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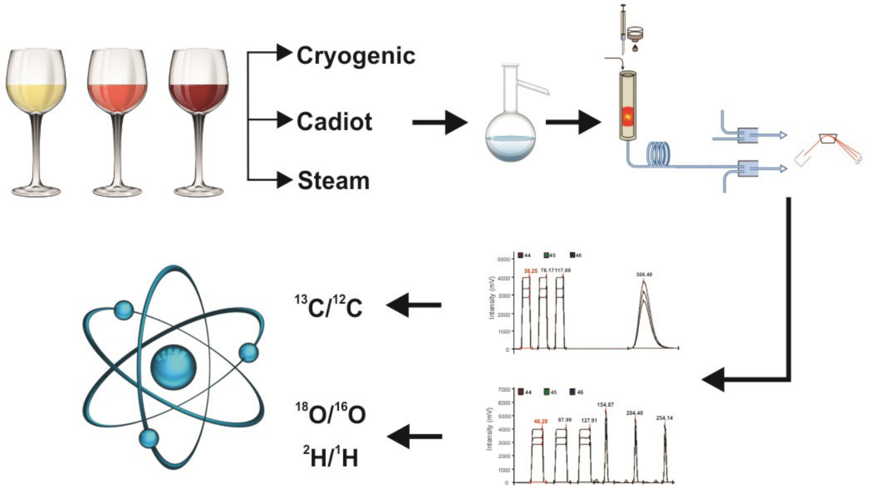
ARTICLE

Optimizing Distillation Techniques for Isotopic Determinations in Beverages

Susiane Leonardelli[1,2*], Júlia Panozzo[1,2], Júlia Daneluz[1], Letícia Leonardelli[1], Gilberto João Cargnel[1], Regina Vanderlinde[1]

[1]Universidade de Caxias do Sul, Instituto de Biotecnologia. Rua Francisco Getúlio Vargas, 1130, 95070-560 Caxias do Sul, RS, Brazil

[2]Laboratório de Referência Enológica Evanir da Silva (LAREN/SEAPI). Avenida da Vindima, 1855, 95084-470, Caxias do Sul, RS, Brazil



Isotopic analysis offers insights into the authenticity, tracing origin, and detecting adulteration in beverages, with applications in various legal contexts. This research addresses challenges in the distillation process to identify the optimal method for preparing samples for isotopic determinations. This study aimed to compare three distillation systems: cryogenic, Cadiot column and steam for carbon, hydrogen and oxygen determinations in ethanol. Furthermore, to explore the benefits of cryogenic method in beverage distillation and to perform the method validation. Additionally, to evaluate the efficiency of the steam distillation for carbon isotopic determination. The ethanol obtained through cryogenic distillation exhibited $\delta^{13}C$ ranging from -29.54 to -24.60‰, slightly lower than those observed in the Cadiot column (-29.12 to -24.64‰). However, trueness analysis revealed comparable results for both methods, supported by satisfactory Z-score and relative error. The outcomes for hydrogen and oxygen exhibited considerable disparities with significant differences. The results of $\delta^{13}C$ found by steam distillation and cryogenic were similar and the mean difference found between the systems was 0.05‰ and the relative error was 0.20%. The study further explored the advantages of cryogenic distillation in ethanol extraction, demonstrating its efficiency for carbon determinations with notable benefits in terms of time and volume required for extraction. Steam distillation proved feasible for carbon determinations, showing similar results to cryogenic distillation. These findings not only present options for laboratories but also highlight the efficiency of cryogenic distillation in ethanol extraction, offering practical applications for isotopic analysis.

Keywords: beverages, stable isotope, cryogenic distillation, Cadiot column, steam distillation

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INTRODUCTION

The capability to discern the origin of chemical substances has established isotopes as an indispensable tool in several disciplines, particularly in forensic science.[1] Regulation and food information to the consumers in the labels are an obligation. The label must include compulsory information following the legislation and can be added optional information. Nonetheless, sometimes the label is correct but the product is adulterated, in this case we have to look for techniques and compounds to control tampering. The major compounds of the alcoholic beverages to control adulteration are ethanol and water.[2]

The origin of alcoholic beverages can be traced back to antiquity, and they have been part of the human culture for many centuries. Over the years, the elaboration oh these beverages have grown significantly with different types of products and development of scientific fundamentals process and legislation.[3] These beverages have parameters that must be performed according to specific legislation, but adulteration is frequent.[4,5]

Considering to work with the ethanol molecules with the purpose to determine stable isotopes to control adulteration, the first step of the preparation is the sample purification, which in beverages are done by distillation. This technique is a robust yet challenging separation with some critical points that comes with drawbacks, such as the interference in the volatile compounds, as well as considerations related to high cost and time constraints.[6,7]

The challenge in the distillation process using high temperatures arise from the formation of an azeotrope, in which is a mix of ethanol and water. The main factor is due to ethanol has a boiling point of 78 °C, while the azeotrope begins to form at 0.2 °C above, for this type of distillation using temperature the critical point is to control the collection of the distillate to avoid contamination. In addition, certain methods for isotopic determination require careful to avoid isotopic fractionation in the distillation process.[8]

In the industry there are various systems available for the recovery and extraction of ethanol from liquid solutions. Techniques using membrane permeation, vacuum stripping, gas stripping, solvent extraction, adsorption, steam distillation and several hybrid methods.[9] The method using Cadiot column is the one internationally recommended for this purpose. However, the official method from International Organisation of Vine and Wine specifies that all the technique available can be used since it meets the specifications and avoids isotopic fractionation.[10]

Considering the established distillation systems, the cryogenic method has been employed for more than six decades, mainly to recover and refine ethylene and propylene from olefin plants.[11] It is a process of separation of mixture, using simple distillation, at high pressure and low temperature. It is used, for example, in the separation of carbon dioxide, where the gas is cooled up to the desublimation temperature and subsequent to the isolation of solid carbon dioxide.[11] This cryogenic method with vacuum is also regarded to a standard method for water extraction in plant tissues for isotope determinations.[12]

The official method recommended for isotopic determinations in beverages in the international compendium takes at least five hours for the extraction[13] and needs substantial expense, so to study and compare other extraction methods is important to improve the sample preparation for the isotopic determinations. Thus, the objective of this research was to evaluate the use of cryogenic, Cadiot column and steam distillation systems for isotopic determinations to provide laboratories with more options. It was also aimed to explore the advantages of cryogenic distillation and validate the parameters. And finally, to compare the efficiency of the steam and cryogenic distillation for carbon isotopic determination.

MATERIAL AND METHODS

Samples

A comprehensive study was conducted on a set of 22 samples harvested in the mountain region of Brazil in 2019, resulting in 76 isotopes determinations aimed at comparing the three distillation systems. The effectiveness of cryogenic distillation for stable carbon isotope ^{13}C was specifically scrutinized in relation to the Cadiot column method, consisting for evaluation seventeen samples: five white and six red wines; four distillates from apple, orange, persimmon and watermelon; as well as one distillate of rice and one of sweet potato.

For the evaluation of hydrogen ^{2}H and oxygen ^{18}O, two white and three red wines were prepared with ethanol extraction conducted through both cryogenic and Cadiot methods. Furthermore, a comparative study involving steam and cryogenic distillation encompassed the preparation and determination of 10 wine samples analysed in triplicate.

Sample preparation

Cryogenic distillation system (CDS)

The CDS setup employed in this research comprised a sealed system regulated by a mechanical device and containing collectors in the end of the extraction line. Figure 1 illustrated the method, which the equipment consists of a diffuser pump, a mechanical pump, a glass tube of 1 m, two liquid nitrogen traps (number 5 and 6) and an extraction line demonstrated for A (Vidropin, São Paulo, Brazil).

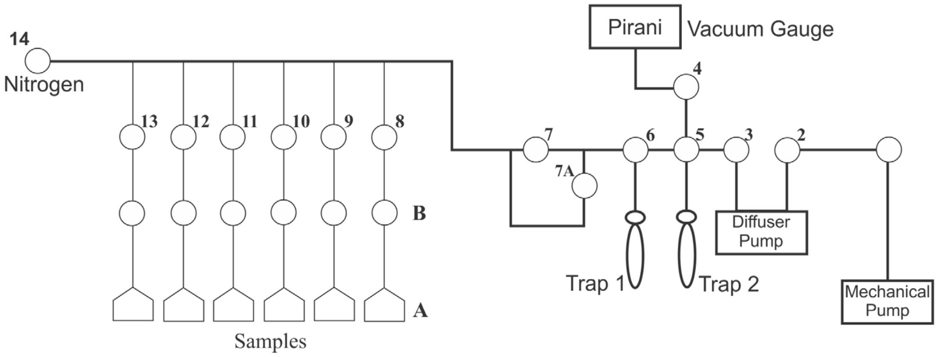

Figure 1. Cryogenic system (CDS) for ethanol distillation.

A volume of 0.0015 L of the sample was placed into a 0.035 L flask, connected to manifold in the end of the extraction line (8 to 13). The liquid was trapped in a vial of 9 mL and immediately cooled, in temperature at -196 ºC and pressure at one Pa. The complete procedure spanned one hour, remaining the distillation for 20 minutes. The ethanol in the samples was vaporizes and gathered in a liquid nitrogen cold trap on side B. After the extraction were completed, the collected analyte was thawed for subsequent analysis.[13,14]

Cadiot column system (CCS)

The distillation process using a Cadiot column (Eurofins Analytics France, Nantes, France) was conducted at the Uruguay National Wine Institute (INAVI) in accordance with the community methods for the analysis of wines in the European Union.[14] Around 0.3 L of the sample were introduced inside a volumetric flask (0.5 L) and heated within the extraction system. The liquid after distillation was trapped in a pre-calibrated erlenmeyer flask (0.12 L), the temperature in the distillation ranging from 78 to 78.2 ºC and lasted approximately 5 h. The round-bottom flask is placed in the heating mantle connected to the extractor and water for the condenser is provided.

Steam distillation system (SDS)

Steam distillation is a method to determine the volume of the alcohol in wine by densimetry using hydrostatic balance. The distillation procedure followed in accordance to the International Compendium of Wine and Musts Analysis.[10] It was measured out 0.1 L of wine sample with a graduated flask between 15 and 25 ºC. The flask was rinsed three times with distilled water and transferred to the distillation tube. Then, 8 mL of the suspension of calcium hydroxide 2 M and antifoam agent were added to the tube and it started the distillation. The distillate was collected in another flask.

Determination of $^{13}C/^{12}C$

The determination of the carbon isotope ratio ($^{13}C/^{12}C$) of ethanol followed the international methodology OIV-MA-AS312-06.[10] The ethanol extracted from sample by distillation was carried out by on-line analysis using an elemental analyser (Flash EA 1112, Thermo Scientific, Bremen, Germany) with an autosampler (AS 1310), coupled to a Delta Plus XL through a Conflo III (Thermo Scientific, Bremen, Germany). The sample of ethanol was injected into a combustion reactor at 900 ºC, all the carbon of the sample was oxidized, passed through a chromatographic column at 45 °C to separated CO_2 from other gas generated and then goes to an isotopic ratio mass spectrometer by a helium flow at 100 mL min^{-1}.

Sample volume was set up by the mass to charge ratio (*m/z*) signal 44 at 3 Volts. Analysing the intensity, it was found that sample volume of 1 µL was employed for ethanol extracted using CDS, while a volume of 0.2 µL was used with the CCS. The analytical error remained below 0.05‰ and the samples were analysed in triplicate. The results were calibrated on the V-PDB scale (Vienna Pee Dee Belemnite) in per mil (‰) against the international reference material Sucrose (NIST 8542, $\delta^{13}C$ value of -10.45‰) and the values were expressed in $\delta^{13}C$.

Performance of the extraction method for carbon using CDS

Linearity was assessed through the repeated analysis of various enrichment levels. Experiments involve the addition of ethanol from sugar cane to a wine sample at concentrations 0, 1, 10, 30, 50, 70, 90, 99 and 100%. The ethanol was extracted from each enriched sample using the CDS, and the analysis were performed in seven replicates.

The accuracy was performed through recovery tests conducted at high, medium, and low analyte concentrations, following the evaluating criteria outlined in the Codex Alimentarius.[15] The precision was gauged through both repeatability and reproducibility assessments in accordance with the Laboratory Guide to Method Validation and Related Topics.[16] The robustness was evaluated doing small intentionally changes in the method, it was considered change in volume and time, the data were statistically evaluated.

Determination of $^{2}H/^{1}H$ and $^{18}O/^{16}O$

Oxygen ($^{18}O/^{16}O$) and hydrogen ($^{2}H/^{1}H$) determinations were carried out using a high temperature conversion elemental analyser connected to an isotopic ratio mass spectrometer (Delta V Advantage) through a Conflo IV (Thermo Scientific, Bremen, Germany). The pyrolysis process took place in a ceramic tube with glassy carbon at 1450 ºC, under a continuous flow of helium at 1.5 bar to generated H_2. Prior to entering into the mass spectrometer, a 1.4 m molecular sieve chromatographic column at 80 ºC was employed to separate H_2 from CO. Sample injection was facilitated by an autosampler (AS 3000).

The isotopic composition is expressed in delta notation, expressed as $\delta^{2}H$ and $\delta^{18}O$, with reference to a primary standard VSMOW (Vienna Standard Mean Ocean Water) with value of 0‰. The analytical error for hydrogen and oxygen remained below to 2‰ and 0.4‰, respectively.

Statistical analysis

All statistical tests were performed using SPSS software, version 26.0 (Chicago, Illinois, USA). To assess the normality of the data the Kolmogorov Smirnov was employed. Data were analysed by ANOVA at 5% significance level.

RESULTS AND DISCUSSION

CDS and CCS distillation for carbon $\delta^{13}C$

The ethanol obtained through distillation using CCS and CDS did not show a statistically significant difference. The $\delta^{13}C$ values can be observed in Figure 2.

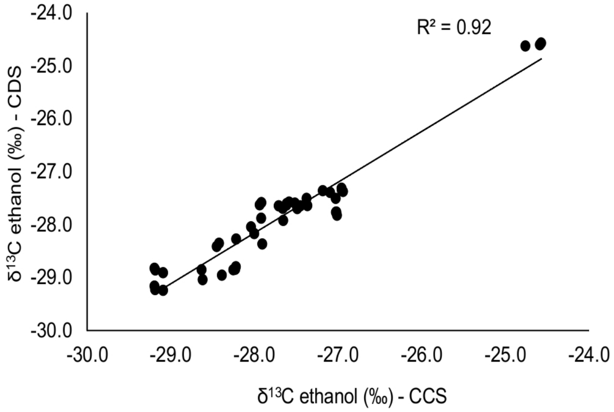

Figure 2. The relationship between $\delta^{13}C$ values of ethanol extracted by CCS (x-axis – Cadiot Column System) and CDS (y-axis – Cryogenic Destilation System) in different beverages.

The $\delta^{13}C$ exhibited a slight variation between the distillations employed to obtain the ethanol. The mean difference identified was 0.14‰, a variance smaller than the findings reported by Rossmann et al.[17] In their study, the authors employed equal method for extraction in two laboratories and they concluded that the variations in $\delta^{13}C$ were attributed to the nature of the material investigated and not exceed the calibration impact.

CDS extraction in general yielded $\delta^{13}C$ values lower than those obtained with CCS. Discrepancies between the systems were also noted in previous studies.[18] In those studies, the researchers noticed a gradual increase in the carbon with 1.2‰ of effect and concluded that there is an influence of the plates, reflux and equilibrium liquid-vapor fractionation.[21] In our investigation, the greatest effect identified was 0.81‰, significantly lower than 1.2‰ as reported in the preview study.

Results reported by Baudler et al.[19] similarly exhibited more negatives values when employing the CCS. Their study involved comparing two extraction systems concerning the starting material, revealing that the distillation process occurs with an inverse vapor pressure isotopic effect, leading to a decrease in $\delta^{13}C$ values.

The mean difference among values is deemed acceptable and falls below the reproducibility limit (R) outlined in the official method for carbon isotopic determination by the OIV. Where for alcohol, the official method sets a reproducibility limit of 0.87‰, while for white wines, it is 0.76‰ and for red wines, it is 0.64‰.[5]

The evaluation of the accuracy of the CDS equipment was conducted and quantitatively calculated. This value concerning $\delta^{13}C$ related to CCS, yielding a mean difference between the systems of 0.57‰ and Z-score of 0.83‰. The outcomes were deemed acceptable, given that the absolute of Z is below 2. This indicated that the $\delta^{13}C$ obtained from the sample distillate through CDS methods are equivalent to those obtained by CCS.

The main benefits of the CDS equipment are concern to the short period to complete the distillation and the sample volume used. Distillation by CCS with 8 columns took approximately 5 h, whereas distillation by the CDS with 6 traps required only one hour. The CDS demonstrated superior speed compared to the CCS, streamlining ethanol extraction for subsequent analysis.

Furthermore, the volume needed for the ethanol extraction in CDS equipment is just 0.0015 L, significantly less than CCS, which varies based on the ethanol percentage in the beverages. Beverages with alcohol exceeding 10%, 0.3 L was required, while samples with alcohol content below 10% needed 0.4 L. Assessing the volume requirements between the distillation methods, CCS necessitates 200 times more sample volume than CDS. Consideration of volume is crucial, especially when dealing with limited availability or expensive and rare samples.

Performance of the CDS for carbon determination δ^{13}C

Giving the applicability of CDS to prepare samples for carbon determinations, the method showed satisfactory parameters for the purpose. The dataset showed a great linear range, characterized by a high determination coefficient (R^2) of 0.99. The working range, being linear and directly proportional to the concentrations, proves well suited for the intended purpose. Moreover, the method demonstrated accurate results for carbon isotope parameters, meeting the standards set by Codex Alimentarius, with a recovery rate of 99%, within the set range of 98% to 102%.[15]

Precision as indicated by repeatability and reproducibility showed values of 0.21‰ and 0.30‰, respectively. Yielded results closely aligned with previous studies on wine and fruit juices that employed similar methods.[12,20]

Another crucial aspect to consider is the robustness, which entails intentionally modifying some parameters and examining the impact on the results. The robustness is shown in Table I.

Table I. Robustness considering variations in volume and time

Parameter	**Condition**	**δ^{13}C (‰)** **Mean** ± SD
Volume (mL)	1.0	$-29.27^{a} \pm 0.08$
	1.5	$-29.27^{a} \pm 0.05$
	2.0	$-29.30^{a} \pm 0.04$
Time (min)	10	$-29.31^{a} \pm 0.06$
	15	$-29.27^{ab} \pm 0.08$
	20	$-29.25^{ab} \pm 0.06$
	25	$-29.29^{ab} \pm 0.05$
	30	$-29.24^{b} \pm 0.11$

Means followed by different letters in the column differ significantly by ANOVA followed by Tukey test at 5% of significance.

The robustness of the CDS was affirmed as the analysed parameters exhibited no statistically meaningful distinctions concerning δ^{13}C values. Unique contrast was found for 10 and 30 minutes. Therefore, it is fundamental to adhere and regulate the recommended extraction time of 20 minutes.

CDS and CCS for hydrogen δ^{2}H and oxygen δ^{18}O

There is also a continuous interest in hydrogen and oxygen characterization for beverages, with a major focus on wines. Influences of the distillation on the δ^{2}H values can be observed in Figure 3.

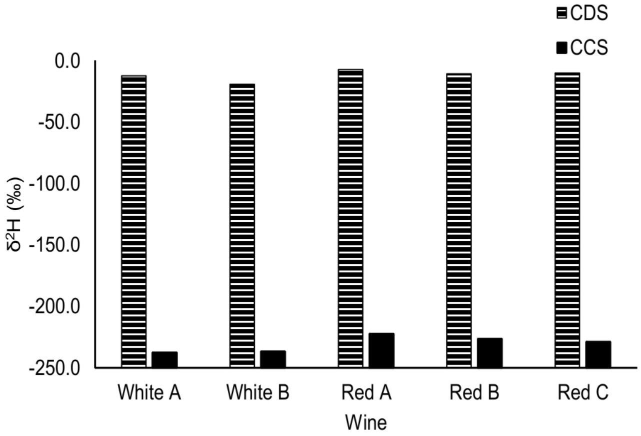

Figure 3. δ^2H values of ethanol distillate by CDS and CCS in wine.

A substantial difference was observed for δ^2H, with distillation using CCS resulting in a notable decrease in δ^2H. Conversely, for $\delta^{18}O$, there was an increase in the values obtained through the CCS, as shown in Figure 4.

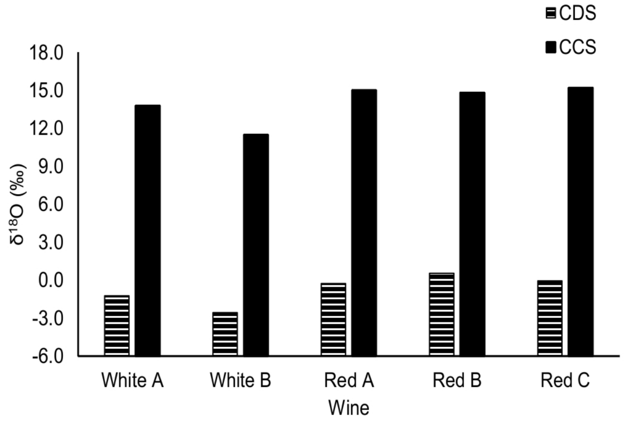

Figure 4. $\delta^{18}O$ values of ethanol distillate by CDS and CCS in wine.

These effects can be attributed to the mixing of water and ethanol, as the CDS does not completely isolate all of the water in the beverage, and water remains in the extracted ethanol after distillation. The values obtained through the CDS closely resembled values found in the atmospheric water reported in previous studies,[21] varying between -146.0 to -11.5‰ for δ^2H and -19.9 to - 2.25‰ for $\delta^{18}O$, as well as values in Germany varying between -170‰ and +18‰ for δ^2H and -24‰ to +2‰ for $\delta^{18}O$.[22] These authors explained that water values are variable due to latitude, altitude and are strongly influenced by seasonal temperatures.

Considering the results above, CDS proved to be ineffective for δ^2H and $\delta^{18}O$ due to water influence on the ethanol extracted. In order to address this limitation, it is crucial to reevaluate the equipment, incorporating steps to capture the water and reduce its impact in this specific application.

CDS and SDS for carbon $\delta^{13}C$

Steam distillation (SDS) is a widely employed method for gauging the alcoholic strength in wine. This technique involves the determination of percentage of ethanol through densimetry using a hydrostatic balance. It is a prevalent system in laboratories for determining alcohol percentage in beverages, forming a routine aspect of laboratory procedures. Employing this distillate for additional analysis, such as isotopic determinations, offers a practical means of streamlining laboratory process.

The carbon isotopic composition ($\delta^{13}C$) of ethanol extracted from wines through both CDS and SDS is presented in Table II.

Table II. $\delta^{13}C$ values of ethanol extracted by CDS and SDS

CDS (‰)	SDS (‰)	Relative error (%)
-22.88 ± 0.09	-22.87 ± 0.05	-0.04
-22.48 ± 0.18	-22.64 ± 0.09	0.69
-22.25 ± 0.08	-22.13 ± 0.04	-0.51
-21.85 ±0.12	-22.00 ± 0.12	0.67
-22.61 ± 0.11	-23.66 ± 0.15	4.44
-22.27 ± 0.11	-22.74 ± 0.13	2.09
-22.76 ± 0.14	-22.37 ± 0.03	-1.73
-23.11 ± 0.12	-22.72 ± 0.13	-1.72
-23.46 ± 0.17	-23.20 ± 0.13	-1.11
-24.27 ± 0.08	-23.08 ± 0.16	-0.82

ANOVA, significance level of 5%.

The $\delta^{13}C$ values of the ethanol samples exhibited no significance differences between the two systems under consideration. The mean difference observed between the systems was 0.05‰, with a mean relative error of 0.20‰, both falling within the previously established range deemed as low and satisfactory for carbon determination in this study. As a result, the SDS exhibited $\delta^{13}C$ values similar to those of the CDS, demonstrating its effectiveness in distilling wines for carbon isotope determinations.

This research has effectively met its objective by comparing the distillation systems for isotopic determination. It has not only fulfilled its goal but also presented choices for laboratories to consider in the ethanol extraction for isotopic determination.

CONCLUSION

In conclusion, this study successfully achieved its objective comparing cryogenic, Cadiot column, and steam distillation systems for isotopic determinations. The research not only provided additional options for laboratories but also explored the benefits of cryogenic distillation in ethanol extraction revealing that cryogenic is efficient for carbon isotopic determination, yielding comparable results to the official method. Furthermore, cryogenic demonstrated a significant advantage in terms of time and volume required. On the other hand, cryogenic proves unsuitable for hydrogen and oxygen analyses due to substantial water interference in the extracted ethanol.

The steam distillation system produced carbon isotope values comparable to those obtained through the cryogenic method. These results underscore feasibility of using steam distillation for $\delta^{13}C$ in beverages. These findings provide valuable insights to the field of isotopic determinations.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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REFERENCES

(1) Matos, M. P. V; Jackson, G. P. Isotope Ratio Mass Spectrometry in Forensic Science Applications. *Forensic Chem*. **2019**, *13*, 100154, 1-75. https://doi.org/10.1016/j.forc.2019.100154

(2) Coldea, T. E.; Mudura, E.; Socaciu, C. Advances in Distilled Beverages Authenticity and Quality Testing. In: Stauffer, M. (Ed.) *Ideas and Applications Toward Sample Preparation for Food and Beverage Analysis*. InechOpen, 2017, Chapter 6, p 182. https://doi.org/10.5772/intechopen.72041

(3) Núñez-Caraballo, A.; García-García, J. D.; Ilyina, A.; Flores-Gallegos, A. C.; Michelena-Álvarez, L. G.; Rodrígues-Cutiño, G.; Martínez-Hernández, J. L.; Aguilar, C. N. Alcoholic Beverages: Current Situation and Generalities of Anthropological Interest. In: Grumezescu, A. M.; Holban, A. M. (Eds). *Processing and Sustainability of Beverages*. Woodhead Publishing, 2019. Vol. 2: The Science of Beverages, pp 37-72. https://doi.org/10.1016/B978-0-12-815259-1.00002-1

(4) Neto, A. G. C.; Costa, M. M. A.; Schuler, M. S. V.; Azevedo, E. P. P. Study of The Main Types of Beverages Adulteration Seized in Recife City. *Braz. J. Forensic Sci*. **2016**, *5* (2), 133-145. https://doi.org/10.17063/bjfs5(2)y2016133

(5) International Standard for the Labelling of Wines. OIV publications, 2022, Paris, France. Available at: https://www.oiv.int/public/medias/8175/en-oiv-wine-labelling-standard-2022.pdf (accessed Dec. 2023).

(6) Hattoi, R.; Yamada, K.; Hasegawa, K.; Ishikawa, Y.; Ito, Y.; Sakamoto, Y.; Yoshida, N. An improved method for the measurement of the isotope ratio of ethanol in various samples, including alcoholic and non-alcoholic beverages. *Rapid Commun. Mass Spectrom.* **2008,** *22* (21), 3410-3414. https://doi.org/10.1002/rcm.3753

(7) Onuki, S.; Koziel, J. A.; Leeuwen, J. V.; Jenks, W. S.; Grewell, D. A.; Lingshuang, C. Ethanol production, purification, and analysis techniques: a review. *Annual International Meeting - American Society of Agricultural and Biological Engineers* **2008**. https://doi.org/10.13031/2013.25186

(8) Kumar, S.; Singh, N.; Prasad, R. Anhydrous ethanol: a renewable source of energy. *Renew. Sust. Energ. Rev*. **2010**, *14* (7), 1830-1844. https://doi.org/10.1016/j.rser.2010.03.015

(9) Offeman, R. D.; Stephenson, S. K.; Franqui, D.; Cline, J. L.; Robertson, G. H.; Orts, W. J. Extraction of ethanol with higher alcohol solvents and their toxicity to yeast. *Sep. Purif. Technol*. **2008**, *63* (2), 444-451. https://doi.org/10.1016/j.seppur.2008.06.005

(10) Compendium of international methods of wine and must analysis. OIV publications, 2022, Paris, France. Available at: https://www.oiv.int (accessed Dec. 2023).

(11) Llewellyn, P. L.; Rodrigues-Reinoso, F.; Rouqerol, J.; Seaton, N. (Eds). *Characterization of Porous Solids VII: Proceedings of the 7th International Symposium on the Characterization of Porous Solids* (COPS-VII), Aix-en-Provence, France. Elsevier, 2006, Vol. 160, 1-734.

(12) Muhammad, A. H.; Nadeem, F.; Tariq, R.; Rashid, A. *Renewable and Alternative Energy Resources. Renew and Alternative Energy Resources*. Academic Press, 2022. https://doi.org/10.1016/C2018-0-03161-8

(13) Camin, F.; Simoni, M.; Hermann, A.; Thomas, F.; Perini, M. Validation of the 2H-SNIF NMR and IRMS Methods for Vinegar and Vinegar Analysis: An International Collaborative Study. *Molecules* **2020**, *25* (2932), 1-11. https://doi.org/10.3390/molecules25122932

(14) Commission Regulation (EEC) No 2676/90 of 17 September 1990. Official Journal L 272, **1990**, pp 1-192. Available at: https://www.fao.org/faolex/results/details/en/c/LEX-FAOC036018/ (accessed Dec. 2023).

(15) FAO/WHO. Codex Alimentarius Commission Procedural Manual. Food and Agriculture Organization of the United Natios and World Health Organization. **2018** (26th ed.), Rome, Italy. Available at: www.codexalimentarius.org. (acessed Dec. 2023).

(16) Magnusson, B.; Örnemark, U. *Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics*. **2014**, 2nd ed. ISBN 978-91-87461-59-0. Available at: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf (acessed Dec. 2023).

(17) Rossmann, A.; Schmidt, H. L.; Reniero, F.; Versini, G.; Moussa, I.; Merle, M. H. *Z.* Stable carbon isotope content in ethanol of EC data bank wines from Italy, France and German. *Z. Lebensm. Unters. Forsch*. **1996**, *203*, 293-301. https://doi.org/10.1007/BF01192881

(18) Moussa, I.; Naulet, N.; Martin, M. L.; Martin, G. J. A Site-Specific and Multieiement Approach to the Determination of Liquid-Vapor Isotope Fractionation Parameters. The Case of Alcohols. *J. Phys. Chem*. **1990,** *94*, 8303-8309. https://doi.org/10.1021/j100384a056

(19) Baudler, R.; Adam, L.; Rossmann, A.; Versini, G.; Engel, K.-H. Influence of the distillation step on the ratio stable isotopes of ethanol in cherry brandies. *J. Agric. Food Chem.* **2006,** *54* (3), 864-869. https://doi.org/10.1021/jf052043a

(20) Thomas, F.; Jamin, E. ^{2}H NMR and ^{13}C-IRMS analyses of acetic acid from vinegar, ^{18}O-IRMS analysis of water in vinegar: International collaborative study report. *Anal. Chim. Acta* **2009**, *649* (1), 98-105. https://doi.org/10.1016/j.aca.2009.07.014

(21) Boschetti, T.; Cifuentes, J.; Iacumin, P.; Selmo, E. Local meteoric water line of Northern Chile (18° S-30° S): An application of error-in-variables regression to the oxygen and hydrogen stable isotope ratio of precipitation. *Water* **2019**, *11* (4), 791. https://doi.org/10.3390/w11040791

(22) Stumpp, C.; Klaus, J.; Stichler, W. Analysis of long-term stable isotopic composition in German precipitation. *J. Hydrol*. **2014,** *517*, 351-361. https://doi.org/10.1016/j.jhydrol.2014.05.034