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Potentials and caveats with oxygen and sulfur stable isotope analyses in authenticity and origin checks of food and food commodities

Nicole Krivachy (Tanz), Andreas Rossmann, Hanns-Ludwig Schmidt*

Isolab GmbH Laboratory for Stable Isotopes, Woelkestrasse 9, D-85301 Schweitenkirchen, Germany

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ABSTRACT

Analyses of stable isotope ratios are officially accepted methods in food authenticity and origin determination. They are routinely practiced by empirical comparison of the unknown samples' δ values with those of authentic material. However, as the isotope characteristics of food are influenced by many parameters, it is desirable to also study and use causal correlations of isotope fractionations for the interpretation of experimental data. Corresponding potentials and limits are outlined for oxygen and sulfur stable isotopes.

In the natural water cycle, plant leaf and animal cell water are the most important sources for food integrated water and organically bound oxygen. The way from sea water to fruit juice water and to organic matter and the integrated isotope fractionations are shown and the possibilities and limits for the assignment of juices and wine to their geographical origin, history and authenticity are deduced. The oxygen flux and isotopic balance in animals and the sources and drains of animal body water are outlined and the problems and limits for its suitability as a bioindicator for origin assignments of animal food products are discussed. The potential of the $\delta^{18}O$ value of organically bound oxygen is demonstrated.

The sulfate reduction in plants is accompanied by isotope fractionation but as normally no sulfur is excreted, the $\delta^{34}S$ value of bulk plant matter is identical to that of the primary local source. Small differences in the $\delta^{34}S$ values of plant compartments are often due to differences in their abundance of main S-containing ingredients, as residual sulfate and cysteine or methionine containing proteins. This is similar with animal tissues and products. Therefore, the sulfur isotope analysis of the bulk matter or of defined fractions of plant and animal samples is an ideal and reliable tool for food origin and authenticity proof and for archaeological and animal migration research.

1. Introduction

In recent years the European Community/Union has defined indications for the quality characterization of food and food commodities like "Protected Denomination of Origin (PDO)", "Protected Geographical Indication (PGI)", and "Certification of Specific Character (CSC)" (cited in Schmidt, Rossmann, Rummel, & Tanz, 2009; further information see Calderone & Guillou, 2008). The criteria and the methodology to be used for conferring or applying these indications on a food sample are indicated in corresponding regulations. Among the officially recommended methods, stable isotope ratio determinations of the bioelements are occurring in the first range. Factors determining these isotope ratios of a natural sample are those of the primary material in question, thermodynamic and kinetic isotope effects, geographical and climatic conditions of the sample's origin, physiological and anatomic properties of plants and animals' positions in food chains. Vice versa, corresponding parameters can be deduced from the isotopic properties of a sample. Furthermore, most important is the individual dependence of the bioelements on the different external influences, their "indicator function" (Schmidt, Roßmann, Stöckigt, & Christoph, 2005). And as the informations implied in the isotope ratios of the individual elements are complementary, many recent investigations on food origin and authenticity use multielement isotope ratio determinations (Bahar et al., 2008; Camin et al., 2007; Schlicht, Roßmann, & Brunner, 2006).

Carbon isotope ratios are predominantly indicative for food (ingredients) assignments to their origin from the photosynthetic plant types C_3, C_4 and CAM plants. One application is the proof of an adulteration of beverages of C_3 plants with C_4 products and vice

* Corresponding author. Prielhofweg 2, D-84036 Landshut, Germany. Tel.: +49 871 44497.
E-mail addresses: nicole.tanz@gmx.de (N. Krivachy (Tanz)), hlschmidt@web.de (H.-L. Schmidt).

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versa. (Jamin, Gonzalez, Remaud, Naulet, & Martin, 1997). In food chains, carbon δ values increase by ~2‰ for each trophic level (Michener & Schell, 1994; for fundamentals of isotope abundance and fractionation indications see Box 1). Nitrogen isotope ratios are correlated to agricultural fertilization practices and hence often used to discriminate between compounds from conventional and "organic" production, respectively (Camin et al., 2011; Rapisarda et al., 2010; Schmidt, Roßmann, et al., 2005; Schmidt, Rossmann, et al., 2005; Sturm & Lojen, 2011). The trophic shift of nitrogen isotope indications can attain 2–4‰ per level (Michener & Schell, 1994). Hydrogen and oxygen are preferably originating from plant leaf or animal drinking water, respectively, the isotope characteristics of which depend on local precipitation and climate, but are modulated by biochemical, physiological and anatomical influences (White, 1989). Although hydrogen and oxygen isotope ratios of meteoric water are correlated among each other by the "meteoric water line" ($\delta^2H\,[‰]_{V\text{-}SMOW} = 8 \times \delta^{18}O[‰]_{V\text{-}SMOW} + 10$, Craig, 1961), the indicator function of hydrogen is more important for individual metabolites, as many isotope fractionations of this element in the secondary metabolism lead to characteristic hydrogen isotope patterns of defined plant compounds. This is the basis for origin and authenticity investigations on spices, aromas and fragrances by means of ^2H NMR measurements (deuterium positional or pattern analysis, Schmidt, Werner, Rossmann, Mosandl, & Schreier, 2007). Trophic levels in context with food chains are of minor importance with hydrogen and oxygen, as the primary source water is globally available in form of an infinite pool with large turnover rate.

In food analysis, oxygen isotope ratios are often determined on the bulk sample. These $\delta^{18}O$ values of biomass provide average data of cell water and organically bound oxygen. However, in most cases of food quality investigations, the $\delta^{18}O$ value of the water is analyzed, even when it is not particularly indicated. The reason is that the corresponding analytic procedure is extremely easy (Horita, Ueda, Mizukami, & Takatori, 1989) and can often, especially with wine and fruit juices, be performed on the untreated sample itself. As the method is based on the isotopic equilibration between the sample water and CO_2, the sample should not be in a state of fermentation, producing additional CO_2. Further artifacts can occur in context with separation of the water from the sample (e.g. meat). This equilibration method has recently been supplemented by the water isotope ratio analysis by IR methodology (Brand, 2010; Lis, Wassenaar, & Hendry, 2008; West, Goldsmith, Brooks, & Dawson, 2010). In any case, as will be shown in this contribution, the interpretation of the results has to take into account the history of the original primary water. On the other hand, most organically

Box 1
Isotope effects and isotope fractionation in closed and open systems.

The indication of isotope concentrations of bioelements is performed in the δ value scale as relative differences to international standards of the International Atomic Energy Agency (IAEA) in Vienna (V). The standard for oxygen is Vienna Standard Mean Ocean Water (V-SMOW) with the isotope ratio $R = [^{18}O]/[^{16}O] = 2005.2 \times 10^{-6}$, that for sulfur is Vienna Canyon Diablo Troilite (V-CDT) with the isotope ratio $R = [^{34}S]/[^{32}S] = 44150.9 \times 10^{-6}$. According to IUPAC rules, delta values are defined as (Coplen, 2011)

$$\delta^{18}O = \left(R_{Sample} - R_{V-SMOW}\right)\Big/R_{V-SMOW} \quad \text{and} \quad \delta^{34}S = \left(R_{Sample} - R_{V-CDT}\right)\Big/R_{V-CDT}$$

However, for practical reasons and because of the general application in the cited references, we are using in the present paper the old formula with the factor 1000, leading to permill [‰]:

$$\delta^{18}O[‰] = \left(R_{Sample} - R_{V-SMOW}\right)\Big/R_{V-SMOW} \times 1000 \quad \text{and} \quad \delta^{34}S[‰] = \left(R_{Sample} - R_{V-CDT}\right)\Big/R_{V-CDT} \times 1000$$

For the determination of the δ values, organic matter is converted by techniques of the elemental analysis into CO ($\delta^{18}O$) and SO_2 ($\delta^{34}S$), respectively, water is equilibrated with CO_2 ($\delta^{18}O$). The isotope ratio of the gases is analyzed, relative to that of a (laboratory) standard, in an isotope ratio mass spectrometer (Sieper et al., 2010; Werner, 2003).

Thermodynamic isotope effects or fractionation factors α are the ratio of physical properties or equilibrium constants K and K^* of isotopologue molecules, e.g. of the vapor pressures of $H_2^{16}O$ (p) and $H_2^{18}O$ (p^*): $\alpha = p/p^*$. Kinetic isotope effects α_{kin} are the ratio of velocity constants k of such molecules in chemical reactions; the element in question is indicated, e.g. for oxygen as $\alpha_{kin} = k_{16}/k_{18}$. Normally, the "lighter" isotopologues react faster and α_{kin} is > 1.0; for the bioelements except hydrogen, it is between 1.00 and 1.07. The basis for kinetic isotope effects on enzyme-catalyzed reactions is the Michaelis–Menten theory. All these considerations concern systems in equilibrium or closed systems, respectively.

Most natural systems are open with infinite pools of isotopically constant substrates (e.g. ground water or soil sulfate). This has for consequence a constant isotope fractionation between substrate and product. Thus, the fractionation constant is defined as $\alpha = R_{Substrate}/R_{Product}$ and the isotope fractionation or discrimination as $\Delta = \alpha - 1 = R_{Substrate}/R_{Product} - 1$. The combination of this equation with the above correlation between R and δ permits to eliminate $R_{Standard}$ and yields, as $\delta_{Product} << 1$:

$$\Delta = (\delta_{Substrate} - \delta_{Product})/(1 + \delta_{Product}) \sim \delta_{Substrate} - \delta_{Product}$$

This provides the possibility to use directly mass spectrometric results of practical measurements for interpretations. All systems discussed in the present paper are open with the exception that most plants do not excrete sulfur in any form and hence the total assimilated S remains in the system. Yet the following prerequisites concerning S-isotope fractionations have to be taken into account (see Fig. 4). A kinetic isotope effect on a defined reaction becomes only efficient in case of a partial substrate conversion, normally realized by formation of at least two products (metabolic branching, MB), one of them depleted, the other enriched in the heavy isotope (isotopic balance). The reverse is realized by metabolite channeling (MC): In a sequence of reactions the substrate and the subsequent products are quantitatively converted (e.g. by a multi-enzyme-complex). Kinetic isotope effects on any step cannot become efficient.