

Multi-isotope Analysis of Tartaric Acid Derivative: Potassium Bitartrate

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Abstract: Tartaric acid is naturally obtained from many fruits, principally grapes. Several tartaric acid derivatives are commercialized, especially salts and esters. They are usually applied in food, pharmaceutical and viniculture industry. The rise in production of synthetic tartaric acid has increased the production of synthetic derivatives consequently. Potassium bitartrate (KHT), also known as cream of tartar, is an important derivative used in food and pharmaceutical industries. At the moment there is no specific regulation for identification and characterization of synthetic tartaric acid derivatives, it is exclusive for naturals. In this study isotope ratio mass spectrometry (IRMS) was applied in order to discriminate KHT production method and geographical origin. The measurements of $\delta^2\text{H}$, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were performed by isotope-ratio mass spectrometry. A clear differentiation between synthetic and natural samples is observed. However, the same was not observed for classification regarding geographical origin. This is a promising technique that combined with a larger database that could be applied in quality control of KHT.

Key words: Tartaric acid derivatives, multi-isotope analysis, KHT, isotopic mass spectrometry.

1. Introduction

Tartaric acid is a natural organic acid and is produced naturally in the fermentation of fruits, such as grapes and tamarind. It is one of the main compounds used to control acidity in wine. Several products from tartaric acid structure are commercialized in food and pharmaceutical industries. Salts and esters are the most produced derivatives, with emphasis on potassium bitartrate ($\text{KC}_4\text{H}_5\text{O}_6$).

Potassium bitartrate, also known as cream of tartar, is formed in wine tanks and spontaneously precipitates in the form of small orthorhombic crystals. This salt is soluble in water and alcohol, and has a slightly acidic taste. Known since antiquity, cream of tartar has its greatest production from lees and grape marc. It is used in the food, pharmaceutical and chemical industries. In food, it is applied as stabilizer, anti-caking and thickener. In winemaking, it acts as a crystallization inducer of tartaric acid salts [1, 2].

Increasing competition in the world market between natural and synthetic products means there is a need for new tools for monitoring the quality of tartaric acid and its derivatives. Although chemical methods can be used to detect contamination, they provide limited information about the geographical origin of a material or the identification of synthetic material of chemically identical composition. Natural abundance of stable isotope is measured by isotope ratio mass spectrometry (IRMS) [3]. This technique is applied frequently for food authentication and determination of geographical origin [4, 5].

The measurements of the stable isotope ratios of hydrogen and oxygen are strongly latitude dependent and related to precipitation water, which depends on the region and climate. This provides important information about geographical origin. Natural tartaric acid carbon isotopes ratio is restricted to a short range, since they come from a plant with a restricted climate. However, synthetic product measurements have a wide range of variation, due to its different sources [6, 7]. A previous study [8] showed the discrimination of

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tartaric acid samples by geographical origin and production method, applying the method IRMS.

Tartaric acid derivatives also need specification regarding its production method and geographic origin. Starting from these considerations, the aim of this research is to make an isotopic characterization of tartaric acid derivative, potassium bitartrate, in order to determine their production method and geographical origin.

2. Method and Materials

2.1 Samples

For this study samples of potassium bitartrate (KHT) were collected from different suppliers and diverse countries. A set of 26 samples of KHT were obtained, of which 19 samples are from natural origin and 7 samples are synthetic.

All samples were cryogenically broken to ensure homogeneity in the size distribution. They were kept in a desiccator under an inert atmosphere to avoid errors due to environmental conditions. No special preparation for isotopic analysis was needed due to high level of purity and homogeneity of samples.

2.2 Isotope Analysis

Isotopic ratio of carbon, oxygen and hydrogen was determined by IRMS. The measurement of $^{13}\text{C}/^{12}\text{C}$ ratio was performed in an Elemental Analyzer (EA) Flash EA1112 coupled to an IRMS Delta V Plus, both from Thermo Scientific. Samples were introduced into a combustion unit maintained at 1,000 °C under a stream of oxygen and helium. Carbon was converted to CO_2 and the signals received on the masses 44, 45 and 46 were used to measure the carbon isotope ratio. Analysis of $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratio was carried out in a high temperature conversion elemental analyzer (TC/EA) from Thermo Finnigan, linked to IRMS Delta V Plus. Samples were deposited into a pyrolysis unit at 1,400 °C containing carbon black in a ceramic tube, filled itself of carbon black. The hydrogen and oxygen from the samples were converted to H_2 and

CO . The $^2\text{H}/^1\text{H}$ ratio was calculated based on the measured signals of m/z 2 and 3 of H_2 . Then $^{18}\text{O}/^{16}\text{O}$ ratio was determined using the signals collected for m/z 28 and 30 of CO .

The stable isotope compositions were reported in the delta (δ) notation as isotope ratio variations relative to an international measurement standard, as per mil (‰), as in Eq. (1).

$$\delta = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} - 1] \times 1,000 \quad (1)$$

where R_{sample} and R_{standard} are $^{13}\text{C}/^{12}\text{C}$ or $^{18}\text{O}/^{16}\text{O}$ or $^2\text{H}/^1\text{H}$ ratios of the sample and standard, respectively. International standard used for ^{13}C was Pee Dee Belemnite (PDB) and for ^2H and ^{18}O was Standard Mean Ocean Water (SMOW).

2.3 Statistical Analysis

The data were evaluated with multivariate statistical techniques by using the software Minitab® Statistical version 17.1.0 (Minitab Inc., State College, USA). Principal components analysis (PCA) was used to simplify data matrix based on the correlation between the variables. The results were compared with the information obtained from the suppliers and sample labeling concerning natural or synthetic production and geographical origin. It enables to demonstrate differences between the various samples or groups of samples through new variables formed, referred as principal components. In addition, discriminate analysis was applied to find mathematical functions to distinguish samples in groups, verifying predetermined and predicted groups by cross validation.

3. Results and Discussions

The $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and $\delta^2\text{H}$ isotopic ratio of the potassium bitartrate samples is presented in Table 1. The $\delta^{13}\text{C}$ data, obtained for KHT, show similar range for natural and synthetic samples. For the samples naturally produced, the $\delta^{18}\text{O}$ range varies from 25.63 to 30.93, while for the synthetic samples the range varies from 20.37 to 22.29, lower than natural ones.

Table 1 Stable isotope ratios of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and $\delta^2\text{H}$ for different production methods of potassium bitartrate.

	$\delta^{13}\text{C}$				$\delta^{18}\text{O}$				$\delta^2\text{H}$			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
KHT natural ($n = 19$)	-23.04	0.61	-23.91	-22.06	28.28	2.65	24.50	34.30	-96.55	29.64	-159.00	-8.40
KHT synthetic ($n = 7$)	-22.39	0.29	-22.78	-22.00	21.33	0.96	19.70	22.40	-141.86	4.26	-147.00	-133.00

The $\delta^{13}\text{C}$ data, obtained for KHT, show similar range for natural and synthetic samples. For the samples naturally produced, the $\delta^{18}\text{O}$ range varies from 25.63 to 30.93, while for the synthetic samples the range varies from 20.37 to 22.29, lower than natural ones. Therefore, $\delta^{18}\text{O}$ values alone allow a first discrimination between potassium bitartrate from different production origins. Despite the high standard deviation for natural KHT, the $\delta^2\text{H}$ data also show a good differentiation of the samples by the range of values since there is no overlap of values.

PCA was used here as a pattern recognition method that calculates new variables (principal components) as a linear combination of the original variables. The data set for the stable carbon, oxygen and hydrogen isotopes of KHT that were analyzed by PCA are shown in Fig. 1. A clear separation is visible between the two groups of KHT. Discriminate analysis with cross-validation indicated 92.3% of correct classification. Two samples identified as natural were reclassified as synthetic, the two most to the left of Fig. 1 close to the region of the synthetic samples. In Fig. 2 the samples were classified according to their geographical origins. Some samples did not have their origins identified by the suppliers; this hindered the complete analysis of this parameter. No significant discrimination was associated with the KHT samples for this criterion.

3.1 Comparison of the $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and $\delta^2\text{H}$ Values of Potassium Bitartrate and Tartaric Acid

We compared $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, and $\delta^2\text{H}$ isotopic ratio measurements of tartaric acid using the same set of potassium bitartrate that was studied previously [8]. In

Fig. 3 potassium bitartrate and tartaric acid samples were classified according to their production method.

It is observed that natural and synthetic KHT are

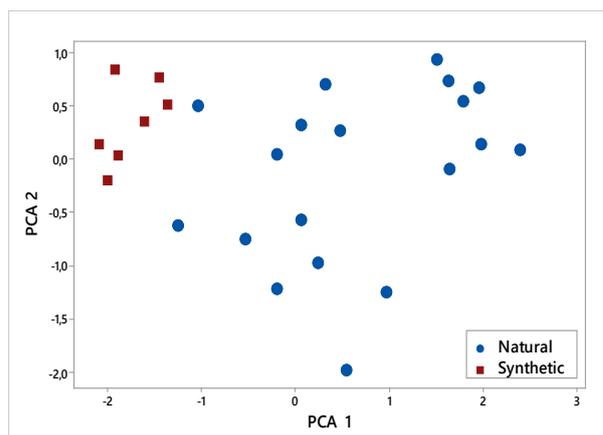


Fig. 1 PCA plot for production method using the first two principal components for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ data for potassium bitartrate.

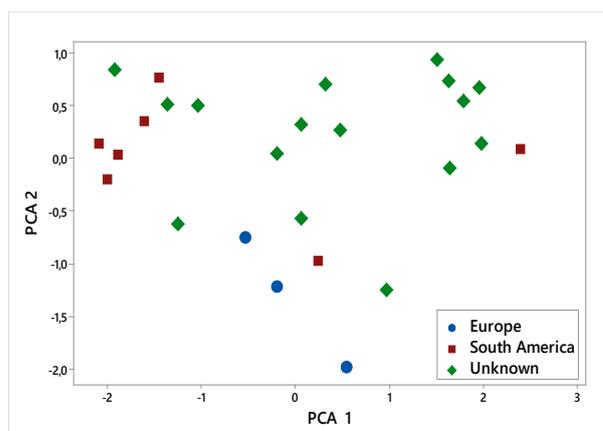


Fig. 2 PCA plot for geographical origin using the first two principal components for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ data for potassium bitartrate.

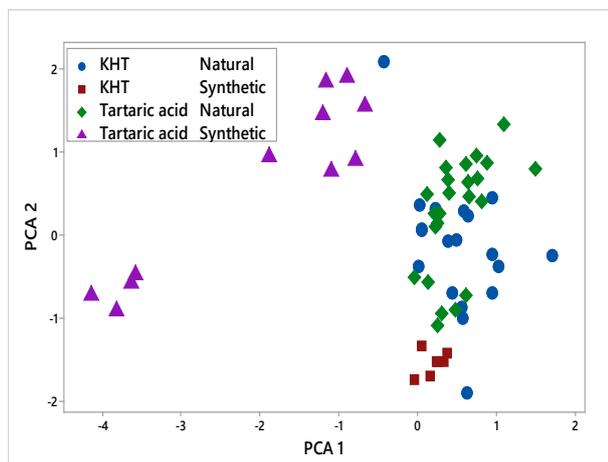


Fig. 3 Comparison PCA plot for production method using the first two principal components for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ data for potassium bitartrate (KHT) and tartaric acid.

more similar and they are closer than natural and synthetic tartaric acid. Unlike tartaric acid, synthetic KHT samples are in a narrow region with similar values of isotopic ratio. Two samples of natural KHT are far from their original set. These were the reclassified samples by the discriminate analysis possibly due to a failure of supplier information. Nevertheless, the comparison of the stable isotope analyses of tartaric acid and KHT suggests the possibility of differentiation of samples by production method.

4. Conclusions

The isotopic analysis is effective to classify the production method and geographical origin of various products, such as tartaric acid. The application of this technique to characterize a derivative of tartaric acid is promising. The method proposed in this study is relatively fast, without extraction or pre-processing methods. IRMS along with multivariate statistical analysis can discriminate KHT from different production methods. To continue this study, it will be necessary to collect a larger number of samples by region, in order to have a sufficient statistical distribution of geographical origin by country.

The carbon, oxygen and hydrogen isotope

composition of potassium bitartrate determined by IRMS is a reliable indicator of the production method, and thus, can be applied for quality control of this product.

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References

- [1] Kassaian, J. M. 2000. "Tartaric Acid." In *Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.
- [2] Yalcin, D., Ozcalik, O., Altioek, E., and Bayraktar, O. 2008. "Characterization and Recovery of Tartaric Acid from Wastes of Wine and Grape Juice Industries." *Journal of Thermal Analysis and Calorimetry* 94 (3): 767-71.
- [3] Rossmann, A. 2001. "Determination of Stable Isotope Ratio in Food Analysis." *Food Reviews International* 17 (3): 347-81.
- [4] Förstel, H. 2007. "The Natural Fingerprint of Stable Isotopes—Use of IRMS to Test Food Authenticity." *Analytical and Bioanalytical Chemistry* 388 (3): 541-4.
- [5] Kokkinofa, R., Fotakis, C., Zervou, M., Zoumpoulakis, P., Savvidou, C., Poulli, K., Louka, C., Economidou, N., Tzioni, E., Damianou, K., Loupasaki, S., and Kefalas, P. 2017. "Isotopic and Elemental Authenticity Markers: A Case Study on Cypriot Wines." *Food Analytical Methods* 10 (12): 3902-13.
- [6] Katerinopoulou, K., Kontogeorgos, A., Salmas, C. E., Patakas, A., and Ladavos, A. 2020. "Geographical Origin Authentication of Agri-Food Products: A Review." *Foods* 9 (4): 489.
- [7] Kelly, S., Heaton, K., and Hoogewerff, J. 2005. "Tracing the Geographical Origin of Food: The Application of Multi-element and Multi-isotope Analysis." *Trends in Food Science & Technology* 16 (12): 555-67.
- [8] Leirose, G., Grenier-Loustalot, M.-F., and Oliveira, A. 2017. "Investigation of Geographical Origin and Production Method of L(+)-Tartaric Acid by Isotopic Analyses with Chemometrics." *Journal of Chemistry and Chemical Engineering* 11 (2017): 45-50.