

# Stable Isotope Ratio Analysis (Sira): A Tool for Isotopic Analysis for Food Authentication and Traceability in Dairy Industry

Ashritha B<sup>1</sup>, N. Veena<sup>2</sup>, Avinash C. Gautham<sup>1</sup> and Somveer<sup>3</sup>

<sup>1</sup>PhD scholar, Department of Dairy Technology, ICAR-NDRI, Karnal

<sup>2</sup>Associate Professor, Department of Food Safety and Quality, DSLD College of Horticultural Engineering and Food Technology, UHSB, Devihosur, Haveri

<sup>3</sup>PhD scholar, Department of Dairy Engineering, ICAR-NDRI, Karnal

\*Corresponding Author: [veena.ndri@gmail.com](mailto:veena.ndri@gmail.com)

The area of food authentication is experiencing tremendous growth as a result of the growing public awareness regarding food quality and safety. Consumers have been showing a growing preference for food labels that provide detailed information on the origin and authenticity of the product. Typically, the food label or description includes information on the ingredients, geographical origin, processing methods, manufacturing dates, and genetic characteristics. An authentic food is defined as one that conforms precisely to the specifications set forth by the producer. The food label description provides vital information for consumers to make informed judgments on their dietary choices and food purchases.

Authenticating food involves identifying instances of mislabeling, where food have been falsely labeled with a specific name, as well as the use of cheaper but similar ingredients, undisclosed processes, or the addition of adulterants to increase the quantity of food. It also entails verifying the accuracy of information regarding the food's origin, species, and production method. Currently, the accurate evaluation of food authenticity is crucial due to consumers' frequent exposure to a diverse range of food products. Undoubtedly, globalization entails the increasing exchange of food products around the globe. Traceability has become a fundamental aspect of food safety policy. It serves as a risk-management tool that allows the food sector or authorities to remove or recall items that have been identified as dangerous. The growing intricacy and elongation of the food chain, in addition to recent food-related concerns, have increased public awareness about the source of food and emphasized the necessity of implementing measures to guarantee that foods are of superior quality and free from harm when they are consumed by individuals (Mania et al., 2018).

Developing a proficient food traceability system offers a method for safeguarding the reputation of a specific region's brand, guaranteeing fair competitiveness in trade, and enhancing consumer trust. Various analytical techniques employed in the determination of food authenticity. Stable isotope ratio analysis (SIRA) is one of the highly accepted approaches for providing information on the traceability of food. This method often encompasses multiple aspects of food production. SIRA is an analytical technique that fulfills these requirements and has been employed to verify the geographical origin of various food items, such as wine (Christoph et al., 2015), meat (Bontempo et al., 2023), milk, and dairy products (Gregorcic et al., 2020). It is also used to compare isotopic results with typical samples from official wine databases, such as the one established by the European Commission in Regulation (EC) No 479/2008. It is possible to create a comparable database to support assertions of geographical origin found on dairy product labels. Isotope analysis offers the benefits of exceptional precision and efficiency and has broad relevance in various domains, including food authenticity, medical sciences, geology, and biology, resulting in its widespread use (Schauble, 2004).

## Stable isotope ratios

Isotopes are atoms that possess different numbers of neutrons while maintaining an identical number of protons. Isotopes of a specific element hold the identical position on the periodic table, thereby exhibiting identical chemical properties but varying in mass. In general, isotopes can be categorized into two types: stable isotopes and radioactive isotopes (also known as unstable isotopes). In contrast to unstable isotopes, which decay and lose mass over predictable time periods, stable isotopes' nucleus properties remain constant throughout time. (Vanhaecke & Degryse, 2012).

Stable isotope ratios show the relationship between heavy and light isotopes. Isotopic data are reported in two ways: absolute ratios and relative to standard (delta notation) (Laursen et al., 2016).

$$\alpha_B^A = \alpha_{A-B} = \frac{R_A}{R_B}$$

' $\alpha$ ' is the factor used to report isotope differences between two phases fraction.

In order to determine the ratio reliably, the actual ratio measured in research samples are compared to a standard isotope ratio, and are given as permil (‰)

$$\delta_{Sample} = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

**Table 1. Isotopic composition of the primary reference standard**

Primary reference material	Isotope ratio	Accepted value (x 10 <sup>6</sup> ppm) (with 95% confidence interval)
VSMOW	<sup>2</sup> H/ <sup>1</sup> H	155.76 ± 0.10
	<sup>18</sup> O/ <sup>16</sup> O	2005.20 ± 0.43
	<sup>17</sup> O/ <sup>16</sup> O	373 ± 15
VPDB	<sup>13</sup> C/ <sup>12</sup> C	11237 ± 9.0
	<sup>18</sup> O/ <sup>16</sup> O	2067.1 ± 2.1
	<sup>17</sup> O/ <sup>16</sup> O	379 ± 15
AIR	<sup>15</sup> N/ <sup>14</sup> N	3676.5 ± 8.1
VCDT	<sup>34</sup> S/ <sup>32</sup> S	45004.5
	<sup>33</sup> S/ <sup>32</sup> S	8100.0

### Different isotopes for food authenticity and traceability

#### Carbon

The primary and largest natural source of carbon is atmospheric carbon dioxide (CO<sub>2</sub>), which is utilized by plants for the process of photosynthesis. During the process of photosynthesis, carbon isotopes undergo fractionation. Plants exhibit a preference for the lighter <sup>12</sup>C over the heavier <sup>13</sup>C in enzymatic and transport reactions (Marshall et al., 2007). However, the extent of this preference differs among plants that

employ different photosynthetic routes. Due to the uniqueness of enzymes associated with the three primary photosynthetic pathways (Calvin cycle, Hatch Slack cycle, and Crassulacean acid metabolism), which exhibit varying preferences for <sup>13</sup>C and <sup>12</sup>C isotopes, the isotopic characteristics of different plant groupings may be reliably differentiated. C<sub>3</sub> plants, such as temperate grasses, barley, and sugarbeet, exhibit  $\delta^{13}\text{C}$  values that fall within the range of -35 to -21‰. On the other hand, C<sub>4</sub> plants, including tropical grasses, maize, and sugarcane, display  $\delta^{13}\text{C}$  values that are less negative, ranging from -14 to -10‰. Hence, the impact of grazed feedstuffs on  $\delta^{13}\text{C}$  values of animal derived foods, like milk, is significantly influenced by geographical factors. This is because C<sub>3</sub> plants are predominantly found in regions with temperate climates and abundant rainfall, while C<sub>4</sub> plants are more prevalent in equatorial regions with less rainfall and greater sunlight exposure (Still et al., 2003)

#### Nitrogen

Atmospheric dinitrogen (N<sub>2</sub>) is the primary natural source of nitrogen. Nevertheless, herbivores obtain their nitrogen from the plants they consume, and the  $\delta^{15}\text{N}$  values vary among different plant functional groups. Legumes have the ability to form a symbiotic relationship with certain bacteria that allows them to convert atmospheric nitrogen into a usable form. This process is associated with a  $\delta^{15}\text{N}$  value of 0‰ (Molkentin & Giesemann, 2010). In contrast, other plants reflect the nitrogen composition of the soil they grow in, which is influenced by agricultural practices such as the application of fertilizers. Additionally, the nitrogen in the soil gradually becomes enriched with <sup>15</sup>N due to the preferential loss of <sup>14</sup>N as nitrate or nitrous oxide. In general,  $\delta^{15}\text{N}$  values tend to be elevated (reaching up to 30‰) in organic fertilizers such as slurry, as a result of selective loss of <sup>14</sup>N during their production. On the other hand, synthetic fertilizers typically exhibit lower values (ranging from 4‰ to 4‰), which is indicative of their origin from N<sub>2</sub> (defined as 0‰) (Gautam & Iqbal, 2010)

## Hydrogen and oxygen

According to Marshall et al. (2007), water serves as the primary reservoir of hydrogen and oxygen atoms in plant and animal systems. Dairy cows typically obtain water through drinking and consuming plant water, both of which are predominantly influenced by precipitation water. Consuming groundwater directly can affect the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  levels of cows' drinking water, serving as a reliable diagnostic of their geographic origin. Due to the irregular replacement of groundwater compared to precipitation water, dairy products from places where deep groundwater is the primary source of drinking water for dairy cows do not experience the same impact on their isotope ratios as products from animals ingesting precipitation water. The reference standard (V-SMOW) utilized in the computation of hydrogen ( $\delta^2\text{H}$ ) and oxygen ( $\delta^{18}\text{O}$ ) reading has a precisely defined value of 0‰ for both ratios (Gonfiantini, 1978). As ocean water evaporates, it loses the heavier isotopes ( $^{18}\text{O}$  and  $^2\text{H}$ ). So when the vapor condenses to form clouds, the isotopic composition of the cloud is lighter (with more of the lighter isotopes  $^{16}\text{O}$  and  $^1\text{H}$ ) compared to the ocean water. This means that the cloud has more negative  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values (Bowen, 2010). Consequently, when water condenses from rain clouds, the heavier isotopes precipitate at a somewhat faster rate. Nevertheless, the seasonal variation significantly influences the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of precipitation water. Hence, it is imperative to take into account seasonality while interpreting data.

## Principle of stable isotopes analysis

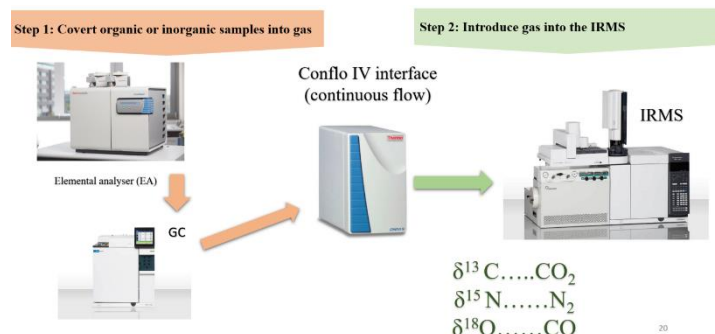
Isotope fractionation is the primary mechanism that influences the proportional distribution of isotopes in natural substances. The chemical behavior of two isotopes of an element is comparable due to their same electronic configuration. However, the variation in mass between the isotopes leads to differences in chemical effects, such as reaction rates and binding strength. The variation in the strength of chemical bonds and the rate at which chemical reactions occur between different isotopes results in a modification of the distribution of heavy and light isotopes (Kendall and Caldwell, 1998). Plant-

based food products frequently exhibit distinct isotopic fingerprints as a result of fractionation processes. Furthermore, researchers have explored isotopic signals as a means of evaluating the nutritional history in animal ecology and thereby for authenticating animal products such as meat, eggs, and dairy products.

Isotope ratio mass spectrometry (IRMS) is a valuable technique for quantifying the relative proportions of isotopes in natural substances. The device consists of three fundamental components: an ion source, a magnetic analyzer, and an ion collector. The light isotope in IRMS exclusively analyzes gases, so the analytes must be converted into a gaseous state (such as  $\text{H}_2$ ,  $\text{N}_2$ ,  $\text{CO}$ ,  $\text{CO}_2$ , and  $\text{SO}_2$ ) that is suitable for ionization. In this scenario, carbon dioxide is generated from organic components by undergoing oxidation with copper oxide. Hydrogen is produced through the reductive conversion of water by reacting it with a metal such as zinc. The ions are spatially segregated by either a fixed magnet or an electromagnet (Kelly, 2003). The radius of deflection is determined by the mass and charge. Ions with identical mass-to-charge ratios exhibit equivalent deflection radii, with heavier ions experiencing less deflection compared to lighter ions. The deflection of ions concentrates them into many beams that subsequently reach the ion collectors. The quantification of each beam is performed using Faraday cups in ion collectors. The ion currents generate the voltage within the cup, which is then converted into a digital signal.

In the Continuous flow-IRMS technique (shown in figure 1), a solid or liquid biological sample is inserted into a high-temperature combustion chamber of an elemental analyzer, where it undergoes conversion into a gaseous state. The gaseous byproducts of combustion, such as  $\text{CO}_2$ ,  $\text{N}_2$ , and  $\text{H}_2\text{O}$ , are transported by a continuous flow of helium gas, passing through a purification process, and then analyzed using a gas chromatography-isotope ratio mass spectrometry apparatus. The gases are separated using gas chromatography and then directed to an ion source, where individual ion beams are created for each combustion gas. Within the magnetic sector of the

mass spectrometer, the beam is divided into many beams based on the molecular weights of the different isotopes present in the gas (Brenna et al., 1997). IRMS has been applied to authenticate numerous food products, including honey, fruit juices, wine, and milk.



**Fig 1: Continuous flow-IRMS working principle**  
**Applications in dairy industry**

Isotope analysis is frequently employed to ascertain the origin of dairy output. The effectiveness of SIRA as an authentication tool for milk and dairy products stems from the inherent characteristic that isotope ratios undergo predictable and replicable changes in response to various factors, such as: (i) the animal's diet, (ii) the geographic origin of the animal (which affects its diet), (iii) the seasonal variations in milk production, and (iv) the manufacturing procedures employed in product production (O'Sullivan et al., 2022).

## Conclusions

SIRA is an independent, reproducible method of authenticating dairy products providing specific information of value to regulators, dairy producers and ultimately consumers. The techniques of isotope analysis have advantages such as high precision, small sample size and same technique can be used for almost any type of food productions. Future food authentications will be increasingly based on multivariate datasets generated by several analytical techniques (SIRA, NMR, metabolomics, elemental fingerprinting, etc.).

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**Table 2. Different isotopes and their fractionation process**

Elements	Isotopes	Natural Abundance	Delta Notation	Fractionation Process	Information
Hydrogen	$^1\text{H}$ $^2\text{H}$	99.98 0.02	$\delta^2\text{H}$	Evaporation, condensation, precipitation, transpiration	Geography, water usage, fertilization rate
Carbon	$^{12}\text{C}$ $^{13}\text{C}$	98.9 1.1	$\delta^{13}\text{C}$	$\text{CO}_2$ assimilation, stomatal conductance	Water use efficiency, fertilization rate
Nitrogen	$^{14}\text{N}$ $^{15}\text{N}$	99.6 0.4	$\delta^{15}\text{N}$	N transformation processes, atmospheric loss	Fertilizer type and rate
Oxygen	$^{16}\text{O}$ $^{17}\text{O}$ $^{18}\text{O}$	99.76 0.04 0.2	$\delta^{18}\text{O}$	Evaporation, condensation, precipitation, transpiration	Geography, water usage, fertilization rate

**Table 3. Applications of SIRA in dairy foods**

Sl. No.	Applications	Isotopes involved	References
1	Distinguish milk between 2 different geographical areas in southern Italy	$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$	Brescia et al., 2003
2.	Characterization of French, Italian, and Spanish Cheeses	$\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ , and $\delta^{34}\text{S}$ of casein and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of glycerol (obtained from lipid hydrolysis)	Camin et al., 2005
3.	Discrimination of organic milk from conventional milk in Korea	$\delta^{13}\text{C}$ of protein in combination $\delta^{15}\text{N}$	Chung et al., 2014
4.	Differentiation of organic and conventional milk (raw, pasteurized, and ultraheat treated).	$\delta^{13}\text{C}$	Erich et al., 2015
5.	Concentrations of some trace elements in goat milk powder	$\delta^{13}\text{C}$ , $\delta^{15}\text{N}$	McLeod et al., 2015
6.	Detection of water addition (>15%)	$\delta^{18}\text{O}$	Gregorcic et al., 2020
7.	Italian cheese (Grana Padano PDO and Parmigiano Reggiano PDO) characterized for the different regions and zones	$\delta^{34}\text{S}$	Pianezze et al., 2020

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