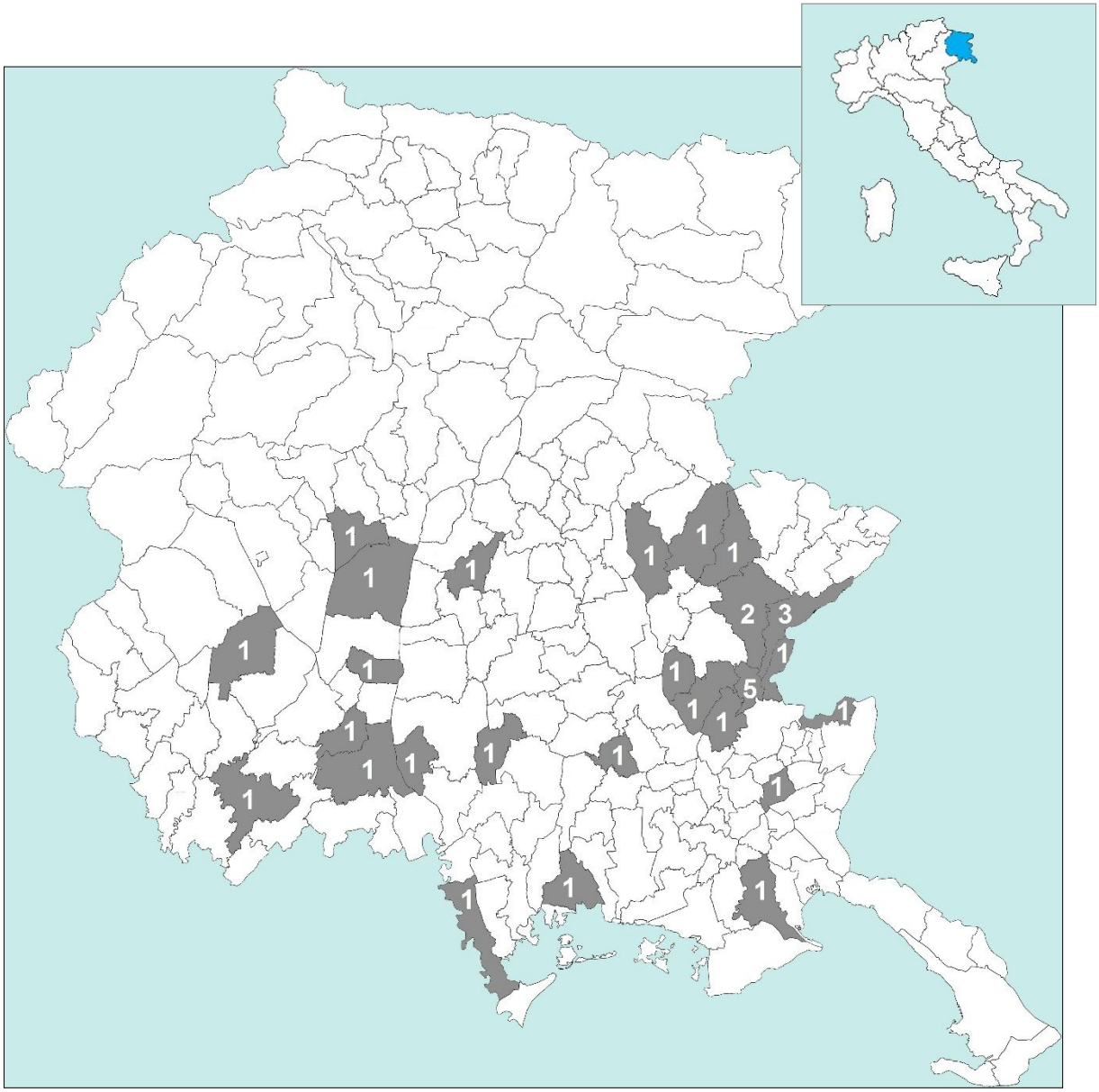

Fig. 1.