REVIEW

ISOTOPE ANALYSIS AS A MEANS OF TRACING AQUATIC PRODUCTS AUTHENTICITY, SOURCE AND GEOGRAPHIC ORIGINS

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ABSTRACT

Aquatic products provide good sources of high-quality protein for humans. Tracing the origin of aquatic products is of critical importance both for consumers and suppliers. In recent years, isotope analysis is becoming a key instrument in food products authentication. This work reviews the use of isotope analysis to trace the production sources (wild or farmed) and geographic origins of aquatic products. Extensive research has studied the isotope values of freshwater fish to Atlantic salmon, sea bass, rainbow trout, and other commercial fish and shellfish. Generally, the ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes were successfully investigated in aquatic products in order to identify the production methods or geographic origins. However, the predictable confidence of isotope analysis can be enhanced in combination with other analytical techniques, such as fatty acids and multi-element profiling. Moreover, future research to combine isotope analysis with data fusion and multivariate data evaluation is recommended.

Keywords: food fraud, provenance, fish, seafood, isotopic abundance

1. INTRODUCTION

Aquatic products provide good sources of high-quality protein for humans (SAPKOTA *et al.*, 2008). According to the Food and Agriculture Organization's annual State of World Fisheries, total world fish production (capture and aquaculture, excluding aquatic plants) peaked at about 171 million tons in 2016, and is expected to reach 201 million tons in 2030 (FAO, 2018). However, the growing number of seafood production and economic globalization have exerted serious pressure on the variety of food products, resulting in food fraud and adulteration (DANEZIS *et al.*, 2016a). In addition, consumers are interested in knowing the origin of aquatic products. Numerous cases have been reported with fish products labelled falsely to increase the chances of marketing and sales (FOX *et al.*, 2018).

In recent years, tracing the origin of fish has become significantly important both for consumers, producers and regulators (POSUDIN *et al.*, 2015). In particular, verifying fish origin and its label description are in compliance is given high importance (DANEZIS *et al.*, 2016b). Traditionally, food authentication has been verified using several methods including genomics and proteomics techniques (CERUSO *et al.*, 2019, ORTEA *et al.*, 2016), chromatographic techniques (GRANATO *et al.*, 2018), isotopic and elemental techniques (GOPI *et al.*, 2019b), vibrational and fluorescence spectroscopy (COZZOLINO, 2015, DANKOWSKA, 2016), nuclear magnetic resonance spectroscopy (STANDAL *et al.*, 2010), sensory analysis (KIANI *et al.*, 2016), immunological techniques (CARRERA *et al.*, 2014) and others (DANEZIS *et al.*, 2016a, DANEZIS *et al.*, 2016b, GOPI *et al.*, 2019a). Amongst them, isotope analysis is one of the prominent analytical techniques (DANEZIS *et al.*, 2016b), that has not been commonly used previously but gaining momentum. In particular, isotope analysis could trace the production methods (wild or farmed) and geographic locations of various species; and it is relatively cost-effective (GOPI *et al.*, 2019a).

During the last several decades, research on food authentication has focused on wine, fruit, vegetables, cereals, meat, dairy products, oils, honey, and eggs (DANKOWSKA, 2016). However, there had been little interest in fishery products, but number of studies on fishery authentication are on the rise since 2007 (DANEZIS *et al.*, 2016a). Moreover, the investigations on isotope analysis of seafood products have been propelled to the forefront due to its advantages compared with other relevant methods, such as DNA, fatty acid and elemental profiling. For instance, the results of DNA profiling can be affected by the removal or degradation of DNA into small fragments in various treatments (NOVAK *et al.*, 2007, ŞAKALAR *et al.*, 2012). Fatty acid compositions depend on variability of seasons and diets (GRIGORAKIS, 2007), and it is difficult to distinguish the fatty acids profiles of wild and cultured samples (OSTERMEYER *et al.*, 2014), or between wild and organic samples (MOLKENTIN *et al.*, 2015). On the other hand elemental profiling needs more time to prepare samples and large database to discriminate the provenance of each species (GOPI *et al.*, 2019a).

In this paper, we review isotope analysis used in aquatic products authentication, particularly focusing on the production source (wild or farmed) and geographic origins. Our emphasis here is placed on applications, methods, accuracy and productivity of current studies attempting to elucidate the traceability of aquatic food products through isotope analysis.

2. ISOTOPE RATIO ANALYSIS

Isotopes are the atoms of the same element, which have equal numbers of electrons (and protons) but different numbers of neutrons (COPLEN, 2011, KELLY *et al.*, 2005). Different isotopes of the same element possess different masses. Isotopes have two specific types: stable and unstable (radioactive isotopes). Stable isotopes do not decay into other elements. In contrast, radioactive isotopes are unstable and decay into other elements. Stable isotopes can be grouped into light and heavy elements isotopes, depending on atomic mass (DANEZIS *et al.*, 2016b).
The less abundant stable isotope(s) of an element have one or two additional neutrons than protons, and thus are heavier than the more common stable isotope. The stable isotope abundance of an element is presented in ratio form as the ratio of the heavy-to-light isotopes (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$). Since this ratio is small, isotope ratio analysis is normally expressed by the ratio of the heavier and the lighter isotopes to a reference compound of normal isotope ratio, which is reported in standard delta (δ) as parts per thousand (per mil, ‰) (COPLEN, 2011, KELLY *et al.*, 2005) as Eq (1):

$$\delta_{ref} = \left(\frac{R_{samp} - R_{ref}}{R_{ref}}\right) \quad (1)$$

Where δ_{ref} is the isotope ratio of the sample expressed in delta units relative to the reference material.
R_{samp} and R_{ref} are the isotope ratios of the sample and reference material, respectively.
For bio-elements ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$) isotope measurements, isotopic ratios are generally investigated by isotope-ratio mass spectrometry (IRMS) (DRIVELOS and GEORGIOU, 2012). For Sr, Pb and other heavy isotopes, thermal ionization mass spectrometry (TIMS), multi-collector-inductively couple plasma mass spectrometry (MC-ICP-MS), and dynamic reaction cell- inductively couple plasma mass spectrometry (MC-ICP-MS) are used (DRIVELOS and GEORGIOU, 2012).

3. ISOTOPE ANALYSIS IN FOOD AUTHENTICATION

The increasing global trade has challenged the guarantee of food safety, transparency and protection of human health (DANEZIS *et al.*, 2016b, KELLY *et al.*, 2005). In addition, aquatic food products are highly perishable commodities and traded worldwide, which give certain difficulties for characterizing its provenance (SCHRÖDER, 2008). Therefore, it is important to verify the authenticity of the aquatic products before entering markets.
Isotopic ratios have been known to be of extreme use in food authentication because food ingredients have variety of isotopes abundance that can reflect its trophic position, food sources, geographic origin, pedology and archaeological sites (DANEZIS *et al.*, 2016b, FULLER *et al.*, 2012). Stable isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ are natural biomarkers to evaluate the effects of different preservation methods on isotopic signatures of fish tissues (ARRINGTON and WINEMILLER, 2002, KELLY *et al.*, 2006, SYVÄRANTA *et al.*, 2008), trophodynamics and food sources in time and space (FULLER *et al.*, 2012, WYATT *et al.*, 2012). Similarly, geographic origin can be recognized by hydrogen, oxygen, sulphur and strontium isotope ratios (KELLY *et al.*, 2005).
There are various issues concerning the traceability and authentication of fishery and aquatic products. Among them, species of origin (fish species), production sources (wild

or famed), and geographic origins (locations) are desirable in traceability of fishery and aquatic products (MORETTI *et al.*, 2003). Consequently, isotope ratios can be used in tracing the authenticity of production sources (wild or famed), and geographic origins of aquatic products.

3.1. Wild and farmed

To date, there are numerous studies focusing to distinguish farmed and wild seafood products, especially since the beginning of the 21st century, due to the increased concerns amongst consumers, who wants to know the origin of fishes (DANEZIS *et al.*, 2016b). The feasibility of using stable isotopes to distinguish recently escaped farmed Atlantic salmon (*Salmo salar*) to wild specimen was investigated by DEMPSON and POWER (2004). Their results showed that muscle tissue of wild salmon had significantly enriched nitrogen $\delta^{15}N$ but depleted lipid corrected carbon $\delta^{13}C'$ (the residual $\delta^{13}C$ values) than those of escaped farmed salmon. In addition, those authors assumed that the differences of isotope fractionation between farmed and wild fish could be retained depending upon the time of year that farmed fish was escaped relative to the June to August rapid growth period. Moreover, their study also supported the fact that adipose tissue can be used as non-invasive utility to determine isotope values in salmonid fishes, as the average $\delta^{13}C'$ and $\delta^{15}N$ of white muscle and adipose fin tissue varied in absolute amount by only 0.5%.

The combined measurement of $\delta^{13}C$ and $\delta^{15}N$ can be useful to differentiate the origin, farmed or wild, Brazilian fresh water fish cachara (*Pseudoplatystoma fasciatum*), but seasonal variations need to be concerned (SANT'ANA *et al.*, 2010). Farmed cachara was found to have significantly enriched $\delta^{15}N$ in rainy but not dry season, whereas $\delta^{13}C$ was found to be enhanced in both seasons. Therefore, the authors assumed that $\delta^{13}C$ is a better indicator for cachara traceability.

The basic of isotope analysis in discrimination of wild (England) and cultured (Scotland and Greece) sea bass (*Dicentrarchus labrax*) was provided by BELL *et al.* (2007). The isotopic data indicated that $\delta^{13}C$ of individual fatty acids 16:0, 18:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, and 20:4n-6 were significantly lighter in cultivated sea bass than those of wild specimen. In addition, total flesh oil of farmed sea bass had the lighter $\delta^{13}C$ compared to that of the wild specimen, but not for the $\delta^{18}O$ of the flesh oil. It was explained by the commercial aquafeed formulations contained more terrestrial-derived raw materials such as wheat, soybean, sunflower, maize, peas and rapeseed meals. Although wild bass had a higher choline nitrogen content than cultivated bass, higher $\delta^{15}N$ of the flesh lipid total glycerol/choline fraction was observed in cultivated bass than that of wild counterpart. It may be due to the differences in growth rate and maturity of wild (1690 g) and cultivated (386 g) sea bass or seasonal variations of $\delta^{15}N$. Nevertheless, those authors stated that because their study only discriminated fish origins from three geographic locations, it warrants further studies combining isotope analysis with other analytical methodologies such as the flesh fatty acids profiles.

The study of FASOLATO *et al.* (2010) highlighted that the utility of $\delta^{13}C$ and $\delta^{15}N$ abundance to discriminate the farmed to wild European sea bass. Because $\delta^{13}C$ abundance can be affected by the variability of tissue lipid content or intramuscular fat (FOCKEN and BECKER, 1998), the $\delta^{13}C$ abundance in this study was analyzed from free-fat muscle. Similar to previous study of BELL *et al.* (2007), the $\delta^{13}C$ values of farmed sea bass were

found to be lower than wild specimens. The $\delta^{15}N$ abundance showed higher values in wild specimen owing to the higher trophic level of fish feed from the Mediterranean Sea.
In an attempt to extend more heterogeneous range of samples, sea bass (*Dicentrarchus labrax*) from 18 different Italian and southern European sources was analyzed for its production sources, wild and cultivated (intensively, semi-intensively, and extensively) by determination of $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions (FARABEGOLI *et al.*, 2018). However, the results of isotopic abundance were less satisfying as there were merely slight differences in isotopic abundance of $\delta^{13}C$ and $\delta^{15}N$ between cultivated and wild fish, as well as Italian and foreign intensively reared fish. According to the authors, $\delta^{13}C$ can be affected by dietary nutrients and habitat shifts; in addition, $\delta^{15}N$ can be influenced by the trophic level of fish feed formulations. These may affect results of isotopic abundance to distinguish between wild and cultivated sea bass.
It must be noted that the specific choice of isotopic abundance element in particular fatted/defatted samples is important. For example, the sole $\delta^{13}C$ abundance in defatted dry matter could not differentiate organic from wild salmon (MOLKENTIN *et al.*, 2015). In this case, the combination of $\delta^{13}C$ in lipid samples and $\delta^{15}N$ in defatted dry matter were needed in the differentiation of organic, conventional and wild fish (MOLKENTIN *et al.*, 2015).
To search for more authenticated method, WANG *et al.* (2018) suggested to use compound-specific amino acid $\delta^{13}C$ fingerprints ($\delta^{13}C_{AA}$) on large-numbered of salmon samples, to (1) discriminate organically, conventionally aquaculture to wild fish from Pacific to Atlantic regions; and (2) detect subtle diet changes by macroalgae or insects with controlled feeding experiments. The bulk isotope values of $\delta^{13}C$ and $\delta^{15}N$ could be used to trace the salmon origins. Those authors found that bulk isotope values resulted in poor discrimination between wild and organic salmon. However, the multivariate analysis of $\delta^{13}C_{AA}$ data could separate the wild, organic and conventional salmon with high certainty, as well as distinguish diets changes among lab-cultured experimental groups, even between the green (*Ulva rigida*) and red (*Palmaria*) macroalgae inclusion groups. In addition, $\delta^{13}C$ of essential amino acids (His, Phe, Val, Ile and Leu) in salmon tissue can reflect the dietary sources, therefore they can satisfactorily differentiate fish origins.
The research of VASCONI *et al.* (2019) used protein carbon and nitrogen isotope analysis to differentiate the wild and famed European eel (*Anguilla anguilla*) from Netherland, Denmark and Italy; and different farming modes (pond, recirculating aquaculture system, lagoon and wild). Multivariate data were performed by principal component analysis and sparse partial least squares discriminant analysis. The results showed that $\delta^{13}C$ and $\delta^{15}N$ abundance can predictively differentiate lagoon and wild eels, but cannot discriminate male and female eels from Netherland and Denmark. In addition, the stable isotope analysis results can ratify only the partial of what has been demonstrated by using the fatty acids profile in this study.

3.2. Geographic origin

In recent years, there has been an increasing interest in isotope analysis to authenticate the geographic origin of fish and shellfish. In particular, the study of ORTEA and GALLARDO (2015), using stable isotope ratio and/or multi-element (Pb, Cd, As, P, S) analyses, has shed some light on not only geographic origin, but also production method, and species authentication of commercially relevant shrimps. The shrimp samples were constituted by 45 individuals of seven different species in nine different geographical

origins. Multivariate analysis were used for data classification, including principal component analysis, cluster analysis, κ-means hierarchical classification and discriminant analysis (DA). The results showed that both stable isotope ratio and multi-element analyses can enhance the prediction capabilities of chemometric technique to discriminate shrimp samples into wild/ farmed, different geographical origins or even biological species, whilst cluster analysis was not appropriate to discern the farm origins. On the contrary, it is of interest to note that KIM *et al.* (2015) stated that isotope analysis is a reliable tool to trace the origin of commercial fish (mackerel, yellow croaker and pollock). Biplot of $\delta^{13}C$ and $\delta^{15}N$ values showed that Australian and Norwegian mackerel had different spatial and trophic position than those of Chinese and Korean counterparts (KIM *et al.*, 2015). The $\delta^{13}C$ of pollock from Japan and Russia, as well as the $\delta^{15}N$ between yellow croaker from Korea and China were likely similar because the two areas are close in their geographical distance (KIM *et al.*, 2015). Those authors also reported that $\delta^{13}C$ signature can be more effective in discrimination of geographic origin due to the distinct values of $\delta^{13}C$ of the three commercial fish.

The geographic origins of commercial hake species (n=60) were evaluated by the isotopic abundance of $\delta^{13}C$ and $\delta^{15}N$ using bivariate scatter plot, principal component analysis and Euclidean hierarchical clustering analysis (CARRERA and GALLARDO, 2017). The results facilitated a clear classification of hakes from six geographic coasts: Europe, North Africa, South Africa, North America, South America, and Australia. Most importantly, the $\delta^{13}C$ signature can corroborate the clear discrimination hake species according to latitude. For example, North African and South American hakes were in the adjacent range of $\delta^{13}C$ (-14 to -16), whilst Australian and North American hakes were in the range of -18 to -20 values of $\delta^{13}C$.

To study the carbon cycle at the molecular level, compound-specific isotope analysis is used as the combination of gas chromatography and isotope-ratio mass spectrometry (LIU *et al.*, 2017, RIELEY *et al.*, 1991). Compared to the conventional isotope analysis of bulk organic carbon, this technique can reflect the material source more accurately (LIU *et al.*, 2017) and understand the carbon fluxes within bio-geochemical systems (RIELEY *et al.*, 1991). Previous studies have applied this technique concerning with source of nutrients in aquatic and terrestrial food webs (LARSEN *et al.*, 2013) and discrimination of organically, conventionally aquaculture to wild fish (WANG *et al.*, 2018). However, few studies have demonstrated the traceability of compound-specific isotope analysis on geographic origins in seafood. One of the first attempts to trace the geographic origin of seafood by this technique was investigated on sea cucumber (*Apostichopus japonicas*) in the coastal area of China (LIU *et al.*, 2017). In this study, principal component analysis (PCA) and discriminant analysis (DA) were used to support the discrimination. Although a total of 28 fatty acids was detected in the fatty acid profiles, but stable carbon isotope compositions were only obtained from 26 fatty acids. The $\delta^{13}C$ values of fatty acids in both November 2015 and April 2016 were relatively enriched in Rushan, Wafangdian and Pikou, and depleted in the Danzi Island, the Changhai Island and Muping. Principal component analysis (PCA) and discriminant analysis (DA) allowed researchers to discriminate between different geographic locations of sea cucumber; except for the Changhai and Zhangzi Island in April 2016, because the two islands are both located in the Dalian sea and have similar environmental conditions. This study was followed up with the amino acids carbon stable isotope analysis of sea cucumber in an attempt to distinguish the sub-regions that are close together (ZHAO *et al.*, 2018). Because the $\delta^{13}C_{AA}$ fingerprint can supply

the information of biosynthetic origin and carbon acquisition (SCOTT *et al.*, 2006), as well as the environmental conditions and food sources (GANNES *et al.*, 1998, MCMAHON *et al.*, 2010); this method resulted in 100% of overall correct classification rate and cross-validation rate to discriminate 8 locations of wild samples and 3 locations of cultured sea cucumber. Especially, the sole $\delta^{13}C$ values of Gly and Ser could similarly discriminate the production method (wild versus cultured) and geographic provinces of sea cucumber, respectively.

Conservation efforts aimed at tracing the seafood geographic origins also involved the multi-element isotope analysis. For tracing the geographic origins (two northern Italian regions and other Italian regions) and type of feed (high or low fish content), the relationships between $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$ and $\delta^{18}O$ values of proteins and fat fractions of rainbow trout (*Oncorhynchus mykiss*) fillet and those of feed and tank water were evaluated (CAMIN *et al.*, 2018). Compared to the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of feed, those isotopic values of fillet proteins were enriched; and $\delta^{13}C$ values of fish fat were depleted. The partial square regression showed that the C, N and S isotopic values of fillet and fish feed were positively correlated within and between material matrixes, and negatively correlated with isotopic values of H and O of feed and H of fillet. Whereas, the isotopic signature of environment water $\delta^{18}O$ was positively correlated with $\delta^{2}H_{protein}$ and $\delta^{18}O_{protein}$ of fillet. Besides that, $\delta^{18}O_{fat}$ of fillet was less significant correlated with other isotopic ratios. In addition, the partial least squares-Discriminant analysis was applied to check the traceability of two geographic origins and feed type. Fish from Friuli Venezia Giulia region was predictably traced by $\delta^{15}N_{protein}$ and $\delta^{18}O_{protein}$; the Trentino fish was marked out by $\delta^{2}H_{protein}$ and $\delta^{18}O_{protein}$; whilst fish fed with high and low fat-feed were discriminated by $\delta^{34}S_{protein}$. The discriminant multiclass model reached the average accuracy of 94%. Furthermore, the authors suggested that geographical signature is extensively influenced by the local forage of diets. Previous studies have also shown that isotope analysis is informative variable to distinguish fish products between different farms (KIM *et al.*, 2015, TURCHINI *et al.*, 2008). To be more precise, however, isotope analysis would be a perfect complement to combine with other analytical techniques (CARTER *et al.*, 2015, GOPI *et al.*, 2019b, ORTEA and GALLARDO, 2015, TURCHINI *et al.*, 2008). One possibility is the combination with fatty acids profile. In an attempt to distinguishing the geographic traceability of sea cucumber (*Apostichopus japonicas*) in seven locations of northern China sea, the stable isotopes of carbon and nitrogen compositions partially overlapped in some areas, whilst fatty acids profile alone could not discriminate all of the origins (ZHANG *et al.*, 2017). However, the combination of $\delta^{13}C$ and 14:1n-5, or $\delta^{15}N$ and 16:0 content could be used to surmount the overlap areas. Additionally, the thorough separation of seven sampling locations were achieved when stable isotopes and fatty acid compositions combined with discriminant analysis and the recognition ability was 89.1%. ZHANG *et al.* (2019) extended the isotopic analysis of scallops (*Patinopecten yessoensis, Chlamys farreri,* and *Argopecten irradians*) of fatty acid $\delta^{13}C$ fingerprinting with fatty acid profile in seven sites of China. The results showed that all scallops of 75 samples were discriminated the geographic origins in combination of principal component analysis with the accuracy rate of 100%.

To trace the geographic origins of seafood, isotope analysis can be associated with trace metal compositions or elemental profiling (CARTER *et al.*, 2015, GOPI *et al.*, 2019b). For instance, CARTER *et al.* (2015) identified that the utility of $\delta^{2}H$ and $\delta^{13}C$ values in meat component of prawns could distinguish between Australian prawns and those imported from neighboring Asian countries. In addition, the data of potassium, zinc and arsenic

concentrations in prawn meat resembled the results obtained in isotope analysis with minor overlapped areas. Therefore, those authors surmised that the association of stable isotope and trace metal analysis would improve the accuracy of classification; however, the discriminant analysis for this combination had not been investigated.

To determine the geographic origins and production method (wild or farmed) of Asian seabass (*Lates calcarifer*), stable carbon and nitrogen isotope analyses and elemental profiling (31 different elements) were conducted in 38 samples from two Australian and one Malaysia regions (GOPI *et al.*, 2019b). Three statistical and ordination methods were used, including univariate (ANOVA) and multivariate (principal component analysis), linear discriminant analysis, and random Forest (R package). The accuracy of stable isotope, elemental profiling and the combination of two methods were 84, 72 and 81%, respectively. The incorrect predictions were two, one and none, respectively for three models. It was suggested that the combination of stable isotope and elemental profiling can be accommodated for seafood authentication. However, this study did not cover the seasonal variations of $\delta^{15}N$ and had limited sample size and species. To extend the provenance of geographic origins (17 different European areas located in Mediterranean Sea basin), 144 wild and farmed specimens of European sea bass were analyzed for the carbon and nitrogen isotope and rare earth elements (lanthanum, europium, holmium, erbium, lutetium, and terbium) (VARRÀ *et al.*, 2019). Data were anatomized by principal component analysis and orthogonal partial last square discriminant analysis (OPLS-DA). The results showed that the satisfactory classification can be achieved in tracing both for geographical origin and production method by OPLS- DA analysis.

In general, isotope analysis has been limited by the absence of reference database on a large scale of different species so that it can be officially applied. While establishing the reliable database, we need to pay attention to some drawbacks of isotope analysis. Particularly, isotope fractionations can be influenced by the environmental factors, e.g. growth conditions (LIU *et al.*, 2017, ZHAO *et al.*, 2018), and diet quality, e.g. high versus low dietary protein contents (FARABEGOLI *et al.*, 2018, WANG *et al.*, 2018). In addition, the results of isotope analysis can be overlapped due to the seasonal variability (SANT'ANA *et al.*, 2010), as well as the inappropriate utility of multivariate statistics and chemometrics methods (VARRÀ *et al.*, 2019).

4. CONCLUSIONS

Despite the limited studies, this review demonstrated that isotope analysis has been very promising in tracing aquatic food products provenance, especially in production sources and geographic origins. However, considerably more work will need to be done to authenticate the provenance of aquatic products. For example, the studies of isotopic abundance should be extended to various types of fish, seafood and aquatic products. The accuracy in analytical instrumentation and methods need to be firmly established and characterized. In addition, seasonal and environmental effects should be considered in isotopic values of food samples. More broadly, multidisciplinary approach and other analytical techniques, such as chemical characterization, fatty acids profile and multi-element profiling, combined with multivariate data evaluation and chemometrics can be further associated with isotopic analysis to improve the level of predictable confidence.

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