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Identification of the geographical origins of sea cucumber (*Apostichopus japonicus*) in northern China by using stable isotope ratios and fatty acid profiles

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ABSTRACT

Geographic traceability is an important issue for food quality and safety control of seafood. In this study, $\delta^{13}C$ and $\delta^{15}N$ values, as well as fatty acid (FA) content of 133 samples of *A. japonicus* from seven sampling points in northern China Sea were determined to evaluate their applicability in the origin traceability of *A. japonicus*. Principal component analysis (PCA) and discriminant analysis (DA) were applied to different data sets in order to evaluate their performance in terms of classification or predictive ability. $\delta^{13}C$ and $\delta^{15}N$ values could effectively discriminate between different origins of *A. japonicus*. Significant differences in the FA compositions showed the effectiveness of FA composition as a tool for distinguishing between different origins of *A. japonicus*. The two technologies, combined with multivariate statistical analysis, can be promising methods to discriminate *A. japonicus* from different geographical areas.

1. Introduction

Sea cucumber (*Apostichopus japonicus*), which belongs to the family Stichopodidae (Aspidochirotida: Holothuroidea) (phylum Echinodermata), is a typical temperate benthic animal. *A. japonicus* is mainly distributed in the northern waters of the western Pacific, including the Bohai Sea and Yellow Sea of China, as well as the eastern coast of Russia and the coasts of Japan and South Korea (Yanagisawa, 1998). Sea cucumber is a major seafood worldwide, having a vast market in China. Production of this species has reached 171,000 tonnes, with annual output exceeding 20 billion yuan in 2012 (Ministry of Agriculture, China [MOAC], 2013). With the continuous development of sea cucumber aquaculture, food safety issues have become increasingly prominent. Illegal activity, namely, adulteration and selling of fake and inferior-quality produce and mislabeling has increased in frequency; it disrupts the market, severely damages the interest of consumers, and infringes the credibility and reputation of the famous high-quality products, resulting in negative economic and social impacts. The uncertainty or misrepresentation of the food source is an important food safety issue. Thus, evaluation of the authenticity and origin of sea cucumber is an important requirement for ensuring quality, providing adequate security controls, and developing effective regulations. A possible solution to the above problem is establishing an origin traceability and identification system using existing technology.

Currently, several ways have been applied to the determination of geographical origin of seafood products. DNA technology is an effective tool to identify the geographical origin of seafood (Ibañez & Cifuentes, 2001). However, this technique is mainly used for species identification from different geographical origins, and it does not apply to the same species which was farmed or has gene flow. Furthermore, the polymerase chain reaction (PCR) is used widely to identify the origin of seafood (Luykx & Van Ruth, 2008), and is a sensitive method that provides a product for several other molecular techniques. For instance, PCR-DGGE of bacterial communities living on the tissue of seafood products has been the molecular tool extensively used for the determination of geographical origin (Tatsadjieu et al., 2010). However, this technique may be effective for fresh seafood but not applicable for processed products, as the processing may destroy the microbial community structure. Trace element fingerprinting (TEF) is a geochemical tool which uses the elemental profile of mineral structures, the compositions of which are affected by local environmental conditions, to identifying the geographical origin of seafood (Reis-Santos et al., 2012). TEF is considered to be a reliable and accurate method to distinguish specimens from geographically close populations (Sorte, Etter, Spackman, Boyle, & Hannigan, 2013) but the

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limitation of TEF is that hard mineralized structures need to be present in the products, and processed products which have been previously separated from shells or fish bones cannot be assessed by this method. Other methods, such as nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), atomic absorption spectroscopy (AAS) and electronic nose (EN), are not widely used for geographical origin traceability of seafood, due to the limitations of each technology (Luykx & Van Ruth, 2008).

Isotope ratio mass spectrometry (IRMS) and FA profile are the major methods for the study of traceability and authentication of food products. The stable isotope composition of organisms is an ideal indicator for their living environment because it is mainly dependent on the food intake. Isotope ratio mass spectrometry (IRMS) is a powerful tool for the detection of adulterated and counterfeit food products (Calderone et al., 2009), which has been widely used in studying the geographical origin and authenticity of biological constituents of food products such as honey, juice, wine, meat, aquatic products, and cereals (Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Ortea & Gallardo, 2015; Raco, Dotsika, Poutoukis, Battaqlini, & Chantzi, 2015). It is recognized as an official method for analyzing the authenticity of food products (Martin & Martin, 1995). IRMS has been applied to the determination of the origin traceability and in authenticity evaluation of seafood such as fish and shrimp (Ortea & Gallardo, 2015; Tanaka, Ohshimo, Takagi, & Ichimaru, 2010). Nevertheless, a study of the origin traceability of sea cucumber has not been reported. FAs are indispensable to the growth and survival of organisms, playing an important role in structure and function maintenance of cell membranes (Alfaro, Thomas, Sergent, & Duxbury, 2006). FAs mainly derive from foods, essentially remaining unaltered during the transfer process through the food chain (Parrish et al., 2000). Therefore, they can be used as biomarkers for tracing relationships in the food web (Iverson, 2009). Studies have been carried out on bivalves and fish (Bachok, Mfilinge, & Tsuchiya, 2003; Fernandez-Jover et al., 2007). The FA composition of organisms can reflect their living environment, as the characteristics of FAs are unchanged during their transfer process. It is believed that the FA composition of organisms varies with their living environment (Nerot et al., 2015). FA composition has been used to establish the origin traceability of various seafoods, including fish and shellfish (Ricardo et al., 2015; Thomas et al., 2008). However, such research on sea cucumber has not been reported.

The combined use of IRMS and FA profile can be more effectively applied to the geographical origin traceability and authentication of seafood. Thomas et al. (2008) reported that the combined use of stable isotope ratios and fatty acid composition can be successfully used to discriminate between fish of authentic wild and farmed origin. Busetto et al. (2008) reported that the combination of fatty acids and isotopic measurements to be a promising method to discriminate between wild and farmed fish and between wild fish of different geographical origin. Studies have suggested that the combined use of fatty acids and stable isotope can effectively trace the geographical origin and seasonality of croaker (*Micropogonias furnieri*; Chaguri et al., 2015). Studies carried out have mainly focused on fish but could be applied to other species of seafood.

In the present study, the $\delta^{13}C$ and $\delta^{15}N$ values, as well as the FA content, of *A. japonicus* samples from northern China Sea were determined and compared to evaluate the applicability of two technologies to the determination of origin traceability and the identification of *A. japonicus*. The aim of this study was to provide a theoretical basis for authenticity identification of the geographic origin of *A. japonicus*.

2. Materials and methods

2.1. Sample collection

Sample collection of wild sea cucumber (*A. japonicus*) was carried out in November 2015. The sampling locations included are distributed in the major producing areas of the northern China Sea (Fig. 1).

All 133 samples were collected from seven sampling locations (LZ, 26; DZ, 18; RS, 16; WFD, 20; PK, 21; CH, 24; ZZ, 8). All samples of *A. japonicus* were 3-year-old adult individuals with average length of 16.3 ± 1.9 cm and average weight of 115 ± 12.7 g. All samples were stored in aseptic food-grade plastic bags after collection and kept refrigerated during sampling and during the two-day transport to the laboratory.

2.2. Sample pretreatment

The samples were dissected immediately, and the body wall was obtained, washed with ultrapure water, and then frozen at −20 °C for 24 h. Completely frozen samples were freeze-dried at −50 °C for 48 h. The dried samples were pulverized by using a glass mortar and pestle, passed through a 80-μm mesh sieve, and then stored under dry conditions.

2.3. Stable isotope ratio analysis

Each 0.5 mg sample was placed in a tin cup. $\delta^{13}C$ and $\delta^{15}N$ values were measured on an elemental analyzer (Flash EA 1112; Thermo Fisher Scientific, Waltham, MA) and a stable isotope ratio mass spectrometer (Delta V Advantage; Thermo Fisher Scientific). The carbon or nitrogen stable isotope natural abundance is expressed as

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3‰$$

where X represents ^{13}C or ^{15}N, and R represents the $^{13}C/^{12}C$ ratio for C or $^{15}N/^{14}N$ ratio for N. The $\delta^{13}C$ value is relative to the Vienna Pee Dee Belemnite standard (PDB), and the value for $\delta^{15}N$ is the relative abundance of N_2 in air. Accuracies of $\delta^{13}C$ and $\delta^{15}N$ analyses were <0.10‰ and <0.20‰, respectively.

2.4. FA analysis

2.4.1. Total lipid extraction

The Folch method of lipid extraction (Folch, Lees, & Sloane-Stanley, 1957) was used in this study for total lipid extraction from *A. japonicus*. The powdered sample (0.4 g) was added to 10 mL of chloroform–methanol solution (2:1, v/v), and the resulting mixture was extracted with shaking for 30 min at 37 °C, extracted overnight, and then filtered. The extract was washed with 1/5 vol of 0.88% NaCl solution and left to stand to allow separation. The chloroform layer was collected, dried over anhydrous sodium sulfate, and then concentrated under a nitrogen stream to obtain the total lipids fraction.

2.4.2. FA methyl esterification

A 20-mg sample of the total lipid was dissolved in 1 mL of 1% sulfuric acid–methanol solution in a 60 °C water bath for 60 min to carry out methylation and then cooled. Subsequently, 1 mL of *n*-hexane was added, and the mixture was oscillated for 30 s and then left to stand. The supernatant was transferred to a sample bottle prior to analysis by gas chromatography/mass spectrometry (GC/MS).