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## From soil to grape and wine: Variation of light and heavy elements isotope ratios

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### ABSTRACT

In the development of a geographical traceability model, it is necessary to understand if the value of the monitored indicators in a food is correlated to its origin or if it is also influenced by 'external factors' such as those coming from its production. In this study, a deeper investigation of the trend of direct geographical traceability indicators along the winemaking process of two traditional oenological products was carried out. Different processes were monitored, sampling each step of their production (grape juice, intermediate products and wine). The results related to the determinations of  $\delta^{18}\text{O}$ ,  $(\text{D}/\text{H})_i$ ,  $(\text{D}/\text{H})_{ii}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $^{87}\text{Sr}/^{86}\text{Sr}$  have been reported. Furthermore, correspondence with the isotopic values coming from the respective soil and vine-branch samples have been investigated as well, showing the optimal traceability power of the monitored geographical tracers.

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### 1. Introduction

The geographical origin or production area of food is acquiring more and more importance for consumers that associate food quality with a well defined or recognizable origin. As a consequence, the production of a food in a particular region or using traditional methods is considered as an added value for the product itself.

Moreover, with the introduction of the product designations, namely Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG), the different labelling systems and the EC 178/2002 Regulation (Regulation EC 178/2002, 2002) the EU has set out the basis for a new control method by defining the terms of traceability and production chain traceability. Unfortunately, many of the actual traceability systems are generally not referred to objective criteria but mostly based on certifications supported by papery declarations. Thus, in a context of globalized market and international food trade, with high risk of imitation, counterfeiting and adulteration, the objective possibility to trace the provenance of food on the basis of objective analytical criteria could be certainly a valuable support for the traditional papery declarations. Therefore,

the definition of criteria for geographical traceability of food, with a particular attention to those awarded with quality marks, such as the PDO, represents a real and challenging task. Generally speaking, from an analytical point of view, there are two approaches that can be adopted in this context: the former is based on a discriminating approach which uses direct and indirect indicators with the aims to differentiate 'homologous' food products but of different geographical origin (Bontempo et al., 2011; Trincerini, Baffi, Barbero, Pizzoglio, & Spalla, 2014); while the latter consists on a more systematic approach, mainly addressed to objectively link the food product to its area of origin and afterwards to discriminate it from the others on the basis of its provenance. The first approach relies on the use of the properties related to the food while the second one considers those related to its territory. In the latter case, it is unavoidable the analysis of matrices such as soils, water and plants (Durante et al., 2013; Marchionni et al., 2013). Starting from these evidences, it is possible to assume that the "territory" contribute to shape the fingerprint of the different products. Therefore, an *a priori* systematic study of the performance of the investigated indicators within the steps that characterise the 'production cycle' of a food, *soil-water-plant-fruit-raw materials-finished food*, represents the holistic approach to develop a model for the geographical origin of food. Only in this case, it is possible to establish a unique link between the final product and its territory of origin.

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This study represents an improvement of the knowledge of the trend of several geographical traceability indicators along the whole winemaking process of two traditional oenological products from soil to grape till the final wine. In particular, the data herein reported are part of a long term research project (<http://www.progettoager.it>) focused on the development of geographical traceability models of oenological products, *Lambrusco* PDO and *Trentodoc*, by means of primary indicators such as elements concentration and isotope ratios of radiogenic and light elements and secondary ones such as NMR spectra polyphenols fingerprint (Papotti et al., 2013) and the aromatic profile respectively. The project is characterised by an innovative analytical methodology due to the use of a systematic approach (Bertacchini et al., 2013; Totaro et al., 2013) which required a deep knowledge of the whole matrices that characterised the investigated systems, namely soils, branches, grape juices, intermediate products and wines.

As far as wine chain is concerned, winemaking process could encompass several steps, which may introduce changes in the element pattern, due to both natural and anthropogenic/extraneous sources. Several studies have been carried out on the variation of some element concentrations during the cellar practices (Cheng & Liang, 2012). In particular, storage tanks, pipes and other wine cellar equipment (brass and stainless steel based tools) increase the content of Cd, Cr and Pb (Kristl, Veber, & Slekovec, 2002) or Fe, Al and Cr (Kment et al., 2005). Variations in the element content occur also as a consequence of the fermentation process (Castiñeira Gómez, Brandt, Jakubowski, & Andersson, 2004). As a consequence, an accurate study to verify whether the strontium and the light isotope ratio are also influenced is necessary in order to finally achieve trustworthy results on the basis of this indicator.

As regard  $^{87}\text{Sr}/^{86}\text{Sr}$ , if no sources of “external” strontium are added (i.e. the use of additives to stabilise the process and eventually to clarify the final product), an increase or decrease in the strontium concentration does not directly affect the isotope ratio. In any case, it is worth to investigate whether and which strontium contaminations occur during the vinification. Generally, an increase of strontium is found in the first steps of the winemaking process, probably released by seeds and skins, whilst a slight decrease during the ageing period is a consequence of strontium precipitation with colloidal particles (Almeida & Vasconcelos, 2004). Therefore, these two phenomena are natural, not being due to external sources, and should not affect the  $^{87}\text{Sr}/^{86}\text{Sr}$  isotope ratio. On the contrary, the addition of bentonites, used for wine clarification, or calcium carbonate, to deacidify, could be the main extraneous sources of strontium (Horn, Schaaf, Holbach, Hiilzl, & Eschnauer, 1993).

In literature, it has been demonstrated that the winemaking process generally does not affect the strontium isotope ratio values. Nevertheless, the rationale of these results relies on the fact that neither additives were used nor relevant difference (around the fourth figure) were present in  $^{87}\text{Sr}/^{86}\text{Sr}$  value. In fact, the few attempts to investigate the influence of the winemaking process on the strontium isotope ratio (Almeida & Vasconcelos, 2001; Marchionni et al., 2016 and references herein reported) reveal no differences in the values measured in the different phases. However, for the former investigation a quadrupole-based ICP-MS was used for the evaluation of the strontium isotope ratio, while in the latter, an internal correction method has been used (Durante et al., 2013 and references herein reported). Indeed, starting from the recent observation that mass discrimination may also occur for heavy elements isotopic systems (Johnson, Beard, & Albarède, 2004) and that these experimental evidences are attained thanking the high sensitivity of the MC-ICP-MS instruments, a detailed study on this topic should be performed.

With regard to isotope ratios of light elements, the variability factors including the oenological ones, of  $\delta^{18}\text{O}$  of water and the

D/H and  $\delta^{13}\text{C}$  of ethanol have been studied since the 1990th in wine, being thenceforth the official analyses for identifying the authenticity of wine in terms of watering down, sugar addition and mislabelling (OIV methods MA-AS311-05, MA-AS312-07 and MA-AS2-12, EU reg 555/2000). Their source are the water, oxygen and the carbon dioxide adsorbed by the plant and their variability is mainly influenced by the geographic and climatic characteristics of the production area (Camin et al., 2015).  $\delta^{13}\text{C}$  of ethanol originates from that of sugar, (D/H)<sub>I</sub> from D/H of the nonexchangeable sites of glucose, whereas (D/H)<sub>II</sub> from the water medium (Martin, Zhang, Naulet, & Martin, 1986). If the yield of fermentation is more than 70%, type of yeast, temperature of fermentation, concentration of sugar, variation in the reaction rates induced by the medium, stopping of fermentation and type of nutrients have not a significant effect on  $\delta^{13}\text{C}$  and (D/H)<sub>I</sub> of ethanol, whereas (D/H)<sub>II</sub> may exhibit significant variations (Fauhl & Wittkowski, 2000; Perini et al., 2014). The must concentration through reverse osmosis was found not affecting the isotopic values of water and ethanol, whereas the concentration through high-vacuum evaporation modifies the  $\delta^{18}\text{O}$  of water and the D/H of ethanol (Guyon, Douet, Colas, Salagoity, & Median, 2006). Very recently it has been studied the effect of addition of Arabic gum as wine additive on the H, O, C and N isotope ratios of colloids. They found that only the  $\delta^{18}\text{O}$  of colloid is shifted, whereas the isotope ratios of ethanol and water are not affected (Sprenger, Meylahn, Zaar, Dietrich, & Will, 2015). Moreover it was shown that wine dealcoholisation has an impact on the  $\delta^{18}\text{O}$  of water and on  $\delta^{13}\text{C}$  of ethanol but not on D/H (Ferrarini, Ciman, Camin, Bandini, & Gostoli, 2016).

The  $^{15}\text{N}/^{14}\text{N}$  ratio has been measured in several foods to trace geographical origin and agricultural systems (Kelly, Heaton, & Hoogewerff, 2005), but not in wine so far. Nitrogen of wine derives not only from soil through plants and grapes, but can derive also from exogenous sources. For example, many winemakers add nitrogen rich substances to the fermenting wine to ensure that the yeast has sufficient nourishment to carry out the conversion of the sugars.

In this study, the results related to the determinations of some of the investigated direct indicators, namely  $\delta^{18}\text{O}$  in water, (D/H)<sub>I</sub>, (D/H)<sub>II</sub>,  $\delta^{13}\text{C}$  in ethanol and  $\delta^{15}\text{N}$  in lyophilised sample and  $^{87}\text{Sr}/^{86}\text{Sr}$  in different oenological production chains have been reported. One of the main aims of this study has been to test the real performance of the studied tracer indicators in the development of geographical traceability model, starting from a deeper analysis of their trend within the production cycle of *Lambrusco* PDO and *Trentodoc*.

Different wine-making processes of several producers of *Lambrusco* PDO and *Trentodoc* wines were monitored. Samples for each step of the process (grape juice, intermediate products, wine) were tracked. Furthermore, for some of the investigated producers, it has been also possible to assess a correspondence with the isotopic values coming from the respective soil and vine-branch samples as well.

## 2. Materials and methods

### 2.1. Reagents and materials

All the sample preparation procedures were carried out under horizontal laminar flow hood, in order to prevent the occurrence of any ambient contamination. Solutions were prepared by using high-purity deionized water TYPE1 (physical and chemical parameters for TYPE1 water comply with ASTM TYPE I and ISO3696 GRADE I purity specifications) obtained from a Milli-Q system (Millipore, Bedford MD) with a resistivity better than 18 M $\Omega$  cm.

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