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Complete List of Authors:	Perini, Matteo; Fondazione Edmund Mach, Strojnik, Lidija; Institut Jožef Stefan Paolini, Mauro; Fondazione Edmund Mach, Food Quality and Nutrition Camin, Federica; Fondazione Edmund Mach Istituto Agrario di San Michele all'Adige,

GC/c-IRMS for improving the detection of authenticity of grape must

M.Perini[a], L. Strojnik[bc], M. Paolini[a*], F.Camin[a]

[a] Fondazione Edmund Mach, via E. Mach 1, 38010 San Michele all'Adige, Italy;

[b]Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia;

[c]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia;

[*] Corresponding Author: e-mail mauro.paolini@fmach.it, tel. num. 0461-615331, fax num. 0461-615288.

Abstract

Since ancient times, grape must and wine have been considered one of the most sophisticated matrices and, in the last years, the continuous rise in volumes and prices of grapes and wine has encouraged frauds and adulterations in the oenological field. One of the most common adulteration is the sugar addition to grape must in the form of cane, beet sugar, or syrup coming from vegetable sources like cereals or fruits. Since 1990, the International Organisation of Vine and Wine (OIV) has issued specific official isotopic methods to fight against this practice, but they are not always effective. With the aim to develop a new method able to identify the sugar addition, we compared the $\delta^{13}C$ value of sugar extracted from grape must and analysed by EA-IRMS to the $\delta^{13}C$ value of proline analysed by GC-c-IRMS, after extraction and derivatization. $\delta^{13}C$ and $\delta^{15}N$ of proline have also been tested as potential geographical markers.

In addition, the carbon isotopic composition of two characteristic grapes must sugars (myo and scyllo – inositol) was measured by GC-c-IRMS after derivatization, in order to identify the illegal correction of their concentration.

On the basis of the obtained results, we can conclude that the compound specific isotope analysis represents a novel analytical tool to support and improve certification and control procedures.

Keywords: GC/c-IRMS, stable isotope analysis, proline, myoinositol and scylloinositol, chaptalization, grape must

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1. Introduction

According to the International Organisation of Vine and Wine (OIV) definition, wine is the beverage resulting exclusively from the partial or complete alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must that is the liquid product obtained naturally or by physical processes from fresh grapes.[1] OIV has established for wine a unique minimum alcoholic strength of 8.5 % vol., with the flexibility to be reduced to 7 % vol to guarantee its stability.[2] To react this limit the addition of sources of sugar to grape must before fermentation is allowed. While the addition of rectified must to grape must or wine before or during fermentation to increase the alcoholic strength is always permitted in all the countries, the use of beet sugar and cane sugar is legal only for specific winegrowing regions and vintages e.g. in Brazil, Canada, Chile, China, France, Germany, Japan, New Zealand, Switzerland, United Kingdom, United States.[3] In Italy the addition of exogenous sugar (beet or cane) is forbidden and it constitutes a fraud (chaptalisation) by unscrupulous wine producers to increase profit.[4]

In 1990 the European Commission (EC) adopted isotopic methods as the first official analytical methods (now OIV MA-AS-311-05, MA-AS-312-06, and MA-AS2-12) to detect and combat these types of grape must and wine frauds.[5] They are based on the analysis of the isotopic ratios of hydrogen (D/H) and carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) in ethanol distilled from wine and after must fermentation. Unfortunately, during the last decades, adulterations became increasingly sophisticated,[6] thus the development of even more powerful analytical methods for must and wine authentication is a great challenge.

The direct stable isotope ratio analysis of single chemical compounds, normally measured by a GC-c-IRMS technique, provide a means of obtaining a more in-depth understanding in respect to the traditionally analysis of bulk products.[7] Examples of application are reported for single amino acids in wheat and durum wheat samples, to discriminate between organically and conventionally agricultural practices,[8] vanillin, to discriminate between natural and

synthetic ones,[9] fatty acid, to identify the adulteration of high value oils with cheaper ones,[9] wine volatile compounds, to reassess the water status in vineyards.[10] In relation to wine, Spitzke at al. developed a GC-c-IRMS method to analyse $^{13}C/^{12}C$ of higher alcohols, for example, 2-methylpropan-1-ol, 2- and 3-methylbutan-1-ol; butan-2,3-diol, 2-phenyl-1-ethanol, and glycerol.[11] By correlating the $\delta^{13}C$ of higher alcohol compounds (such as 2-/3-methylbutan-1-ol) with that of wine ethanol (R^2 = 0.829), they were able to improve the detection of chaptalisation. Other groups,[12,13] have also investigated the isotopic composition of ethanol and glycerol by GC-c-IRMS as alternative techniques to determine adulteration of wines.

This paper illustrates two studies that propose new specific compound methods to improve the identification of grape must authenticity.

In the first one we compared, for the first time, the $\delta^{13}C$ of the sugar fraction extracted from grape must using the official method UNI ENV 12140:1997, with the $\delta^{13}C$ of proline after extraction and derivatization.[14] Among the amino acids the most abundant in wine and grapes is the proline with a content ranging between 30 and 85%,[15] and this makes it possible to obtain a sufficient quantity of it for derivatization and analysis. Moreover, yeast does not require proline as a nitrogen source and it is therefore maintained in wine. The isotopic ratio of nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) was also analysed using proline, as additional marker to trace the geographical origin of grapes. As reported by Paolini et al.[8] the isotopic ratio of nitrogen could be a useful additional marker, because differently from H, O and C, nitrogen in grape compounds derives directly from the soil, and therefore the factors affecting its isotopic variability are different from those affecting the other 3 isotopic ratios.[16]

In the second study we tested two characteristic grape must sugars (myo and scyllo - inositol) after derivatization, to see if the analysis of their $\delta^{13}C$ values could be useful in identifying the illegal correction of their concentration in concentrated and rectificated grape must (CRM). These polyalcohols originate in the grape berry and their quantification has been already

proposed by Monetti et al. to control the authenticity of the CRM, because they are not retained by the resins used for concentration process and they are not present in other purified commercial sugars.[17] A minimum content of myo-inositol (750 mg/kg sugar) and a myo- and scyllo-inositol ratio of 20 or less have been suggested as authenticity indexes.[17] In Regulation 479/2008 this proposal was only partially adopted and today official CRM controls focus on the presence of myo-inositol alone, without considering its commercial availability, its levels in musts, and the relationship between the two isomers.[18] There is the suspect that fraudsters correct the concentration of these two polyalcohols, in particular myo-inositol, by adding commercial pure myo- and scyllo-inositol to fake grape must concentrate originated from other fruits (e.g. date or tapioca) or from a mix of sugars. The $\delta^{13}C$ variability of authentic and fake polyalcool have been explored in this study and tested on samples coming from the market, in order to verify their validity as fraud detectors.

2. Materials and methods

2.1. Reagents and solutions

Proline ≥98%, myo-Inositol ≥99%, scyllo-Inositol ≥98% and cation-exchange resin (Amberlite® IR120) were purchased from Sigma Aldrich. All other solvents (acetone, dichloromethane, ethanol, ethyl acetate and isopropanol) and reagents (acetic anhydride, silylating agent HMDS+TMCS+Pyridine 3:1:9, and triethylamine) used were of analytical grade and purchased from Sigma Aldrich and VWR.

2.2. Sampling

2.2.1 Italian grape must, first study

59 authentic Italian grape musts were sampled during 2016 (N=36) and 2017 (N=23) harvests (Table 1). One to five samples were collected from 15 different Italian regions (Abruzzo, Campania, Emilia Romagna, Friuli Venezia Giulia, Lazio, Liguria, Lombardy, Marche,

Piedmont, Puglia, Sardinia, Sicily, Tuscany, Trentino Alto Adige, Umbria and Veneto), at the usual technological harvest time (early, medium and late harvesting) (Supplementary Figure 1S). 25 different grape varieties were considered in order to describe natural variability (Table 1). The sampling was supervised by the technicians of the Edmund Mach Foundation (San Michele all'Adige - Italy), who personally followed all the harvesting and grape crushing stages for must production (as part of the Italian Project 'Climaitalia2020'). Proline was extracted from all the samples and derivatized and subjected to the analysis of $\delta^{13}C$ and $\delta^{15}N$.

2.2.2 Rectified concentrated grape must, second study

10 authentic rectified concentrated grape musts of the 2018 harvest were collected (Table 2). In addition, 7 commercial CRM samples from Italy and Spain were also considered.

2.3 EA-IRMS analysis

2.3.1 Extraction of sugar fraction,

The sugar fraction was extracted using the official method UNI ENV 12140. In brief, the solid fraction of the must (50 mL) was removed by centrifugation at 1400 g for 10 min. 2 g of powdered calcium hydroxide were added to the supernatant liquid and the solution was heated in a bath at 90°C for 3 min. The precipitate was separated by centrifugation of the hot solution (3 min at 1400 g) and the supernatant liquid was acidified with 0.1 mol/L sulfuric acid in order to obtain a pH value of approximately 5. After a night in the refrigerator (4°C), the supernatant liquid was freeze-dried to obtain the sugar fraction.

2.3.2 EA-IRMS analysis of $\delta^{13}C$

The $\delta^{13}C$ value of sugar fraction samples was measured using an elemental analyser (Flash EA 1112, Thermo Scientific, Bremen, Germany), coupled with an IRMS (DELTA V, Thermo Scientific) through a ConFlo IV dilutor device (Thermo Finningan, Bremen, Germany).

2.4 GC-c-IRMS analysis

2.4.1 Isolation and derivatization of proline

The grape must sample was adjusted to pH 2.3 with HCl 0.01 M and 100 µl of a norleucine solution (8 mg/mL in 0.1M HCl) was added as internal standard. Proline was isolated using an Amberlite IR120 cation-exchange resin, previously saturated with H^+ at all exchange sites as reported by Takano et al.[19] 5 mL of grape must sample were passed through the resin column and washed with water. Finally, proline was eluted with NH_4OH (10% w/w) and then dried under N_2.[20]

Proline was analysed after *N*-acetylisopropyl derivatization, following the method reported by Corr at al.[14] Briefly, proline was esterified with 1 mL of acetyl chloride:isopropanol mixture (1:4 v/v) at 100 °C for 1h and then acylated with 1 mL of acetic anhydride-triethylamine-acetone mixture (1:2:5 v/v) at 60°C for 10 min. The reagents were evaporated under a gentle stream of nitrogen at room temperature, 1 mL of saturated sodium chloride-water solution and 1 mL of ethyl acetate were added and then mixed vigorously. The organic layer containing the derivatized proline was dried under nitrogen, residual water was removed with dichloromethane and finally dissolved in ethyl acetate (200 mL).

2.4.2 Derivatization and quantification of myo and scyllo inositol

Derivatization and quantification of myo- and scyllo-inositol in grape must were performed following the official method RESOLUTION OIV-OENO 419C-2015. Briefly, 5 g of grape must were weighted in a 50 mL volumetric flask, adding 1 mL of xylitol standard solution (10000 mg/L in water) and then brought to volume with water. 100 µl of the final solution were dried under a gentle stream of nitrogen and 400 µl of the derivatization mixture (HMSD + TMCS + Pyridine, 3:1:9) were added. The vial was closed and placed in the oven at 80°C for 60 minutes.

Myo- and scyllo-inositol were quantified using an Agilent Intuvo 9000 GC-FID system, injecting 3 µl in split mode (1:10) into a 30 m HP-5MS Ultra Inert (0.32 mm I.D. × 0.25 µm film thickness; Agilent) with H_2 as carrier gas (2 mL/min). The oven temperature was programmed starting at 100°C, raised to 240°C by 10°C/min, then raised to 260°C by 2°C/min, and finally raised to 310°C by 50°C/min and held at this temperature for 5 minutes. The injector temperature was set at 270°C.

2.4.3 GC-c-IRMS analysis of proline

The $\delta^{13}C$ and $\delta^{15}N$ values of proline were determined using a Trace GC Ultra (GC IsoLink + ConFlo IV, Thermo Scientific) interfaced with an IRMS (DELTA V, Thermo Scientific) and with a single-quadrupole MS detector (ISQ Thermo Scientific). 0.5 µL of each sample was injected in splitless mode and a 60 m HP-INNOWAX capillary column (0.32 mm I.D. × 0.25 µm film thickness; Agilent) was used with He as carrier gas (1.4 mL/min). The injector was at 250 °C and the injector was at 250 °C and the oven temperature program was set as follows: initial 40 °C held for 2 min, ramped to 140 °C at 40 °C/min, followed by ramped to 180 °C at 2.5 °C/min for, then ramped to 220 °C at 6 °C/min and finally ramped to 250 °C at 40 °C/min for 15 min.

The eluted proline was combusted into N_2 and CO_2 in a combustion furnace reactor operated at 1030°C. During $\delta^{15}N$ analysis, a liquid nitrogen trap was added after the reactor to block the CO_2.

In order to monitor derivatization step and instrumental performance, a standard proline was derivatized and the $\delta^{13}C$ and $\delta^{15}N$ values were measured using GC-c-IRMS before and after each analytical run and compared with the isotopic composition measured directly by EA-IRMS ($\delta^{13}C$ = -24.5‰ and $\delta^{15}N$ = +1.1‰) without any derivatization step.

2.4.4 GC-c-IRMS analysis of myo and scyllo inositol

The $\delta^{13}C$ values of myo and scyllo inositol were determined injecting 2µl in splitless mode in a 30 m HP5-MS capillary column (0.32 mm I.D. × 1.00 µm film thickness; Agilent) with He as carrier gas at 1.5 mL/min. The injector was at 250 °C and the oven temperature program used is as follows: held for 20 min at 150°C, increased to 220°C at 10°C/min, and finally ramped to 320°C at 40°C/min and held for 10 min..

In order to monitor the derivatization step and instrumental performance, a standard mix of myo and scyllo-inositol with know carbon isotopic composition (-37.2‰ and -36.9‰ respectively) was derivatized and the $\delta^{13}C$ values were measured using GC-C-IRMS before and after each analytical run.

2.5 Data expression

All the $\delta^{13}C$ and $\delta^{15}N$ values are reported in relation to the known isotopic composition of the reference CO_2 and N_2 gasses introduced into the ion source of the IRMS at the beginning and end of each EA and GC run. All samples were measured three times, and the isotope ratio was expressed in δ‰ versus V-PDB (Vienna - Pee Dee Belemnite) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$ according to equation 1.

$$\delta = \left[\frac{(R_s - R_{std})}{R_{std}} \right] \quad (1)$$

where R_s is the isotope ratio of the sample and R_{std} is the isotope ratio of the internationally accepted standard.

The $\delta^{13}C$ and $\delta^{15}N$ values of pure non-derivatized proline were determined by EA-IRMS. The isotopic values $\delta^{13}C$ and $\delta^{15}N$ were calculated against two working in-house standards (caseins), the first one with $\delta^{13}C$=−21.98‰ and $\delta^{15}N$=7.38‰ while the second one with $\delta^{13}C$=−30.60‰ and $\delta^{15}N$=-3.40‰. They have themselves been calibrated against international reference materials: fuel oil NBS-22 with $\delta^{13}C$=−30.03‰, sucrose IAEA-CH-6 with

220 $\delta^{13}C$=−10.45‰ (IAEA-International Atomic Energy Agency, Vienna, Austria), and L-
221 glutamic acid USGS 40 with $\delta^{13}C$=−26.39‰ and $\delta^{15}N$=−4.52‰ (U.S. Geological Survey,
222 Reston, VA, USA) for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ and potassium nitrate IAEA-NO_3 ($\delta^{15}N$=+4.7‰)
223 from IAEA for $^{15}N/^{14}N$.
224 The $\delta^{15}N$ and $\delta^{13}C$ values of proline in grape must were calculated against the standard proline,
225 analysed before and after each sample. The instrumental data were corrected on the basis of
226 the difference between the $\delta^{15}N$ and $\delta^{13}C$ values of the standard proline in GC-c-IRMS (mean
227 of six results, three before and three after the samples) and in EA-IRMS, which was in any case
228 always lower than 0.5‰ and ±1.6‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Likewise, the $\delta^{13}C$ value
229 of myo and scyllo inositol in must was calculated against the mixture of standard myo and
230 scyllo inositol, analysed before and after each sample.
231 The effective $\delta^{13}C$ values of proline, myo and scyllo inositol were obtained applying an
232 empirical correction in order to remove the contribution of the derivatization reagents.
233 Correction factor was calculated by determining the $\delta^{13}C$ value of the underivatized standard
234 (EA-IRMS) and the $\delta^{13}C$ value of the derivatized standard (GC-c-IRMS):

235 $$n_{cd}\boldsymbol{\delta}^{13}C_{cd} = n_c\boldsymbol{\delta}^{13}C_c + n_d\boldsymbol{\delta}^{13}C_d \quad (2)$$

236 where n is the number of moles of carbon, and the subscripts c, d, and cd represent the
237 compound of interest, the derivative group, and the derivatized compound, respectively.

238

239 **2.6 Repeatability limit and uncertainty**

240 One sample of grape must and one of grape CRM were treated and derivatized one time a
241 month for one year to calculate the within-laboratory reproducibility standard deviation (SR)
242 of $\delta^{13}C$ and $\delta^{15}N$ analysis of proline, myo and scyllo inositol. The analytical uncertainty (U) of
243 the $\delta^{13}C$ and $\delta^{15}N$ of proline, myo and scyllo inositol analysis, expressed as the coverage factor

k=2 multiplied for the SR (within-laboratory reproducibility standard deviation), was 0.4‰, whereas the reproducibility limit expressed as k*rad 2*SR (with k=2) was 0.6‰.[21]

To determine the repeatability limit for $\delta^{13}C$ and $\delta^{15}N$ of proline, 10 replicates of a grape must sample were derivatized, and each of the them were analysed using GC-c-IRMS. For the $\delta^{13}C$ of myo and scyllo-inositol a sample of grape CRM was considered. The standard deviation obtained (1σ) was 0.5‰ for $\delta^{13}C$ of proline and 0.2‰ for all other parameters (Supplementary Table 1S).

2.7 Statistical analysis

The data were analysed with the Statistica software 13.1 (StatSoft Inc., Tulsa, OK, USA). Statistically significant correlations were verified using the Pearson correlation test. Statistically significant differences were observed using a Tukey HSD test. In all the statistical analysis, the cutoff value was set at $p<0,05$, which is associated with a significant difference between groups of values.

3. Results and discussion

Study 1

In plants, the sugar fraction (mainly glucose and fructose) is the result of photosynthesis, that takes place in the green plastids of plant cells using carbon dioxide and water as precursors. Sugars, through glycolysis and the Krebs cycle, are used by the plants to synthesise 2-oxoglutarate that thanks to the action of glutamate synthase is converted in glutamate.[22] Proline comes from glutamate, which is converted to proline by two consecutive reduction steps catalyzed first by pyrroline-5- carboxylate synthase (P5CS) and then by pyrroline-5-carboxylate reductase (P5CR).[23]A strict correlation between the isotopic composition of sugars and amino acids is expected, given the biosynthetic path described above.

To investigate this relationship in real must samples, we considered 59 authentic musts cover all of Italy and from two harvest years. The $\delta^{13}C$ values of both sugar and proline ranged between -30‰ and -23‰, in line with the botanical origin of the matrix. Indeed, the Vitis Vinifera species belongs to plants with a C3 photosynthetic cycle and its $\delta^{13}C$ normally ranges from -29‰ to -25‰.[30]

As reported in Table 1, it seems there is not a big isotopic fractionation between sugar fraction and proline. The $\Delta^{13}C$ ($\delta^{13}C$ sugar - $\delta^{13}C$ proline) varies in a narrow range between -1.7‰ and +1.6‰.

By comparing the $\delta^{13}C$ values of the sugar fraction with the $\delta^{13}C$ of the amino acid proline, we obtained a significant ($P < 0.01$, $R^2=0.71$) linear relationship ($\delta^{13}C$ sugar= 0.70* $\delta^{13}C$ proline - 7.65; Figure 1). We can define a threshold value for the relationship, calculating 95% of the confidence interval of the regression line from the following equation:

$$y = 0.70x - 7.65 - 2*s$$

where "y = 0.70x – 7.65" is the linear regression model obtained from the 59 data points, "2" is the Student t and "s" is the standard deviation of the residues (difference between calculated and observed value), which in this case is 1.59.

As the addition of exogenous cane sugar to must changes only the $\delta^{13}C$ of the sugar fraction and not that of proline, as here demonstrated (Supplementary Table 2S), the fraudulent practice of sugar addition changes this relationship, which could go beyond the threshold value, even if the $\delta^{13}C$ of the sugar fraction is not outside the upper limit defined by the wine databank (EU Regulations 273 and 274/2018) and by the natural grape variability (Guideline for Grape Juice of the Association of the Industries of Juices and Nectars from Fruits and Vegetables (AIJN)) and equal to -23‰.

To demonstrate the possibility of improving chaptalisation detection, we simulated the adulteration of the 59 musts by adding an increasing % of cane sugar ($\delta^{13}C$ = +12‰) and calculated the number of samples identified as adulterated with cane sugar, both on the basis of the $\delta^{13}C$ value of the sugar fraction (which must be lower than -23‰) and that of the relationship between the 2 $\delta^{13}C$ (Figure 2). In all cases, the relationship improved detection of the chaptalisation of must. With an addition of 20% cane sugar this new method made it possible to identify all of our adulterated samples as actually adulterated, while with an addition between 5 and 20% the detection increases from a minimum of 6% (5 samples rather than 1 with 5% addition) to 72% (59 samples rather than16 with 20% addition) (Figure 2).

$\delta^{13}C$ analysis of both the sugar fraction and the amino acid proline and their correlation can therefore be considered as a reliable internal standard for improving detection of the fraudulent addition of sugar to must.

Our results can be used to check the authenticity of both grape must and wine. Indeed, the proline utilization by *Saccharomyces cerevisiae* as source of nitrogen requires the presence of oxygen and therefore, in anaerobic fermentation conditions, as happens in wine production, proline is conserved in wine without isotopic fractionation.[8] Moreover, the $\delta^{13}C$ of wine ethanol is strictly correlated with that of the relevant must sugar with a mean difference of 1.7‰ between them.[24] Therefore we can calculate both regression line and 95% confidence limits also for $\delta^{13}C$ of ethanol vs $\delta^{13}C$ of proline in wine, by predicting $\delta^{13}C$ of ethanol from $\delta^{13}C$ of sugar (Figure 1).

The isotopic ratio of carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) were also analysed in proline, with the aim to evaluate the power of this marker to trace the geographical origin of grapes. We investigated $\delta^{13}C$ of proline patterns in grape must across Italy (Figure 3A). Carbon isotope measurements described a gradient of more depleted values in the north Italy to more enriched values in the south Italy (Figure 3A). This was not surprising

316 since stable carbon isotope ratios of plant materials, are primarily related to the photosynthetic
317 pathway used by a plant, even if $\delta^{13}C$ in foodstuffs exhibits some geographical dependence
318 linked to water stress and humidity during cultivation, although these differences are often very
319 small in comparison to other isotopes.[25]
320 As reported in Figure 3B it is not possible to identify a strictly correlation between $\delta^{15}N$ of
321 proline and the region from which the sample comes from. This could be due to the different
322 agricultural practices adopted even within neighboring areas (e.g. organic or chemical
323 fertilisation) or to the pedological characteristics of the soil. Indeed, inorganic fertilisers have
324 $\delta^{15}N$ values close to those of atmospheric N_2 (from -6‰ to +6‰), whereas manure and other
325 organic fertilisers can be substantially enriched (from +1‰ to +37‰).[26]
326 Paolini et al. reported that, despite nitrogen isotope fractionation through the chain soil - wine,
327 the $\delta^{15}N$ values of leaves, grapes, wine and in particular of proline in must and wine maintain
328 the variability of $\delta^{15}N$ in the growing soil.[8] Samples from Sardinia have the higher $\delta^{15}N$ values
329 (+9.8‰). This is in line with previous data reported for other matrices coming from this region,
330 such as casein from pecorino cheese.[27] The main reason could be the water stress due to the
331 high temperature, which affects the biological turnover of nitrogen isotopes.[28]

332 **Study 2**

333 Myo-inositol, a carbocyclic sugar, is synthesized in grapes from glucose-6-phosphate (G-6-P)
334 in two steps. First, an inositol-3-phosphate synthase enzyme (e.g. ISYNA1) isomerize the G-
335 6-P to myo-inositol 1-phosphate, which is then finally dephosphorylate to give free myo-
336 inositol by an inositol monophosphatase enzyme (e.g. IMPA1). It is normally abundant in must
337 grape (more than 750 mg/kg sugar) while its stereoisomer scyllo-inositol is less abundant (more
338 than 38 mg/kg sugar).[17,29] In addition, a ratio of 20 or lower between myo- and scyllo-inositol
339 has been suggested as authenticity index.[17] These limits of content are a useful routine control
340 tool of CRM, but they can be easily falsified by fraudulent addition to the must of pure

commercial polyalcohols in the right concentration. To describe the natural $\delta^{13}C$ variability of these must components, several samples of authentic CRM were analysed. In all these samples the myo- and scyllo-inositol content was measured and all of them fell within the limits suggested by Monetti et al. (Table 2).[17]

As reported in Table 2 myo-inositol and scyllo-inositol, that differ chemically only in the three-dimensional orientations of their atoms in space, showed a similar $\delta^{13}C$ range between -29.4‰ and -25.0‰, which is typical for plants with a C3 photosynthetic cycle.[30] The maximum difference between the two $\delta^{13}C$ is 2.4‰ and it can be used as a limit beyond which an addition of one of the two polyalcohols can be suspected. In addition, a limit of -25‰ for both the polyalcohols could be proposed (Figure 4). While commercial myo-inositol shows an average value of $\delta^{13}C$ of -29.0±0.3‰ probably due to the origin of the grapes from which the commercial product is extracted at low cost, the $\delta^{13}C$ values of scyllo-inositol is typical of C4 plants (-11.8±0.3‰). Scyllo-inositol is rare and expensive, not being widely available from commercial sources, and for this reason it must be synthesized. Several synthetic approaches to produce scyllo-inositol are known.[31] Rodriguez et al. present a concise synthesis of scyllo-inositol starting from inexpensive D-glucose.[32]

Commercial glucose is normally produced via the enzymatic hydrolysis of starch,[33] that belongs to the C4 plant with a range of variability between −10 and −16‰,[30] and this could justify the values found for scyllo-inositol. The illegal addition of this polyalcohol should be easily identified. A difference between the two $\delta^{13}C$ higher than 2.4‰ or a value of $\delta^{13}C$ scyllo-inositol higher than -25‰ could be interpreted as an index of adulteration. Figure 4 shows the isotopic composition of myo- and scyllo-inositol of commercial CRM samples compared with the authentic one. Only one sample falls within the variability limit, while five samples showed higher $\delta^{13}C$ scyllo-inositol values (higher than -25‰). Sample Q (Table 2) is characterized by

a high $\delta^{13}C$ myo-inositol value. Maybe in this case, too, commercial myo-inositol from a C4 plant source was used.

$\delta^{13}C$ analysis of proline by GC/c-IRMS combine with the analysis of the sugar fraction represents a novel analytical tool to support and improve the detection of fraudulent addition of cane sugar to must and wine. While $\delta^{13}C$ of proline could be useful as geographical indicator while $\delta^{15}N$ of proline seems too correlate with the agronomic practices adopted. Moreover, the compound-specific analysis of $\delta^{13}C$ of myo and scyllo-inositol represents a useful tool to identify the illicit addition of these two polyalcohol in must concentrated obtained not from grape (e.g. from cereal) to mime the composition of an authentic grape CRM.

Abbreviation used

CRM – concentrated rectified must

GC - gas chromatographyI

RMS - Isotopic Ratio Mass Spectrometry

Supporting information description

Table 1S: Repeatability of methods ($\delta^{13}C$ data corrected)

Table 2S: Effect of addition of sugar cane to a grape must on the $\delta^{13}C$ and $\delta^{15}N$ value of proline

Figure 1S: Map of sampling

Novelty statement

Bibliography

(1) Office international de la vigne et du vin. *Code International Des Pratiques œnologiques*; 1996.

(2) International Organisation of Vine And Wine. *International Standard for the Labelling of Wines*; 2015.

(3) Robinson, J.; Johnson, H. *The World Atlas of Wine 8th Edition*; Mitchell Beazley, 2019.

(4) Holmberg; Holmberg. Wine Fraud. *International Journal of Wine Research*. 2010, p 105. https://doi.org/10.2147/ijwr.s14102.

(5) Union, E. Regulation EC N. 2676/90 of the Commission Regulation (EEC) No 000/90 of 17 September 1990 on Determining Community Methods for the Analysis of Wines. *Off J Eur Commun* **1990**, *272*, 1–192.

(6) Schieber, A. Introduction to Food Authentication. *Modern Techniques for Food Authentication*. 2018, pp 1–21. https://doi.org/10.1016/b978-0-12-814264-6.00001-3.

(7) van Leeuwen, K. A.; Prenzler, P. D.; Ryan, D.; Camin, F. Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry for Traceability and Authenticity in Foods and Beverages. *Comprehensive Reviews in Food Science and Food Safety*. 2014, pp 814–837. https://doi.org/10.1111/1541-4337.12096.

(8) Paolini, M.; Ziller, L.; Bertoldi, D.; Bontempo, L.; Larcher, R.; Nicolini, G.; Camin, F. δ15N from Soil to Wine in Bulk Samples and Proline. *Journal of Mass Spectrometry*. 2016, pp 668–674. https://doi.org/10.1002/jms.3824.

(9) Perini, M.; Pianezze, S.; Strojnik, L.; Camin, F. C and H Stable Isotope Ratio Analysis Using Solid-Phase Microextraction and Gas Chromatography-Isotope Ratio Mass Spectrometry for Vanillin Authentication. *J. Chromatogr. A* **2019**, *1595*, 168–173.

(10) Spangenberg, J. E.; Vogiatzaki, M.; Zufferey, V. Gas Chromatography and Isotope Ratio Mass Spectrometry of Pinot Noir Wine Volatile Compounds (δ 13 C) and Solid Residues (δ 13 C, δ 15 N) for the Reassessment of Vineyard Water-Status. *Journal of Chromatography A*. 2017, pp 142–155. https://doi.org/10.1016/j.chroma.2017.08.038.

(11) Spitzke, M. E.; Fauhl-Hassek, C. Determination of the 13C/12C Ratios of Ethanol and Higher Alcohols in Wine by GC-C-IRMS Analysis. *European Food Research and Technology*. 2010, pp 247–257. https://doi.org/10.1007/s00217-010-1267-x.

(12) Calderone, G.; Naulet, N.; Guillou, C.; Reniero, F. Characterization of European Wine Glycerol: Stable Carbon Isotope Approach. *J. Agric. Food Chem.* **2004**, *52* (19), 5902–5906.

(13) Jung, J.; Jaufmann, T.; Hener, U.; Münch, A.; Kreck, M.; Dietrich, H.; Mosandl, A. Progress in Wine Authentication: GC–C/P–IRMS Measurements of Glycerol and GC Analysis of 2,3-Butanediol Stereoisomers. *European Food Research and Technology*. 2006, pp 811–820. https://doi.org/10.1007/s00217-006-0274-4.

(14) Corr, L. T.; Berstan, R.; Evershed, R. P. Optimisation of Derivatisation Procedures for the Determination of delta13C Values of Amino Acids by Gas Chromatography/combustion/isotope Ratio Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21* (23), 3759–3771.

(15) Orte, P. H.; Guitart, A.; Cacho, J. Amino Acid Determination in Musts and Wines by HPLC After Derivatization With Phenylisothiocyanate. *Am. J. Enol. Vitic.* **1997**, *48* (2), 229–235.

(16) Santesteban, L. G.; Miranda, C.; Barbarin, I.; Royo, J. B. Application of the Measurement of the Natural Abundance of Stable Isotopes in Viticulture: A Review. *Australian Journal of Grape and Wine Research*. 2015, pp 157–167. https://doi.org/10.1111/ajgw.12124.

(17) Monetti, A.; Versini, G.; Dalpiaz, G.; Reniero, F. Sugar Adulterations Control in Concentrated Rectified Grape Musts by Finite Mixture Distribution Analysis of Themyo-Andscyllo-Inositol Content and the D/H Methyl Ratio of Fermentative Ethanol. *Journal of Agricultural and Food Chemistry*. 1996, pp 2194–2201.

436 https://doi.org/10.1021/jf9502358.
437 (18) Regulation, C. No 479/2008 of 29 April 2008 on the Common Organisation of the Market
438 in Wine, Amending Regulations (EC) No 1493/1999, (EC) No 1782/2003,(EC) No
439 1290/2005,(EC) No 3/2008 and Repealing Regulations (EEC) No 2392/86 and (EC) No
440 1493/1999. *Official Journal of the European Union* **2008**, 1–61.
441 (19) Takano, Y.; Kashiyama, Y.; Ogawa, N. O.; Chikaraishi, Y.; Ohkouchi, N. Isolation and
442 Desalting with Cation-Exchange Chromatography for Compound-Specific Nitrogen
443 Isotope Analysis of Amino Acids: Application to Biogeochemical Samples. *Rapid*
444 *Commun. Mass Spectrom.* **2010**, *24* (16), 2317–2323.
445 (20) Styring, A. K.; Kuhl, A.; Knowles, T. D. J.; Fraser, R. A.; Bogaard, A.; Evershed, R. P.
446 Practical Considerations in the Determination of Compound-Specific Amino Acid δ15N
447 Values in Animal and Plant Tissues by Gas Chromatography-Combustion-Isotope Ratio
448 Mass Spectrometry, Following Derivatisation to theirN-Acetylisopropyl Esters. *Rapid*
449 *Communications in Mass Spectrometry*. 2012, pp 2328–2334.
450 https://doi.org/10.1002/rcm.6322.
451 (21) Bell, S. *A Beginner's Guide to Uncertainty of Measurement*; 2001.
452 (22) Forde, B. G.; Lea, P. J. Glutamate in Plants: Metabolism, Regulation, and Signalling.
453 *Journal of Experimental Botany*. 2007, pp 2339–2358.
454 https://doi.org/10.1093/jxb/erm121.
455 (23) Verbruggen, N.; Hermans, C. Proline Accumulation in Plants: A Review. *Amino Acids*.
456 2008, pp 753–759. https://doi.org/10.1007/s00726-008-0061-6.
457 (24) Rossmann, A.; Schmidt, H.-L.; Reniero, F.; Versini, G.; Moussa, I.; Merle, M. H. Stable
458 Carbon Isotope Content in Ethanol of EC Data Bank Wines from Italy, France and
459 Germany. *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*. 1996, pp 293–301.
460 https://doi.org/10.1007/bf01192881.
461 (25) Hurley, J. M.; West, J. B.; Ehleringer, J. R. Stable Isotope Models to Predict Geographic
462 Origin and Cultivation Conditions of Marijuana. *Sci. Justice* **2010**, *50* (2), 86–93.
463 (26) Bateman, A. S.; Kelly, S. D. Fertilizer Nitrogen Isotope Signatures. *Isotopes in*
464 *Environmental and Health Studies*. 2007, pp 237–247.
465 https://doi.org/10.1080/10256010701550732.
466 (27) Manca, G.; Camin, F.; Coloru, G. C.; Del Caro, A.; Depentori, D.; Franco, M. A.; Versini,
467 G. Characterization of the Geographical Origin of Pecorino Sardo Cheese by Casein
468 Stable Isotope (13C/12C and15N/14N) Ratios and Free Amino Acid Ratios. *Journal of*
469 *Agricultural and Food Chemistry*. 2001, pp 1404–1409.
470 https://doi.org/10.1021/jf000706c.
471 (28) Heaton, T. H. E. The 15N/14N Ratios of Plants in South Africa and Namibia: Relationship
472 to Climate and Coastal/saline Environments. *Oecologia*. 1987, pp 236–246.
473 https://doi.org/10.1007/bf00379365.
474 (29) Sanz, M. Inositols and Carbohydrates in Different Fresh Fruit Juices. *Food Chemistry*.
475 2004, pp 325–328. https://doi.org/10.1016/j.foodchem.2003.12.001.
476 (30) O'Leary, M. H. Carbon Isotopes in Photosynthesis. *BioScience*. 1988, pp 328–336.
477 https://doi.org/10.2307/1310735.
478 (31) Husson, C.; Odier, L.; Vottéro, P. J. A. New Conditions for the Synthesis of Scyllo-
479 Inositol Starting from Myo-Inositol. *Carbohydrate Research*. 1998, pp 163–165.
480 https://doi.org/10.1016/s0008-6215(98)00003-2.
481 (32) Rodriguez, J.; Walczak, M. A. Synthesis of Asymmetrically Substituted Scyllo -Inositol.
482 *Tetrahedron Letters*. 2016, pp 3281–3283. https://doi.org/10.1016/j.tetlet.2016.06.038.
483 (33) Eliasson, A.-C. *Starch in Food: Structure, Function and Applications*; CRC Press, 2004.

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Figure caption.

Figure 1: Correlation between $\delta^{13}C$ of proline and sugar fraction. The limit accepted by the Association of the Industries of Juices and Nectars from Fruits and Vegetables (AIJN) and the limit calculated based on the 95% of the confidence intervals were reported as dotted line.

Figure 2: Improvement in must chaptalization detection by the number of samples identified as adulterated with cane sugar.

Figure 3: Geographical variability of $\delta^{13}C$ (A) and $\delta^{15}N$ (B) of proline in authentic Italian grape must.

Figure 4: Variability of $\delta^{13}C$ of myo and scyllo inositol of authentic grape CRM and samples collected on the market.

Figure 1S: Map of sampling

525 Table 1 Experimental values of the isotope composition $\delta^{13}C$ and $\delta^{15}N$ of proline, $\delta^{13}C$ of the
526 sugar fraction and variation between the two $\delta^{13}C$

Number	Year of Harvest	Italian region	Variety of grape	$\delta^{15}N$ proline (‰. vs AIR)	$\delta^{13}C$ proline (‰. vs V-PDB)	$\delta^{13}C$ sugar (‰. vs V-PDB)	Δ $\delta^{13}C$ sugar - $\delta^{13}C$ proline
1	2016	Veneto	Oseleta	5.6	-24.2	-24.7	-0.5
2	2016	Veneto	Corvinone	4.2	-25.2	-24.9	0.3
3	2016	Veneto	Manzoni	4.7	-27.0	-28.1	-1.1
4	2016	Veneto	Chardonnay	4.8	-27.0	-26.5	0.5
5	2016	Friuli VG	Sauvignon	0.2	-27.6	-27.7	-0.1
6	2016	Friuli VG	Malvasia	0.0	-29.0	-28.2	0.8
7	2016	Piedmont	Nebbiolo	0.6	-28.8	-27.1	1.7
8	2016	Piedmont	Nebbiolo	-0.5	-28.8	-27.4	1.4
9	2016	Piedmont	Nebbiolo	-0.1	-28.8	-27.6	1.2
10	2016	Piedmont	Nebbiolo	-0.3	-26.2	-24.7	1.5
11	2016	Piedmont	Riesling	1.6	-27.8	-26.2	1.6
12	2016	Lombardy	Pinot	4.9	-27.4	-28.2	-0.8
13	2016	Lombardy	Chardonnay	0.5	-28.6	-27.6	1.0
14	2016	Emilia Romagna	Barbera	-1.5	-25.8	-26.1	-0.3
15	2016	Emilia Romagna	Cabernet	-2.5	-26.6	-26.6	0.0
16	2016	Toscany	Sangiovese	4.7	-25.8	-25.7	0.1
17	2016	Toscany	Sangiovese	4.0	-26.2	-25.6	0.6
18	2016	Lazio	Syrah	2.6	-26.2	-25.7	0.5
19	2016	Lazio	Vermentino	0.6	-27.0	-26.0	1.0
20	2016	Lazio	Malvasia	2.4	-25.0	-26.1	-1.1
21	2016	Lazio	Montepulciano	0.8	-28.8	-28.1	0.7
22	2016	Marche	Verdicchio	4.4	-28.8	-27.2	1.6
23	2016	Marche	Verdicchio	6.5	-30.0	-28.4	1.6
24	2016	Marche	Montepulciano	4.5	-29.4	-28.7	0.7
25	2016	Marche	Sangiovese	3.7	-29.8	-28.8	1.0
26	2016	Marche	Verdicchio	2.8	-28.4	-27.7	0.7
27	2016	Abruzzo	Trebbiano	3.2	-28.6	-27.3	1.3
28	2016	Umbria	Grechetto	3.7	-27.6	-27.6	0.0
29	2016	Umbria	Sagrantino	2.4	-28.8	-28.9	-0.1
30	2016	Campania	Aglianico	4.5	-25.2	-25.9	-0.7
31	2016	Puglia	Primitivo	6.5	-26.0	-25.4	0.6
32	2016	Puglia	Primitivo	5.0	-26.0	-25.6	0.4
33	2016	Sicily	Insolia	3.7	-24.2	-25.7	-1.5
34	2016	Sicily	Insolia	2.0	-26.4	-25.4	1.0
35	2016	Sardinia	Vermentino	6.4	-26.6	-25.6	1.0
36	2016	Sardinia	Monica	9.8	-24.2	-23.3	0.9
			Mean	**3.0**	**-27.2**	**-26.7**	**0.5**
			SD	**2.6**	**1.6**	**1.4**	**0.8**
			Min	-2.5	-30.0	-28.9	-1.5
			Max	9.8	-24.2	-23.3	1.7

Number	Year of Harvest	Italian region	Variety of grape	δ^{15}N proline (‰. vs AIR)	δ^{13}C proline (‰. vs V-PDB)	δ^{13}C sugar (‰. vs V-PDB)	Δ δ^{13}C sugar - δ^{13}C proline
1	2017	Trentino AA	Muller Thurgau	8.0	-28.4	-28.7	-0.3
2	2017	Trentino AA	Pinot grigio	6.5	-26.4	-27.5	-1.1
3	2017	Trentino AA	Kerner	9.2	-27.2	-28.4	-1.2
4	2017	Veneto	Chardonnay	2.8	-27.0	-26.6	0.4
5	2017	Veneto	Chardonnay	1.2	-24.6	-25.8	-1.2
6	2017	Friuli VG	Chardonnay	-1.0	-28.4	-27.9	0.5
7	2017	Piedmont	Riesling	2.4	-26.2	-27.2	-1.0
8	2017	Piedmont	Nebbiolo	0.4	-25.4	-26.7	-1.3
9	2017	Piedmont	Nebbiolo	2.9	-27.8	-27.4	0.4
10	2017	Piedmont	Nebbiolo	4.5	-27.2	-26.8	0.4
11	2017	Piedmont	Nebbiolo	5.4	-25.8	-24.9	0.9
12	2017	Lombardy	Pinot	5.8	-27.0	-26.8	0.2
13	2017	Toscany	Sangiovese	2.6	-23.6	-23.4	0.2
14	2017	Lazio	Malvasia	5.2	-27.0	-26.8	0.2
15	2017	Lazio	Montepulciano	2.6	-28.0	-27.7	0.3
16	2017	Marche	Verdicchio	6.0	-27.0	-25.6	1.4
17	2017	Marche	Verdicchio	8.0	-27.2	-25.5	1.7
18	2017	Marche	Verdicchio	3.7	-24.6	-26.2	-1.6
19	2017	Abruzzo	Montepulciano	4.1	-24.6	-24.6	0.0
20	2017	Abruzzo	Montepulciano	6.2	-23.4	-23.0	0.4
21	2017	Abruzzo	Montepulciano	6.2	-25.2	-23.8	1.4
22	2017	Umbria	Grechetto	5.4	-24.4	-25.4	-1.0
23	2017	Campania	Aglianico	2.1	-24.4	-24.2	0.2
			Mean	**4.4**	**-26.1**	**-26.1**	**0.0**
			SD	**2.6**	**1.5**	**1.6**	**0.9**
			Min	-1.0	-28.4	-28.7	-1.6
			Max	9.2	-23.4	-23.0	1.7

Table 2 Experimental values of the isotope composition $\delta^{13}C$ of myo and scyllo inositol and variation between the two $\delta^{13}C$ in authentic samples of CRM and in samples from the market.

	Sample	Geographical origin	$\delta^{13}C$ scyllo inositol (‰. vs V-PDB)	$\delta^{13}C$ myo-inositol (‰. vs V-PDB)	Δ $\delta^{13}C$ scyllo - $\delta^{13}C$ myo
Authentic CRM samples	A	Puglia	-28.6	-28.2	-0.4
	B	Italy	-28.6	-26.2	-2.4
	C	Italy	-28.6	-29.4	0.8
	D	Italy	-28.2	-29.4	1.2
	E	Italy	-27.0	-27.4	0.4
	F	Italy	-27.0	-28.2	1.2
	G	Italy	-26.6	-29.0	2.4
	H	Italy	-25.0	-25.8	0.8
	I	Sicily	-25.0	-26.2	1.2
		Mean	-27.2	-27.8	0.6
		SD	1.5	1.4	1.3
		Min	-28.6	-29.4	-2.4
		Max	-25.0	-25.8	2.4
Market's CRM	L	Italy	-23.4	-25.8	2.4
	M	Italy	-22.2	-27.8	5.6
	N	Italy	-21.8	-27.8	6.0
	O	Spain	-21.8	-27.4	5.6
	P	Spain	-19.0	-23.4	4.4
	Q	Spain	-25.0	-21.0	-4.0
	R	Spain	-25.8	-25.8	0.0

566 Table 1S: Repeatability of methods (**δ^{13}C** data corrected)

	δ^{13}C proline (‰, vs V-PDB)	δ^{15}N proline (‰, vs AIR)	δ^{13}C scyllo inositol (‰. vs V-PDB)	δ^{13}C myo-inositol (‰. vs V-PDB)
1	-24.4	5.3	-28.6	-29.0
2	-25.4	5.5	-28.7	-29.1
3	-25.4	5.2	-29.0	-29.0
4	-25.0	5.3	-28.5	-28.6
5	-24.4	5.3	-28.5	-28.6
6	-24.4	5.7	-29.0	-28.5
7	-25.4	5.2	-28.6	-28.7
8	-24.8	5.3	-28.2	-28.7
9	-25.0	5.4	-28.5	-29.0
10	-25.8	5.5	-28.6	-29.0
Mean	**-25.0**	**5.4**	**-28.6**	**-28.8**
SD	**0.5**	**0.2**	**0.2**	**0.2**

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Table 2S: Effect of addition of sugar cane to a grape must on the $\delta^{13}C$ and $\delta^{15}N$ value of proline

% w/w of cane added to must	$\delta^{13}C$ proline (‰, vs V-PDB)	$\delta^{15}N$ proline (‰, vs AIR)
0	-24.4	5.4
14	-25.0	5.3
28	-24.4	5.5
32	-24.4	5.2

Graphic for table of content

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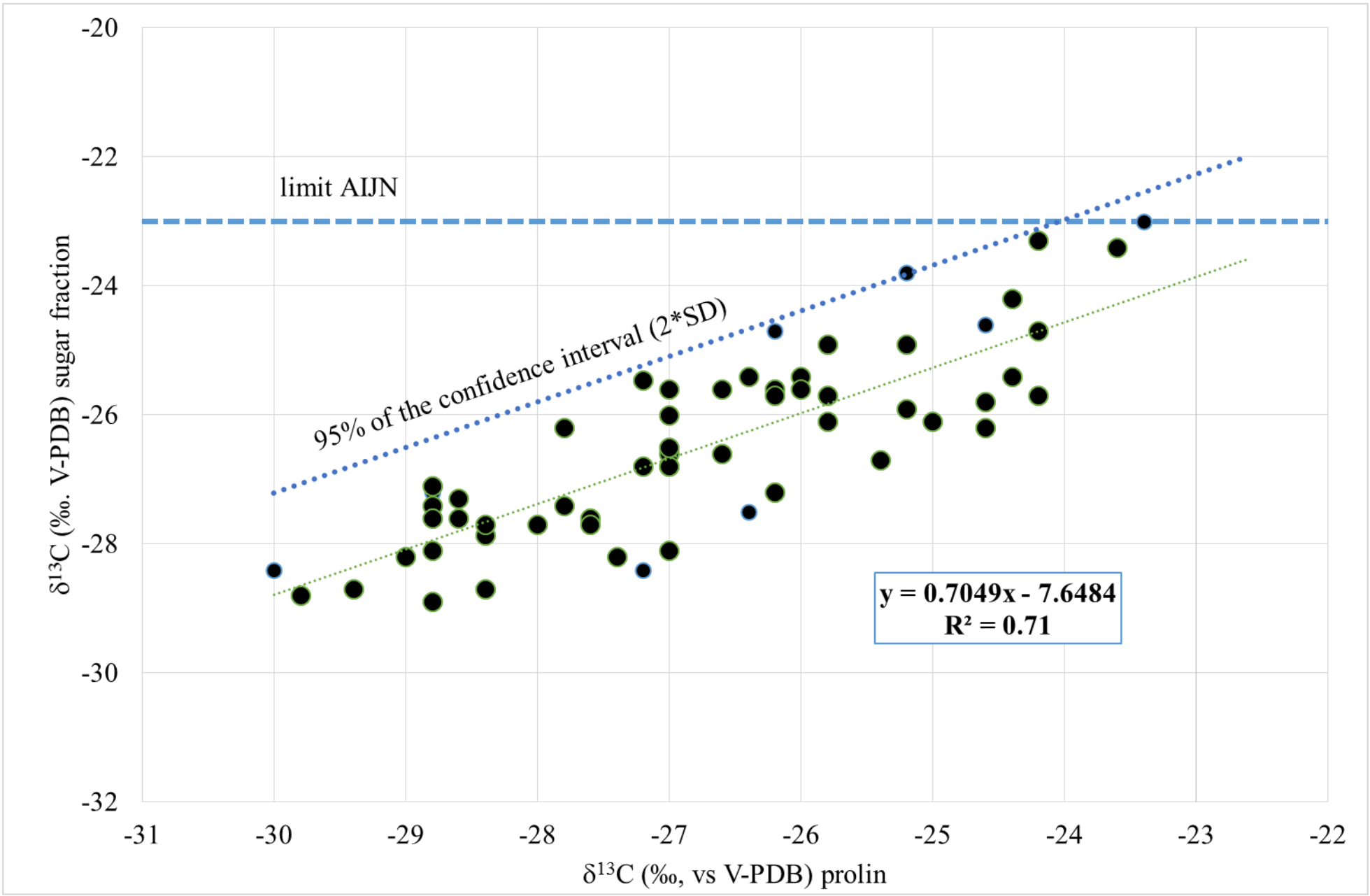
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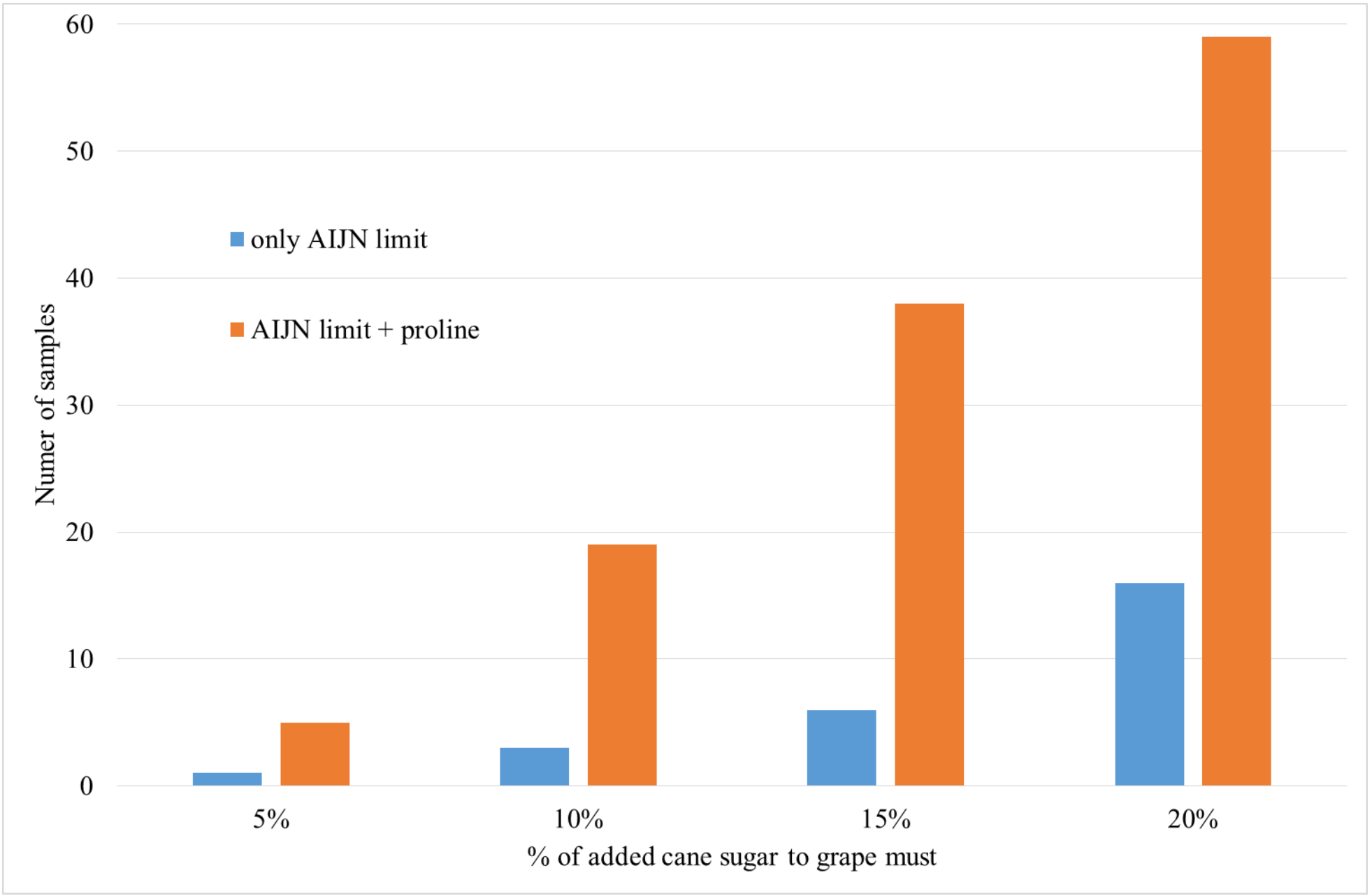
limit AIJN
95% of the confidence interval (2*SD)
y = 0.7049x - 7.6484
R² = 0.71
δ13C (‰. V-PDB) sugar fraction
δ13C (‰, vs V-PDB) prolin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15%
20%
% of added cane sugar to grape must

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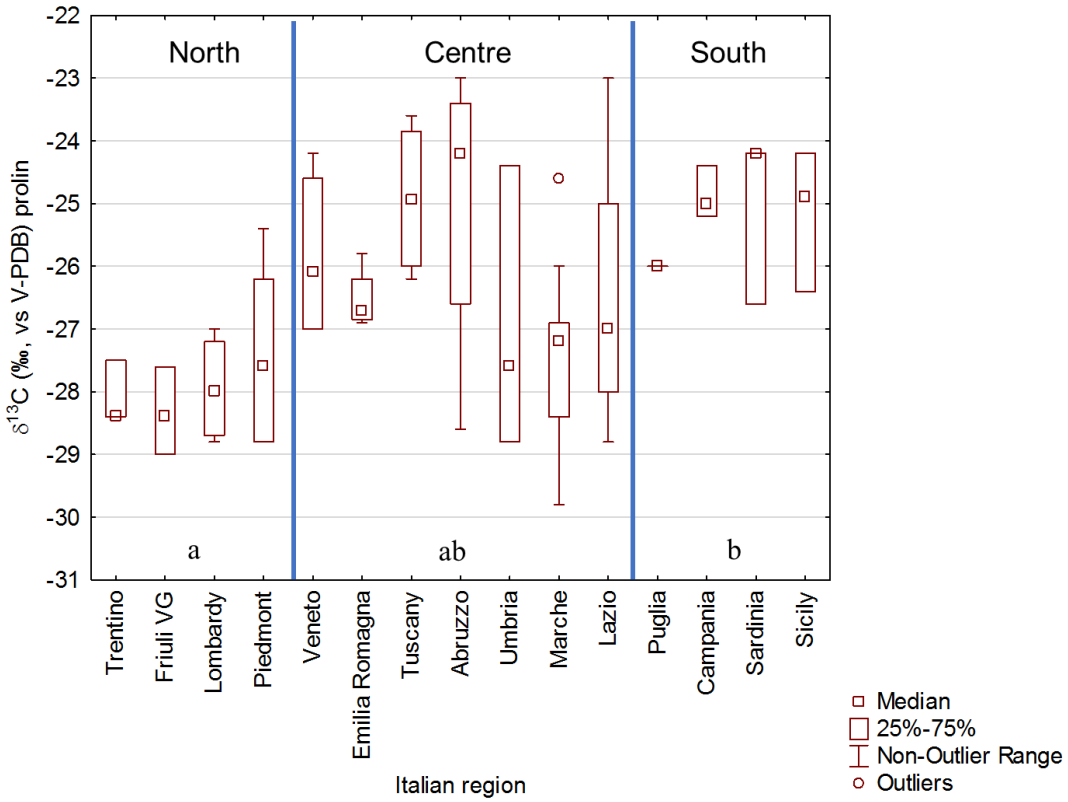
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δ13C (‰, vs V-PDB) prolin
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Trentino
Friuli VG
Lombardy
Piedmont
Veneto
Emilia Romagna
Tuscany
Abruzzo
Umbria
Marche
Lazio
Puglia
Campania
Sardinia
Sicily
Italian region
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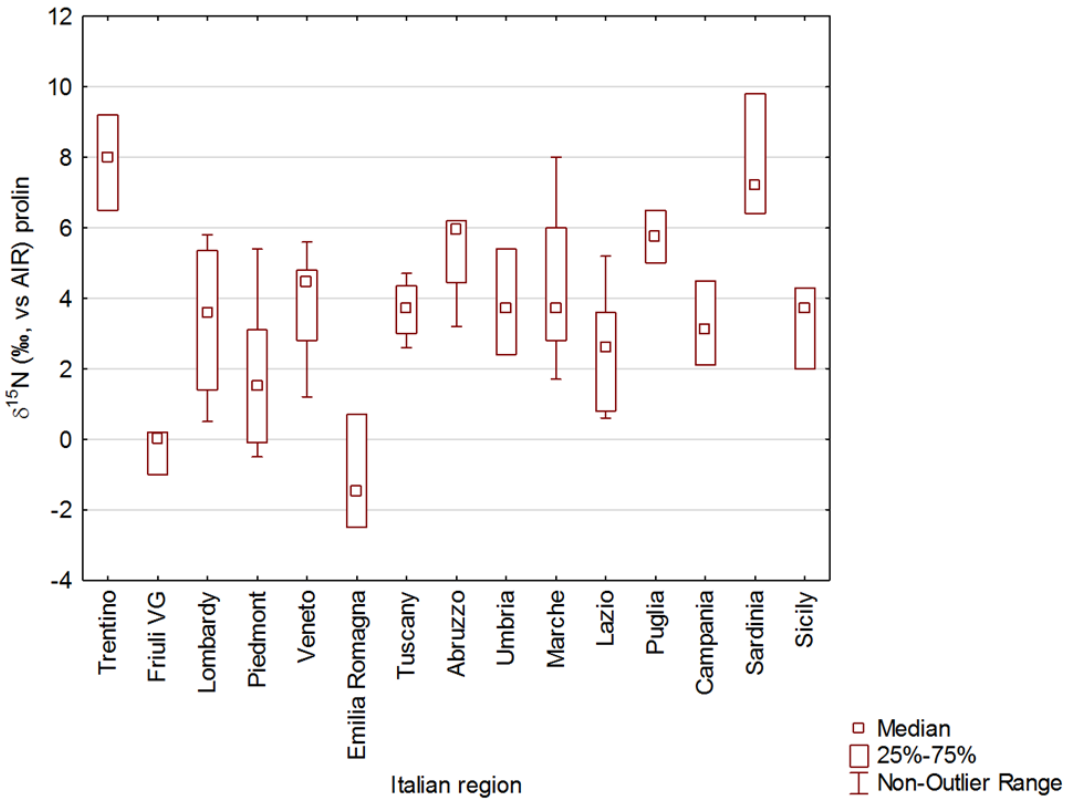
Non-Outlier Range
Outliers

B


δ15N (‰, vs AIR) prolin
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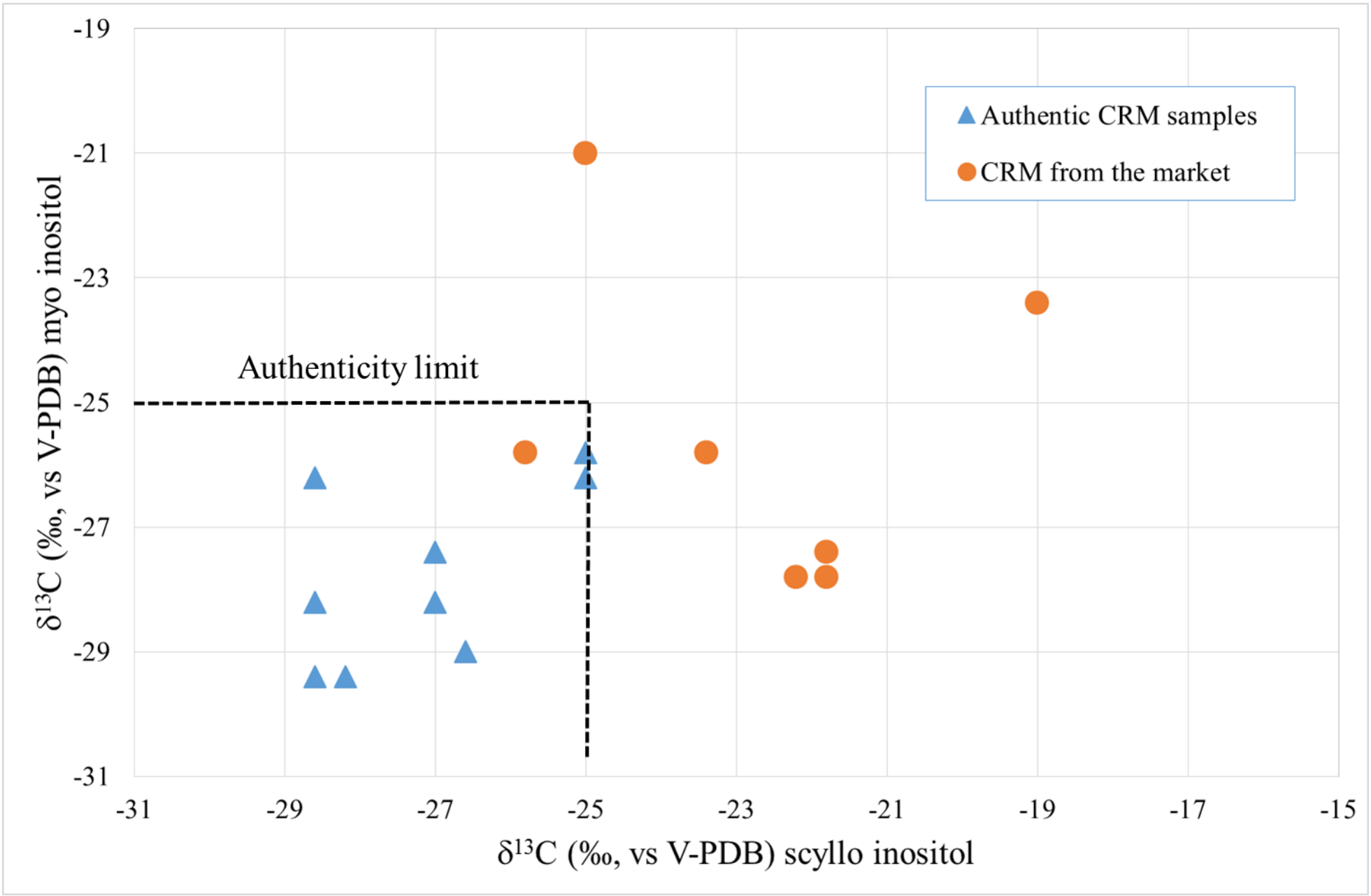
Friuli VG
Lombardy
Piedmont
Veneto
Emilia Romagna
Tuscany
Abruzzo
Umbria
Marche
Lazio
Puglia
Campania
Sardinia
Sicily
Italian region
Median
25%-75%
Non-Outlier Range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δ13C (‰, vs V-PDB) myo inositol
Authenticity limit
Authentic CRM samples
CRM from the market
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δ13C (‰, vs V-PDB) scyllo inositol