

Is your juice truly organic? An isotopic approach for certifying organic grape juice

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Abstract. The sustainability and authenticity of grape juice production have gained increasing attention, particularly regarding the environmental impact and health benefits of organic practices. This study aimed to evaluate the isotopic composition of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) in grape juices produced under organic and conventional farming systems, to determine whether these markers can differentiate production methods. A total of 36 samples (18 organic and 18 conventional) were analysed using elemental analysis and isotope ratio mass spectrometry. Results showed significantly higher $\delta^{15}\text{N}$ values in organic grape juices (mean of 7.78 ‰), consistent with the use of organic fertilisers naturally enriched in ^{15}N . In contrast, conventional juices had significantly lower $\delta^{15}\text{N}$ values (mean of 1.24 ‰), reflecting the use of synthetic fertilisers. No significant differences in $\delta^{13}\text{C}$ values were observed between systems, with all samples falling within the expected range for C3 plants, suggesting that carbon isotope ratios are more influenced by photosynthetic pathway and environment factors than by farming practices. These findings confirm that $\delta^{15}\text{N}$ analysis is a reliable tool for distinguishing organic from conventional grape juices. This approach contributes to food authentication and supports sustainable agriculture verification.

1. Introduction

The sustainability of grape juice production has become an area of growing interest, with life cycle assessment revealing significant environmental impacts associated with viticulture, particularly greenhouse gas emissions resulting from the use of synthetic fertilisers and pesticides [1]. Organic grape production compared to conventional methods is generally associated with a lower environmental footprint. Studies have shown that organic systems reduce carbon, nitrogen, and water footprint, contributing to more sustainable agricultural practices [2, 3]. Moreover, organic farming supports greater soil microbial diversity, which strengthens ecosystems health and resilience. These environmental advantages position organic viticulture as a valuable strategy in reducing the overall impact of grape juice production [2].

Organic grape juice is widely appreciated for its health benefits, especially its strong antioxidant properties. Studies have investigated its nutritional profile, safety, and differences from conventional juice. Organic juice

typically contains higher levels of polyphenols and flavonoids, key antioxidant compounds, largely due to the absence of synthetic pesticides and fertilisers in organic farming [4, 5, 6]. While the antioxidant phenolic compounds in organic juices are similar to those conventional ones, organic juices often have higher flavonoid content, which contribute to their enhanced antioxidant capacity [6].

Nevertheless, the quality of grape juice is strongly influenced by the choice of farming methods and cultivars. Studies highlight Bordô, BRS Violeta and BRS Cora for their high levels of bioactive compounds and antioxidant activity, making them suitable for juice production [7, 8, 9]. BRS Magna also shows good adaptability and juice quality, especially when combined with other aspects [10]. In Brazil, grape juice is produced primarily from *Vitis labrusca* varieties, which are especially known for their high levels of bioactive compounds and potent antioxidant properties. Hybrid cultivars are also used and contribute to the nutritional and functional quality of the juice [7, 8].

The isotopic composition of nitrogen $^{15}\text{N}/^{14}\text{N}$ can reveal information about nitrogen sources and viticultural practices, with higher values commonly associated with organic and biodynamic systems, making it a potential tool for verifying organic certification and assessing nitrogen cycling [11, 12]. Additionally, the carbon isotope ratio $^{13}\text{C}/^{12}\text{C}$ in grape juice serves as a reliable indicator of photosynthetic pathway and plant water status during ripening, reflecting the integrated water conditions experienced by the vine and supporting vineyards zoning and precision viticulture [13, 14].

In this sense, and based on previous studies carried out on grapes, this study aimed to determine carbon and nitrogen isotope ratios in grape juices produced under organic and conventional agricultural practices, to verify whether the patterns observed in the grapes are reflected in the final product.

2. Materials and methods

2.1. Samples

The study was conducted with 36 samples, 18 organic and 18 conventional.

2.2. Experimental design

The experimental design aimed to test the hypothesis that different agricultural management systems produce distinct nitrogen and carbon isotopic signatures in grape juice, allowing the differentiation between organic and conventional products.

2.3. Measurements

The samples were dehydrated in an oven at a temperature of 70 °C for 24 hours for nitrogen determination. Then, 5 mg of the sample was placed in tin capsules and injected in the system using a solid sampler. The samples were introduced into the combustion reactor of the elemental analyser (Flash EA 1112, Thermo Fisher Scientific, Bremen, Germany), where they were converted into gas in a quartz reactor with copper oxide and cobalt and silver oxide, under a continuous flow of ultra-pure helium at a flow rate of 150 ml/min and pulses of oxygen for combustion.

For carbon determination, the grape samples were manually crushed and placed in 2 mL vials. Afterward, 1 μL was injected using a liquid sampler following the method described at OIV-MA-AS312-06 [15].

2.4. Statistical analyses

Results were analysed with ByoEstat 5.3 at a 5 % significance level, followed by Tukey's test.

3. Results and Discussion

Nitrogen supplementation in grape juice is a critical factor in grape cultivation, affecting fermentation dynamics and the sensory attributes of the final products. The choice of nitrogen type, application method, and concentration must be carefully managed to optimise juice quality. The influence of the production system can be observed in Table 1.

Table 1. Results of $\delta^{15}\text{N}$ from organic and conventional grape juice, expressed in ‰.

Juice	Identification	$\delta^{15}\text{N}$ (‰) \pm SD*
Organic	2019	9.30 ± 0.29^a
Organic	2020	6.67 ± 0.17^a
Organic	2021	6.50 ± 0.18^a
Organic	2022	8.65 ± 0.15^a
Organic	Al	3.32 ± 0.18^b
Organic	Sn	4.21 ± 0.15^b
Conventional	133	1.37 ± 0.15^c
Conventional	130	1.02 ± 0.07^c
Conventional	134	0.87 ± 0.13^c
Conventional	135	0.89 ± 0.14^c
Conventional	136	0.62 ± 0.15^c
Conventional	137	0.59 ± 0.18^c
Conventional	138	3.35 ± 0.19^b

*SD = Standard deviation. Means followed by different uppercase letters in the column differ significantly according to ANOVA complemented by Tukey's multiple comparisons test at 5% of significance level.

The $\delta^{15}\text{N}$ values for organic grape juice were significantly higher than those of conventional juice. The $\delta^{15}\text{N}$ mean value for organic grape juice, excluding two outliers (samples Al and Sn), was 7.78 ± 1.4 ‰. In contrast, the conventional samples showed a mean value of 1.24 ± 1.00 ‰.

The significantly higher $\delta^{15}\text{N}$ values observed in organic grape juice compared to conventional samples are consistent with the nitrogen sources typically used in each production system. Organic farming primarily relies on organic fertilisers such as composted manure, which are naturally enriched in ^{15}N due to biological and microbial processing. In contrast, conventional agriculture often uses synthetic nitrogen fertiliser produced via the Haber-Bosch, which are isotopically lighter, with $\delta^{15}\text{N}$ closer to atmospheric N_2 [16].

The mean $\delta^{15}\text{N}$ value of 7.78 ± 1.4 ‰ for organic grape juice in this study is within the range commonly reported for organically grown products, which often reaches values close to 7.5 to 10 ‰, depending on the type of organic inputs and environmental factors [11, 12, 17].

Recent literature continues to support the use of nitrogen isotope ratios as a robust marker for food authentication, especially in the organic vs. conventional context. For example, Camin et al. [16] demonstrated the effectiveness of multi-isotope approaches (including $\delta^{15}\text{N}$) in

distinguishing organically and conventionally produced foods, including grape derivatives. Similarly, Giannioti et al. [18] emphasized the reliability of stable isotope analysis in official controls and regulatory frameworks for organic labelling. Considering that both European and American legislation prohibit the use of synthetic fertilisers in organic production.

The $\delta^{13}\text{C}$ values of both organic and conventional grape juice samples ranged from -27.26 ‰ to -25.77 ‰, as shown in Table 2. All samples fall within the expected range for C_3 plants, such as grapevines, which typically present $\delta^{13}\text{C}$ values between -29.01 ‰ and -28.85 ‰ depending on environmental conditions and water availability [13].

Table 2. Results of $\delta^{13}\text{C}$ from organic and conventional grape juice, expressed in ‰.

Juice	Identification	$\delta^{13}\text{C}$ (‰) \pm SD*
Organic	2019	-27.26 \pm 0.04 ^a
Organic	2020	-26.26 \pm 0.03 ^a
Organic	2021	-26.82 \pm 0.02 ^a
Organic	2022	-25.77 \pm 0.03 ^a
Organic	A1	-25.94 \pm 0.03 ^a
Organic	S1	-25.92 \pm 0.04 ^a
Conventional	133	-26.35 \pm 0.03 ^a
Conventional	130	-26.91 \pm 0.06 ^a
Conventional	134	-27.22 \pm 0.03 ^a
Conventional	135	-26.45 \pm 0.04 ^a
Conventional	136	-26.93 \pm 0.01 ^a
Conventional	137	-26.75 \pm 0.19 ^a
Conventional	138	-26.49 \pm 0.11 ^a

*SD = Standard deviation. Means followed by different uppercase letters in the column differ significantly according to ANOVA complemented by Tukey's multiple comparisons test at 5% of significance level.

No statistically significant differences were observed between organic and conventional samples, suggesting that the cultivation system alone does not strongly influence $\delta^{13}\text{C}$ values in grape juice. This is in line with findings from previous studies that reported $\delta^{13}\text{C}$ as more sensitive to environmental factors (such as water stress, light intensity, altitude) than to fertilisation practices or organic and conventional status [19]. Interestingly, organic juices from different years showed slight variation, with values increasing from -27.26 ‰ (2019) to -25.77 ‰ (2022), which may reflect annual differences in climate conditions, particularly water availability. Less negative $\delta^{13}\text{C}$ values are often associated with reduced stomatal conductance and water stress, which limit discrimination against ^{13}C during photosynthesis [20].

Conventional samples also exhibited intra-group variability, ranging from -27.22 ‰ to -26.35 ‰, with some overlapping the organic values. This reinforces the

idea that $\delta^{13}\text{C}$ alone may not serve as a reliable marker for production method but could be complementary to other isotopic or compositional markers in multi-parameter authenticity assessments [21, 22].

Overall, the $\delta^{13}\text{C}$ values provide insight into the physiological conditions of grapevine growth but do not discriminate between organic and conventional juice under the conditions studied.

4. Conclusion

This study demonstrated that $\delta^{15}\text{N}$ values in grape juice effectively distinguish between organic and conventional production systems, reflecting the patterns previously observed in grapes and confirming the influence of fertiliser type. In contrast, $\delta^{13}\text{C}$ values did not show significant differences between systems, being more influenced by environmental factors. Thus, nitrogen isotope analysis proves to be a reliable marker for verifying agricultural practices in grape derived products, while carbon isotopes may serve as complementary indicators.

Acknowledgments

This study was part of a research project supported by the Coordination for the Improvement of Higher Education Personnel (CAPES Foundation) with the Grant/Award Number: 88887.518542/2020-00, the University of Caxias do Sul (UCS), the Department of Agriculture of Rio Grande do Sul, the Cooperativa Vinícola Garibaldi, and SENS Advanced Mass Spectrometry.

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