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Stable isotope ratio analysis to detect biosynthetic citric acid addition to Italian tomato sauce

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Summary: *An innovative LC-co-IRMS (liquid chromatography-isotope ratio mass spectrometry) method to detect the addition of biosynthetic citric acid to tomato sauce was optimized and validated. Threshold carbon isotopic values for authentic tomato citric acid were established and used to test a group of commercial samples available on the market.*

Keywords: *stable isotopes, liquid chromatography – isotope ratio mass spectrometry, Italian tomato sauce*

Introduction

Tomato sauce is an important product in Italian market and economy. Citric acid (E330) is naturally present in tomatoes and, according to EU laws, it can be added to tomato sauce with no restrictions about its amount and origin [1]. Unlike consumer perception, the citric acid most added to the tomato sauce does note derive from citrus fruits [2], but it is biosynthetically obtained from *Aspergillus Niger* fungus fed on cheap starting materials such as corn and cane. As consumers are willing to pay an extra for products labelled as “100% natural” or “no additives”, a method to detect the addition of biosynthetic citric acid to food and, in this case, to Italian tomato sauce, is required.
Tomato belongs to the group of C3 plants, meaning that inside the plant carbon dioxide (CO2) is fixed via Calvin cycle during the photosynthesis. On the other hand, cheap sources like cane and corn, provided to *Aspergillus Niger* for citric acid synthesis, are classified as C4 plants. These plants adapted to environmental conditions choosing an alternative photosynthetic pathway. This results in statistically different carbon isotopic values ($\delta^{13}C$) for C3 and C4 plants [3], making it possible for the stable isotope ratio analysis (SIRA) to discriminate between different sources.
The AIJN (Association of the Industry of Juices and Nectars) provided reference values for the citric acid of other types of fruits (lemon, orange, grapefruit) but no values are given for tomato citric acid. Reference $\delta^{13}C$ are reported for tomato bulk sugar [4], but to the best of our knowledge, no information about single sugars like glucose and fructose are available.
This work had therefore multiple goals. First, we wanted to validate a method that could provide $\delta^{13}C$ values for tomato citric acid ($\delta^{13}CCA$) and apply it on a wide dataset of authentic Italian tomato sauce samples to establish reference values. Once assessed reference $\delta^{13}CCA$ values, we wanted to apply them to commercial samples available on the market to check whether they were added with biosynthetic citric acid or not. Moreover, since it has been reported that in some cases the ratios between the isotopic values of acids and sugars were more effective than the $\delta^{13}C$ itself to detect additions to the samples [5], ratios between the $\delta^{13}CCA$ and the $\delta^{13}C$ of tomato glucose (RCA/G) and fructose (RCA/F) were considered.

Experimental

A 300 samples dataset has been considered, mainly represented by Italian authentic tomato sauces, sampled by ICQRF (Central Inspectorate for Fraud Repression and Quality Protection of the Agri-Food Products and Foodstuffs), and an additional group of tomato sauce samples available on the market.
The analyses were carried out through a liquid chromatographer coupled with an isotope ratio mass spectrometer (LC-co-IRMS) (Thermo Scientific, Bremen). The column (Rezex ROA- Organic Acid H+ (P/N 00H-0138-K0, Phenomenex, Torrance, USA) selected for citric acid isolation of made it possible to simultaneously measure the $\delta^{13}C$ of citric acid ($\delta^{13}C_{CA}$), glucose ($\delta^{13}C_{G}$) and fructose ($\delta^{13}C_{F}$).
Samples preparative included the centrifugation of the tomato sauce sample and their dilution 1 to 10 with milliQ water. The analytical protocol followed for the analysis and a deeper description of the apparatus selected are described in the literature [6].

Results

To validate the LC-co-IRMS method, the within- and between-days repeatability of both a standard and an authentic tomato sauce sample were measured. The tomato sample within-day repeatability gave standard deviations of 1.5‰, 1.5‰ and 1.2‰ for $\delta^{13}C_{CA}$, $\delta 13C_{G}$ and $\delta^{13}C_{F}$, respectively. The between-days repeatability

gave standard deviations of 1.1‰, 0.8‰ and 0.9‰ for $\delta^{13}C_{CA}$, $\delta^{13}C_G$ and $\delta^{13}C_F$, respectively.
The $\delta^{13}C_G$ and $\delta^{13}C_F$ of Italian tomato sauce samples were in line with the values provided by the AIJN for bulk tomato sugar. The two parameters resulted linearly correlated (Pearsons's coefficient = 0.84), in agreement with previous results on grape glucose and fructose [6].
The $\delta^{13}C_{CA}$ of authentic Italian tomato samples averaged -26.5±1.3‰, ranging from a minimum value of -31.3‰ to a maximum value of -23.6‰ (Figure 1). Considering the standard deviation and the analytical uncertainty, a threshold value for the $\delta^{13}C_{CA}$ for authentic samples was established. As previously mentioned, when evaluating the authenticity of the commercial samples, the $\delta^{13}C_{CA}$ values were used together with the ratios between citric acid values and glucose/fructose ones (RCA/G, RCA/F). Applying the threshold values to the commercial samples, it turned out that 5.2% of them was added with biosynthetic citric acid.

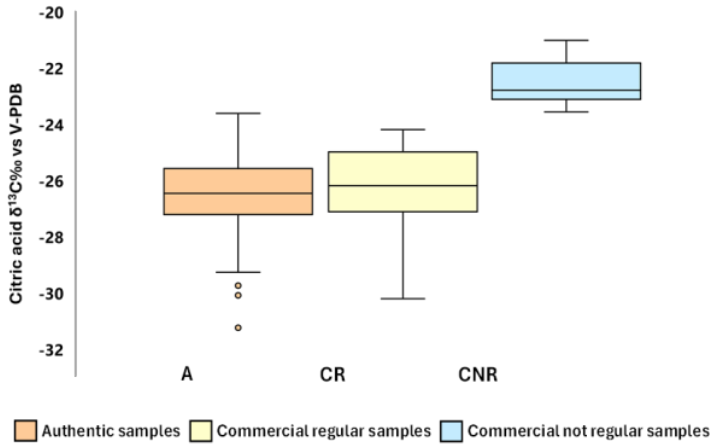

Figure 1. *Citric acid $\delta^{13}C$ of authentic Italian tomato samples and for regular/not regular commercial samples*

Conclusions
In the present study, a wide dataset made up of more than 300 authentic Italian tomato sauce samples was analysed through LC-co-IRMS to simultaneously measure the $\delta^{13}C$ of citric acid, glucose and fructose. For the first time, reference $\delta^{13}C$ values for tomato citric acid were established, giving the opportunity to test a group of commercial tomato sauce samples. The 5.2% of the test group turned out to be added with biosynthetic citric acid. For a broader applicability of the method, authentic tomato sauces having different geographical origins should also be analysed.

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