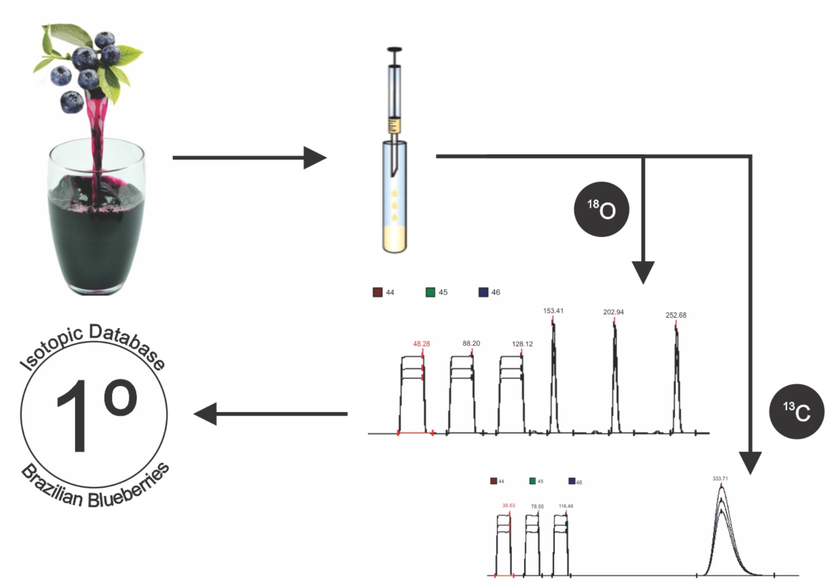
ARTICLE

Carbon and Oxygen Isotope Profile in Brazilian Blueberries (*Vaccinium spp.*)

Letícia Leonardelli[1], Susiane Leonardelli[1,2*], Joséli Schwambach[1]

[1]*Instituto de Biotecnologia, Universidade de Caxias do Sul, Rua Francisco Getúlio Vargas, 1130, 95070-560, Caxias do Sul, RS, Brazil*
[2]*Laboratório de Referência Enológica Evanir da Silva (LAREN/SEAPDR), Av. da Vindima, 1855, 95084-470, Caxias do Sul, RS, Brazil*



The Brazilian blueberry (*Vaccinium spp.*) production has made remarkable progress in developing quality and quantity. The price of the fruit for fresh and processing purposes is linked to its quality and origin. An effective method for authenticity control and traceability is the stable isotopes method. Carbon (^{13}C) and oxygen (^{18}O) of Brazilian blueberries have never been extensively explored. In this work, the results of ^{13}C and ^{18}O (performed by IRMS) of thirty blueberry samples were presented and discussed, being eleven different cultivars produced in the Southern Brazilian region from mountain and high-altitude. The blueberry showed the typical range of carbon isotopes for C_3 plants, with significant differences between Rabbiteye and Southern Highbush. The $\delta^{13}C$ and $\delta^{18}O$ values showed a significant difference between a mountain and a high-altitude region. This study represents the first isotopic database for Brazilian blueberries, and it can be incorporated into a traceability system. In addition, these results can be used to verify the authenticity of the fruit composition declared on the label and as an effective tool for identifying the geographical origin.

Keywords: isotopic composition, quality control, mass spectrometry, food and beverage analysis, geographical discrimination

Cite: Leonardelli, L.; Leonardelli, S.; Schwambach, J. Carbon and Oxygen Isotope Profile in Brazilian Blueberries (*Vaccinium spp.*). *Braz. J. Anal. Chem.* (Forthcoming). http://dx.doi.org/10.30744/brjac.2179-3425.AR-14-2022

Submitted 25 February 2022, Resubmitted 20 May 2022, Accepted 15 June 2022, Available online June 2022.

INTRODUCTION

Blueberry (*Vaccinium spp.*) production and consumption have increased in the last decade and become more popular because this fruit shows excellent sensory properties. Also, it has aroused particular interest of people due to its beneficial properties for human health. Blueberries can be consumed naturally or in products, like juice. However, the benefits will only be achieved if the products from blueberry are genuine, without adulteration with cheaper raw, like sugar cane or apple juice.[1] Three blueberry cultivars are responsible for the worldwide production: Highbush (*Vaccinium corymbosum* L.), Lowbush (*V. angustifolium* Ait.) and Rabbiteye (*Vaccinum virgatum* Ait.).[2]

The gastronomic versatility and the growing demand for blueberry stimulated a significant increase in global production, which more than doubled between 2010 and 2019, rising from 439,000 metric tons to nearly 1.0 million metric tons. The most significant production is in the United States, followed by Canada, Chile, and Peru.[3] In Brazil, the production is still relatively low. It was introduced in the 80's in the South region, motivated by the growing world demand and the attractive fresh fruit prices on the European market. Furthermore, Brazil has some advantages such as the possibility of early production in the Northern Hemisphere off-season and the availability of water and land suitable for cultivation.[4] The main cultivars produced are Rabbiteye (Aliceblue, Bluebelle, Bluegem, Briteblue, Climax, Delite, Powderblue and Woodard) and Southern highbush (Misty, O'Neal and Georgiagem). New varieties of Southern highbush cultivars have been introduced, such as Star, Jewel, Emerald, Millenia, Primadonna and Snowchaser.

Several studies have shown the benefits of blueberry, such as effects on cardiovascular disease, type 2 diabetes mellitus, neurological decline, ophthalmologic disorders, bone protection, decrease blood pressure and cholesterol, anti-diabetic properties, cytotoxic activity, for example. In addition, anti-inflammatory action associated with blueberry intake is supported by clinical, animal and in vitro studies.[5] The bioactivity of blueberry extract is mainly due to phenolics compounds, such as anthocyanins, phenolic acids and proanthocyanidins, making it one of the most desirable and nutritious fruits.[6]

Accordingly, the isotope ratio $^{13}C/^{12}C$ expressed in $\delta^{13}C$, C_3 plants such as blueberry, grape and orange, show $\delta^{13}C$ values ranging from -33 to -22‰, and C_4 plants such as corn, sugar cane and sorghum range from -16 to -10‰. Therefore, knowing the isotopic profile is an important way to control adulteration, and preserve the authenticity and the benefits of products from blueberry fruits.[7,8]

Natural variation in carbon isotope ratio ($^{13}C/^{12}C$) has been immensely investigated. Isotopic composition such as $^{13}C/^{12}C$ is used to reduce the database's variability and to detect relatively small amounts of sugar added to fruit products, even when the isotopic values of investigated samples are close to the natural variation range of the fruit.[7] Plants have lower levels of ^{13}C to ^{12}C, these two carbon isotopes occur naturally in nature, due to the fractionations during the photosynthetic cycle. The photosynthetic cycle is responsible for the most significant difference between the values of ^{13}C in C_3 (Calvin cycle) and C_4 (Hatch-Slack cycle) plants. The CO_2 fixed in the photosynthesis will reflect the botanical origin of carbon in plants, as the metabolic pathway discriminates differently against the heavier isotope ^{13}C present in atmospheric CO_2, for plants C_3 and C_4.[9]

Recent research confirmed a variation in chemical composition in small fruits due to the influence of cultivar, genetic background, climate, growing conditions, maturity and post-harvest handling techniques.[10] So, the factors cited, plant type and agriculture practices reflect the isotopic composition of $^{13}C/^{12}C$ and $^{18}O/^{16}O$.[11] For example, the mild water stress increase berry sugar, early or late.[12] In the same way water stress affects stable isotopes, because changes the gradient between atmospheric CO_2 and intercellular CO_2 concentration during the photosynthesis process in the plant.[13]

In the literature, there are several references for isotopic characterization from different fruits,[14,15] but few studies about different blueberry cultivars. Furthermore, there are no studies for stable isotopes in Brazilian blueberries. Therefore, the study aimed to build the first database for stable isotopes of blueberries, including eleven cultivars: Northern Highbush (Bluecrop, Elliot and Duke), Southern Highbush (Misty and O'Neal) and Rabbiteye (Bluegen, Briteblue, Climax, Delite, Florida, and Power blue), characterizing the range of carbon ($^{13}C/^{12}C$) and oxygen ($^{18}O/^{16}O$) values in different blueberry cultivars. In addition, to assess the effectiveness of the stable isotopes of carbon and oxygen to determine the geographical origin of blueberries.

MATERIALS AND METHODS

Samples

The blueberries were collected during the 2017 and 2018 vegetation seasons from 30 sampling sites in the Southern Brazilian region of Rio Grande do Sul. Samples of different cultivars belonged to three varieties: Northern Highbush (Bluecrop, Elliot and Duke), Southern Highbush (Misty and O'Neal), and Rabbiteye (Bluegen, Briteblue, Climax, Delite, Florida, and Power blue).

Sampling site description

Samples were collected in different private fields located in 7 cities of Rio Grande do Sul. Five from the mountain region: Bento Gonçalves (1 sample Rabbiteye: Bluegen), Caxias do Sul (11 samples: being 10 Rabbiteye: Florida: 3, Briteblue:1, Climax: 3, Bluegen: 2 and Powder Blue:1 and 1 Southern Highbush: O'Neal: 1), Flores da Cunha (3 samples: being Powder Blue:1, Bluegen: 1 and Climax: 1) , Nova Petrópolis (1 sample Florida) and São Marcos (5 samples: Delite: 1, Bluegen: 1, Bluecrop: 1, Climax: 1 and Florida: 1) and two from high-altitude region: Vacaria (8 samples: O'Neal: 2, Climax: 1, Bluecrop: 1, Elliot: 1, Duke: 1, Misty: 1 and Bluegen: 1) and Campestre da Serra (1 sample Florida).

The two regions are about 110 km apart, and the mountain region has an altitude of 750 meters, whereas the high-altitude region has 971 meters. The climatic and environmental data variables, such as the precipitation, the mean annual temperature and the mean annual chill hours, were obtained from National Institute of Meteorology (INMET). Furthermore, information about soil was obtained from the Soil Museum of Rio Grande do Sul (MSRS). The mountain region is marked by a soil well-drained, shallow, developed from volcanic rocks, rocky and sloping. In contrast, the high-altitude region presents the soil poorly drained, deep, developed from basalt, hydromorphic and rocky. Based on the published literature, these variables were the most likely to have significant effects on carbon and oxygen isotope ratio, and the data are relatively accurate for each location. All the samples were collected from irrigated field.

Sample preparation

The juice was extracted from the fruit using a juice maker (Skimsen, Brusque, Brazil), and the samples were manually crushed and after centrifuged at 1750 rpm to separate and remove the seeds and peels from the fruit. The juice was filtered by a membrane with a pore size of 0.8 µm (Millipore Merck, Germany). All samples were subjected to the analyses of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in triplicate.

Stable isotope ratio ($^{13}C/^{12}C$) analysis

The carbon isotopic ratio ($^{13}C/^{12}C$) of each sample was determined in triplicate using an isotope ratio mass spectrometer (Delta Plus XP, Thermo Fisher Scientific, Bremen, Germany) following total combustion in an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific). Juice from blueberry (1 µL) was injected directly into the reactor; the sample was converted into CO_2 gas by combustion in a quartz reactor with copper oxide, and silvered cobaltous-cobaltic oxide. Under a continuous flow of ultra-pure helium (flow rate at 100 mL min^{-1}) to form CO_2 and H_2O. The gases passed through a reduction column at 680 ºC containing reduced copper whilst water was trapped with magnesium perchlorate for the gases to be separated on a Porapak Q chromatographic column at 45 ºC. The resulting gas (CO_2) was then transferred to the isotope ratio mass spectrometer via a universal continuous flow interface (Conflo III, Thermo Fisher Scientific) to determine the $^{13}C/^{12}C$.[16]

The values of $^{13}C/^{12}C$ are denoted in delta related to the intentional V-PDB (Vienna-Pee Dee Belemnite) and the $\delta^{13}C$ values were calculated against the international reference material Sucrose IAEA-CH-6. (RM 8542, from International Atomic Energy Agency, Viena, Austria). A reference gas CO_2 4.8 (Air Liquide, Paris, France) was used to calibrate against the reference material to ensure the results trueness. The analytical instrumental error was lower than ± 0.20‰.

Determination of δ^{18}O of water by equilibration (GasBench-IRMS)

Sample preparation was based on the CO_2 equilibrium obtained through the isotopic exchange reaction between CO_2 (g) and water in the sample (L). Samples (500 µL) were transferred into the vial using a pipette, and then the vial was flushed with a mixture of 0.3% CO_2 in helium at a flow rate of 100 mL min^{-1} for 5 min. The complete exchange between the CO_2 from the sample and the helium-CO_2 gas was reached after 24 h of equilibration at 25 ºC. The headspace formed was injected into the peripheral device (Gasbench II, Thermo Fisher Scientific). The helium flow gently moves the headspace of the vial into the fused silica capillary through the diffusion traps (Nafion®) to remove the water and then to a Valco® loop for injection into the gas chromatograph.

The loop injection set up 100 µL aliquots of the sample gas into the gas chromatography column at 70 ºC, with a helium flow of 20 mL min^{-1} at 10-12 psi. The spectrometer then measured the isotope species with mass 44, 45 and 46. The analytical error was lower than 0.20‰, and the samples were analyzed in triplicate.

Method validation

The method was validated, and the following parameters were evaluated: selectivity, linearity, working range, detection limit, quantification limit, accuracy, recovery and calculation of measurement uncertainty. For selectivity and accuracy, tests were carried out by adding cane sugar to blueberry juice. A solution (100% C_4) with cane sugar in ultra-pure water (80 g L^{-1}) was added to blueberry juice at the following concentrations: 1, 2, 5, 10, 20, 30, 50 and 80%. The accuracy values were evaluated by the AOAC Association of Official Agricultural Chemists criteria. A curve reading seven concentrations in seven replicates was prepared for linearity and working range. The detection and quantification limits were calculated by testing a sample containing the matrix. Finally, the calculation of measurement uncertainty of the methods was performed using GUM Workbench 2.4 software.

Statistical analysis

All statistical analyses were performed by SPSS software, version 22.0 (IBM Corporation, New York, USA) at 5% significance level. The normal distribution of the residuals was evaluated through the Kolmogorov Smirnov. Data were analyzed by ANOVA, and if a statistically significant effect was verified, multiple pairwise comparisons were performed using Tukey's test.

RESULTS AND DISCUSSION

Carbon isotopic composition ($^{13}C/^{12}C$)

The δ^{13}C values for blueberries cultivars are summarized in Table I.

Table I. Isotope composition δ^{13}C (‰) of pulp from eleven different blueberry cultivars, expressed by maximum, minimum, mean and standard deviation (SD)

Cultivar	n	Variety	δ^{13}C Mean ± SD* (‰)	δ^{13}C Minimum (‰)	δ^{13}C Maximum (‰)
Bluecrop	2	Northern highbush	-24.69 ± 0.47	-25.26	-24.18
Bluegen	6	Rabbiteye	-25.86 ± 0.93	-27.38	-24.93
Briteblue	1	Rabbiteye	-25.80 ± 0.07	-25.86	-25.73
Climax	6	Rabbiteye	-26.06 ± 1.12	-27.41	-24.02
Delite	1	Rabbiteye	-24.97 ± 0.08	-25.06	-24.92
Duke	1	Northern highbush	-24.53 ± 0.05	-24.61	-24.44

continues on the next page

Table I. Isotope composition $\delta^{13}C$ (‰) of pulp from eleven different blueberry cultivars, expressed by maximum, minimum, mean and standard deviation (SD) (continuation)

Cultivar	n	Variety	$\delta^{13}C$ Mean ± SD* (‰)	$\delta^{13}C$ Minimum (‰)	$\delta^{13}C$ Maximum (‰)
Elliot	1	Northern highbush	-24.56 ± 0.04	-24.60	-24.52
Florida	6	Rabbiteye	-25.55 ± 0.70	-26.34	-24.15
Misty	1	Southern highbush	-23.21 ± 0.05	-23.26	-23.15
O'Neal	3	Southern highbush	-24.72 ± 1.13	-26.16	-23.44
Powder Blue	2	Rabbiteye	-25.83 ± 0.99	-26.78	-24.86

*SD = Standard Deviation, significance level of 5%.

Blueberries' $\delta^{13}C$ values ranged from -27.37 to -23.21‰, nearly identical to those found by other authors in C_3 plants, as expected for plants with the same metabolic pathway.[17] The $\delta^{13}C$ values for the Northern Highbush variety in this study were similar to the results found in blueberry produced in the Northern Italian region (-24.20 ± 1.30‰) and the Rabbiteye close to found in the Eastern European (-26.20 ± 1.40‰).[7] However, the isotopic ranges of sugar from blueberry pulp classified by varieties showed a significant difference between the varieties Rabbiteye and Southern Highbush, with lower $\delta^{13}C$ values for Rabbiteye. Previous studies have also indicated that genetic diversity can influence the $\delta^{13}C$ values of plant biomass.[18]

The difference is not statistically significant because blueberry cultivars present a natural variation and $\delta^{13}C$ values specific for each cultivar. Furthermore, other influences in $\delta^{13}C$ values are important, such as plant physiology, plant resistance to climatic factors and cultivation practices adopted.[11] Southern Highbush blueberries are hybrids from *Vaccinium corymbosum* (Highbush, tetraploid). This variety shows characteristics like the need for upland soils, short fruit development periods without organic amendment and plants that do not tolerate winter temperatures below freezing.[19]

The lowest $\delta^{13}C$ values were found for Bluegen cultivar and the highest for Misty. In a study developed with samples from Italy, Poland and Romanian, the values of $\delta^{13}C$ for pulp ranged between -28.60 to -22.10‰ comparing different cultivars; the Brigitta Blue showed the highest $\delta^{13}C$ values and Bluecrop showed the lowest $\delta^{13}C$ value.[20] In our study, the cultivar Climax showed the lowest value. Previous studies with apples showed $\delta^{13}C$ and $\delta^{15}N$ values were statistically influenced by the cultivar and geographical origin, and their interactions. Furthermore, they confirmed the existence of a great effect of the geographical origin and a limited influence due to the variety.[15]

The lower standard deviation found for Briteblue, Delite, Duke, Elliot and Misty happened because there is just one producer for those cultivars in the South.

Oxygen isotopic composition ($^{18}O/^{16}O$)

The $\delta^{18}O$ values for blueberries cultivars are summarized in Table II.

Table II. Isotope composition $\delta^{18}O$ (‰) of pulp from eleven different blueberry cultivars, expressed by maximum, minimum, mean and standard deviation (SD)

Cultivar	n	Variety	$\delta^{18}O$ Mean ± SD* (‰)	$\delta^{18}O$ Minimum (‰)	$\delta^{18}O$ Maximum (‰)
Bluecrop	2	Northern highbush	-0.62 ± 0.48	-1.18	-0.08
Bluegen	6	Rabbiteye	-0.27 ± 1.24	-2.62	1.78
Briteblue	1	Rabbiteye	-0.52 ± 0.09	-0.64	-0.37

continues on the next page

Table II. Isotope composition δ^{18}O (‰) of pulp from eleven different blueberry cultivars, expressed by maximum, minimum, mean and standard deviation (SD) (continuation)

Cultivar	n	Variety	δ^{18}O Mean ± SD* (‰)	δ^{18}O Minimum (‰)	δ^{18}O Maximum (‰)
Climax	6	Rabbiteye	-0.83 ± 1.99	-3.01	2.62
Delite	1	Rabbiteye	-0.53 ± 0.09	-0.69	-0.45
Duke	1	Northern highbush	-0.10 ± 0.06	-0.17	-0.01
Elliot	1	Northern highbush	+0.14 ± 0.09	-0.07	+0.23
Florida	6	Rabbiteye	-1.65 ± 1.04	-3.04	+0.09
Misty	1	Southern highbush	+0.86 ± 0.08	+0.73	+1.00
O'Neal	3	Southern highbush	-1.05 ± 1.61	-3.12	+1.01
Powder Blue	2	Rabbiteye	-2.00 ± 0.67	-2.79	-1.20

*SD = Standard Deviation, significance level of 5%.

Blueberries δ^{18}O values ranged from -3.12 to +2.62‰. These results were very similar to those found by Camin et al.[20] in highbush blueberry collected in Italy, Poland and Romania, where the researcher found values ranged from -2.2 to +4.3‰. A variation of δ^{18}O values also was observed by Klavins et al.,[21] working with blueberries from different countries. They observed a pattern based on the ^{18}O water cycle. Duet to the natural water cycle and precipitations, the heavy oxygen isotope (^{18}O) tends to concentrate more in regions close to the ocean, and lower δ^{18}O values indicate a greater distance from the ocean. Considering these factors δ^{18}O values are more characteristic to the geographical location.

Geographical origin

The samples were separated from two regions: mountain and high altitude. The difference between the regions was highly significant at the 95% confidence level for the δ^{13}C and δ^{18}O values. The results can be seen graphically in Figure 1, where the δ^{13}C are plotted concerning the δ^{18}O, differentiating them by region.

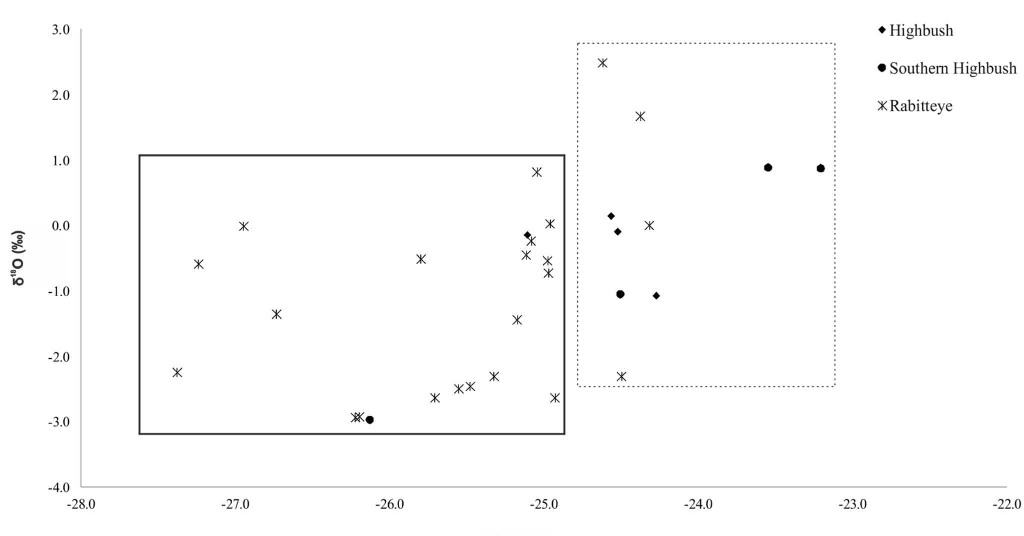


Figure 1. δ^{13}C versus δ^{18}O values of all blueberry samples by region.

Variation in the δ^{13}C values is usually connected to the cultivar, due to the influence of CO_2 fixation during photosynthesis. However, differences in geographical origin considering δ^{13}C were also found in products, such as olive oil. For example, the δ^{13}C allowed to differentiate olive oil from Sicily to other regions of Italy. The influence was assigned to the geographical position, considering the latitude of the olive grove.[22] Significant differences related to δ^{13}C were also found in mangoes cultivated in Mexico from those cultivated in the Ivory Coast, Senegal, and Spain. Meanwhile, δ^{18}O values in the same study allowed to differentiate mangoes produced in Brazil, Equatorial Guinea, Ivory Coast and Spain from those produced in Mexico, Peru, and Senegal.[23]

In this study, the δ^{18}O for samples from high-altitude region (0.42 ± 1.18‰) were significantly higher than the values found for samples from mountain (-1.37 ± 1.21‰). Considering that the mountain region has an altitude of 750 meters whereas the high-altitude region has 971 meters. The results showed an increase in the values with increasing altitudes. Taous et al.[24] also found an influence of geographical position in Argan oil in δ^{13}C and δ^{18}O, with an increased value with increasing altitudes and a negative tendency with latitude, suggesting a continental effect on the coastal oils showing more enriched isotopic values.

Geographical influence, such as latitude, altitude, elevation, and in addition climatic factors like precipitation, hydric stress and light exposure, affect the efficiency of CO_2 fixation. The reason is that δ^{13}C and δ^{18}O of plants are influenced by the availability of water, relative humidity, and environmental temperature.[25] These factors influence the plant's stomata, in which regulate leaf diffusive conductance and influence water loss and carbon gain.[26]

The two regions considered in this study showed different precipitation volumes in 2017, in which mountain region showed 1864 mm during this year, whereas in the high-altitude region, the precipitation volume was 1715 mm in the same year.[27] The difference is reflected in the δ^{18}O values; lower values was found for the mountain region, where the precipitation volume was higher than in the high-alitude region. The volume of precipitation directly influences the oxygen because the heavy oxygen isotope tends to concentrate more in the oceans due to the influence of the natural water cycle and precipitation. As a result, the lower δ^{18}O values are characteristic of regions distant from the ocean.[21] On the other hand, the temperature for the mountain region was higher, showing an annual mean of 18.30 ºC, while in the high-altitude region 16.10 ºC.[27]

Concerning the geographical position, differences in soil type between the mountain and the high-altitude region can also influence the δ^{13}C and δ^{18}O values due to water availability. The soil in the mountain is well-drained, while it is poorly drained in the high-altitude region.[28]

The blueberries can provide excellent nutrients and nutraceutical benefits to human health. However, the region's characteristic and environmental influences are important reasons for the differences found in the δ^{13}C and δ^{18}O values in this study. Furthermore, global warming and associated precipitation change impact many agricultural ecosystems.

Results of validation method

The analytical procedure has been adapted to the case of blueberry juice from a method proposed for other fruit juice. All parameters analyzed for the method validating were considered satisfactory (Table III).

Table III. Results of validation parameters of the method

Parameter	Results
Linear range (%)	0 – 100
R^2	0.99
LOD (‰)	0.27
LOQ (‰)	0.87

continues on the next page

Table III. Results of validation parameters of the method (continuation)

Parameter	Results
Z-score (%)	-1.41
Accuracy (%)	0.31
Repeatability (‰)	0.22
Intermediate accuracy (‰)	0.07
Recovery (%)	100.7
Measurement uncertainty (‰)	0.31

The standard deviation used to calculate the repeatability was 0.09‰. It was very similar to the standard deviation that Jamin et al.[29] found and according to the repeatability recommended by European Committee for Standardization (CEN) for orange and pineapple juice, beet and cane sugar (CEN - ENV12140) particularly on the question whether the ENV can be converted into an European Standard (EN).[16]

The isotopic recovery has been determined using low, medium and high concentrations of a test mixture of sucrose solution in a blueberry juice with known isotopic content. The recovery reached was between 97-103% according to the recommendation by Codex Alimentarius Commission.[30] It shows that the method employed effectivelly applies to blueberry juice and is free of isotopic effects.

CONCLUSION

In conclusion, this study represents the first isotope database for Brazilian blueberries that covers eleven cultivars from three different varieties. The range of $\delta^{13}C$ and $\delta^{18}O$ were characterized and the database could be incorporate into a traceability system, helping future studies about the food industry and authenticity products. In addition, the results showed satisfactory classification performances to discriminate blueberries of the mountain from those of the high-altitude region. The results are rather explorative, and they can be excellent support for the existence of a potential indication of geographical origin for Brazilian blueberries.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research was supported by University of Caxias do Sul and Secretary of Agriculture from Rio Grande do Sul (SEAPDR) in the Oenological Reference Laboratory Evanir da Silva (LAREN) and also for the producers who supplied the blueberry and make this work possible.

REFERENCES

(1) Mengist, M. F.; Grace, M. H.; Xiong, J.; Kay, C. D.; BAssil, N. Hummer, K.; Ferruzzi, M. G.; Lia, M. A.; Iorizzo, M. Diversity in Metabolites and Fruit Quality Traits in Blueberry Enables Ploidy and Species Differentiation and Establishes a Strategy for Future Genetic Studies. *Front. Plant Sci.* **2020**, *11* (370), 1-4. https://www.doi.org/10.3389/fpls.2020.00370

(2) Retamales, J. B.; Hancok, J. F. *Blueberries*. 2nd ed., Bell & Bain Ltd, **2018**.

(3) Protzman, E. *Blueberries around the Globe – Past, Present, and Future. International Agricultural Trade Report. United States Department of Agriculture*, **2021**, 1-6.

(4) Oliveira, I. P.; Marques, L. O. D.; Belarmino, L. C.; Mello-Farias, P.; Canever, M. D. Costs and financial viability of blueberry production in Pelotas. *Rev. Econ. Sociol. Rural*. **2022**, *60* (2), 1-12. https://www.doi.org/10.1590/1806-9479.2021.236746

(5) Kalt, W.; Cassidy, A.; Howard, L.; Krikorian, R.; Stull, A. J.; Tremblay, F.; Amora-Ros, R. Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Adv. Nut.* **2020**, *11* (2) 224-236. https://www.doi.org/10.1093/advances/nmz065

(6) Massarotto, G.; Barcellos, T.; Garcia, C. S. C.; Brandalize, A. P. C.; Moura, S.; Schwambach, J., Roesch-Ely, M. J. Chemical Characterization and Cytotoxic Activity of Blueberry Extracts (cv. Misty) Cultivated in Brazil. *Food Sci*. **2016**, *81* (8), 2076–2084. https://www.doi.org/10.1111/1750-3841.13385

(7) Magdas, D. A.; Cristea, G. Light Isotopes. Isotopic fingerprint. In: Georgiou, C. A.; Danezis, G. P. *Food Authentication: Management, Analysis and Regulation*, 1st ed., John Wiley & Sons, **2017**, 118-128. https://www.doi.org/10.1002/9781118810224

(8) Perini, M.; Giongo, L.; Grisenti, M.; Bontempo, L.; Camin, F. Stable isotope ratio analysis of different European raspberries, blackberries, blueberries, currants and strawberries. *Food Chem*. **2018**, *239*, 48-55. https://www.doi.org/10.1016/j.foodchem.2017.06.023

(9) Taskos, D.; Zioziou, E.; Nikolaou, N.; Doupis, G; Koundouras, S. Carbon isotope natural abundance ($\delta^{13}C$) in grapevine organs is modulated by both water and nitrogen supply. *OENO One*. **2020**, *54* (4), 1183–1199. https://www.doi.org/10.20870/oeno-one.2020.54.4.3600

(10) Yan, Y.; Pico, J.; Sun, B. Pratap-Singh, A.; Gerbrandt, E.; Castellarin, S. D. Phenolic profiles and their responses to pre- and post-harvest factors in small fruits: a review. *Food Sc. Nutr.* **2021**. https://www.doi.org/10.1080/10408398.2021.1990849

(11) O'Leary, M. H. Carbon Isotopes in Photosynthesis: Fractionation techniques may reveal new aspects of carbon dynamics in plants. *BioScience* **1988**, *38* (5), 328–336. https://www.doi.org/10.2307/1310735

(12) Ramos, M. C.; Pérez-Álvarez, E. P.; Peregrina, F.; Toda, F. M. Relationships between grape composition of Tempranillo variety and available soil water and water stress under different weather conditions. *Sci. Hortic.* **2020**, *262*, 1-12. https://www.doi.org/10.1016/j.scienta.2019.109063

(13) Bchir, A.; Escalona, J. M; Gallé, A.; Hernández-Montes, E.; Tortosa, I.; Braham, M.; Medrano, H. Carbon isotope discrimination ($\delta^{13}C$) as an indicator of vine water status and water use efficiency (WUE): Looking for the most representative sample and sampling time. *Agric. Water Manag.* **2016**, 11-20. https://www.doi.org/10.1016/j.agwat.2015.12.018

(14) Bontempo, L.; Caruso, R.; Fiorillo, M.; Gambino, G. L.; Perini, M.; Simoni, M.; Camin, F. Stable isotope ratios of H, C, N and O in Italian citrus juices. *J. Mass Spectrom.* **2014**, *49* (9), 785–791. https://www.doi.org/10.1002/jms.3420

(15) Mimmo, T.; Camin, F.; Bontempo, L.; Capici, C.; Tagliavini, M.; Cesco, S.; Scampicchio, M. Traceability of different apple varieties by multivariate analysis of isotope ratio mass spectrometry data. *Rapid Commun. Mass Spectrom.* **2015**, *29* (21), 1984–1990. https://www.doi.org/10.1002/rcm.7306

(16) CEN - ENV12140. Determination of stable carbon isotope ratio (13C/12C) of sugars from fruit juices, using isotope ratio mass spectrometry. *European Committee for Standardization*, **1997**.

(17) Camin, F.; Bontempo, L.; Perini, M.; Piasentier, E. Stable Isotope Ratio Analysis for Assessing the Authenticity of Food of Animal Origin. *Compr. Rev. Food Sci. Food Saf.* **2016**, *15* (5), 868–877. https://www.doi.org/10.1111/1541-4337.12219

(18) Choi, S.; Shin, W.; Bong, Y.; Lee, K. Effects of climate factors on spatiotemporal variation in carbon and oxygen isotope ratios in Korean rice. *J. Food Compos. Anal.* **2022**, *108*, 1-4. https://www.doi.org/10.1016/j.jfca.2022.104416

(19) Schuchovski, C.; Biasi, L. A. Dormancy of Floral Buds of Rabbiteye Blueberry in a Mild Winter Climate. *Braz. Arch. Biol. Technol.* **2021**, *64*, 1-15. https://www.doi.org/10.1590/1678-4324-2021190755

(20) Camin, F.; Perini, M.; Bontempo, L.; Giongo, L. Multi-element (H, C, N, O) stable isotope characterization of blueberries. *Acta Hort*. **2009**, *810*, 697–704. https://www.doi.org/10.17660/ActaHortic.2009.810.92

(21) Klavins, L.; Maaga, I.; Bertins, M.; Hykkerud, A. L.; Karppinen, K.; Bobinas, C.; Salo, H. M.; Nguyen, N.; Salminen, H.; Stankevica, K.; Klavins, M. Trace Element Concentration and Stable Isotope Ratio Analysis in Blueberries and Bilberries: A Tool for Quality and Authenticity Control. *Foods* **2021**, *10* (3), 567. https://www.doi.org/10.3390/foods10030567

(22) Lacumin, P.; Bernini, L.; Boschetti, T. Climatic factors influencing the isotope composition of Italian olive oils and geographic characterisation. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 448-454. https://www.doi.org/10.1002/rcm.3896

(23) Muñoz-Redondo, J. M.; Bertoldi, D.; Tonon, A.; Ziller, L.; Camin, F.; Moreno-Rojas, J. M. Tracing the geographical origin of Spanish mango (*Mangifera indica* L.) using stable isotopes ratios and multi-element profiles. *Food Control* **2021**, *125*, 1-11. https://www.doi.org/10.1016/j.foodcont.2021.107961

(24) Taous, F.; Amenzou, N.; Marah, R.; Maguas, C.; Bahmad, L.; Kelly, S. Stable isotope ratio analysis as a new tool to trace the geographical origin of Argan oils in Morocco. *Forensic Chem.* **2020**, *17*, 1-13. https://www.doi.org/10.1016/j.forc.2019.100198

(25) Floare-Avram, C. V.; Covaciu, F.; Voica, C.; Puscas, R.; Feher, I.; Marincas, O.; Magdas, D. A. Differentiation of tomatoes based on isotopic, elemental and organic markers. *J Food Sci. Technol.* **2020**, *57*, 2222-2232. https://www.doi.org/10.1007/s13197-020-04258-z

(26) Bertolino, L. T.; Caine, R. S.; Gray, E. Impact of Stomatal Density and Morphology on Water-Use Efficiency in a Changing World. *Front. Plant Sci.* **2019**, *10* (225) 1-11. https://www.doi.org/10.3389/fpls.2019.00225

(27) https://www.embrapa.br. [Acessed Feb. 2022].

(28) https://www.ufsm.br/museus/msrs/unidade-de-solos. [Acessed Feb. 2022].

(29) Jamin, E.; Gonzalez, J.; Remaud, G.; Naulet, N.; Martin, G. G.; Weber, D.; Rossmann, A.; Schmidt, H. L. Improved detection of sugar addition to apple juices and concentrates using internal standard ^{13}C IRMS. *Anal. Chim. Acta* **1997**, *347* (3), 359–368. https://doi.org/10.1016/S0003-2670(97)00189-X

(30) FAO/WHO. Codex Alimentarius Commission Procedural Manual. Food and Agricultural Organization of the United Natios and World Health Organization, **2018** (26th ed.). Rome. www.codexalimentarius.org/ [Acessed May 2022].