



Species identification of dried scallop adductor muscle (*yao zhu*) products sold on the market



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ABSTRACT

Dried scallop adductor muscle, known as *yao zhu*, has been ranked in the list of the eight sea treasures in Chinese cuisine, it has been commonly recommended and consumed in China over many centuries due to its delicious taste and abundant nutrition. However, the true species composition of the dried scallop adductor muscles in the trade is unclear. Indeed, it is difficult to identify the scallop species just based on their morphological characters of processed adductor muscles. In this study, 60 *yao zhu* products commercialized in Chinese market were analyzed in order to authenticate species. Direct sequencing and BLAST method based on a fragment of the 16S rRNA gene were carried out and results showed that 34 products (56.7%) were scallop species *Argopecten irradians*, and other 26 products (43.3%) were the scallop species *Mizuhopecten yessoensis*, indicated the *yao zhu* market were dominated by the both species, which were the introduced aquaculture scallop species. Moreover, a simple and fast PCR method proved to be capable of unequivocal identification between the two species. Therefore, this work facilitates the identification of *yao zhu* products in the market, support of food control and protection of consumers' rights.

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1. Introduction

Dried scallop adductor muscle, known as *yao zhu* (瑶柱) in China, has been ranked in the list of the eight sea treasures (abalone, sea cucumber, shark fin, fish maw, dried scallop adductor muscle, bird's nest, shark's lip and fish roe) in Chinese cuisine. Dried scallop adductor muscle has been commonly recommended and consumed in China over many centuries due to its delicious taste and abundant nutrition. In 2014, world trade of scallops reached 157,200 tonnes, China was the leading buyer, importing about 30,000 tonnes, a sharp increase (24.1%) compared with the year before. Purchases from the USA, the second largest importer, were stable at 27,500 tonnes. In terms of supplies, China was also the top supplier in 2014, exporting 38,000 tonnes, which is a notable rise of

21.9% compared with 2013. Meanwhile, the trade scale of scallop in Europe dominated by France, followed by Spain, Netherlands, Italy, Belgium and Denmark (FAO, 2015).

In international market, scallop adductor muscles are usually sold as frozen, seasoned or canned products (Colombo, Trezzi, Bernardi, Cantoni, & Renon, 2004; Lopez-Pinon, Insua, & Mendez, 2002; Pacheco-Aguilar et al., 2008). In China, dried scallop adductor muscle products are well-known in the market. Dried scallop adductor muscles are usually processed by cutting the scallops to take out the adductor muscles, following boiling them and drying them in the sun, then processed in preserved forms such as dried products and sold directly to merchants.

Nowadays, more than 20 scallop species are commercial exploited or cultivated worldwide (Shumway and Parsons, 2011). However, the true species composition of the dried scallop adductor muscles in the trade is unclear (unlabeled). According to Chinese Regulation of Food Safety (Regulation No.9 of Feb. 28, 2009) for the common organization of the markets in food products, food products must be correctly labeled with the scientific

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name (or commercial name) of the species before retail distribution. Actually, even merchants do not know the species of dried scallop adductor muscle products, not to mention the consumers. Indeed, it is hard to identify the scallop species just based on their morphological characters of processed adductor muscles. Therefore, it is necessary to develop analytical methods for species identification of dried scallop adductor muscle products in order to ensure food safety and prevent frauds.

When taxonomic features have been removed due to processing, methods based on DNA profiles are typically used to identify food products at the species level. Currently DNA sequence-based approaches have been widely employed for testing and confirming food authenticity, such as BLAST (Armani et al., 2015b; Galal-Khallaf, Ardura, Borrell, & Garcia-Vazquez, 2016; Lago, Herrero, Vieites, & Espiñeira, 2011, 2013; Wen et al., 2015), FINS (Ardura, Pola, Linde, & Garcia-Vazquez, 2010b; Armani et al., 2015b; Chan, Ling, Shaw, Chiu, & Pui-Hay But, 2012; Kappel & Schröder, 2016; Wen, Hu, Zhang, & Fan, 2011) and DNA Barcoding (Armani et al., 2015a; Galal-Khallaf, Galal-Khallaf, Ardura, Pola, Ginuino, Gomes, & Garcia-Vazquez, 2010a; Galal-Khallaf, Ardura, Mohammed-Geba, Borrell, & Garcia-Vazquez, 2014, 2016; Xiong et al., 2016). Indeed, in view of the above-mentioned methods, DNA sequence was the basic information. For identification of unknown species, especially the sample is trace amounts of tissues, DNA sequencing is still the most direct and reliable method.

Accordingly, the objective of this study was to achieve the species identification and test a variety of dried scallop adductor muscle products sold on the Chinese commercial market applying direct sequencing and BLAST method based on a fragment of the 16S rRNA gene. This work facilitates the evaluation of the situation of dried scallop adductor muscle products in the market, support of food control and protection of consumers' rights.

2. Materials and methods

2.1. Sample collection

A total of 60 *yao zhu* samples without any reference to a particular species (coded as A1–J6, Fig. 1) were purchased from the Yidelu dried seafood retail market (one of the biggest dried seafood retail markets in China) in Guangzhou and five dried seafood retail markets in Zhanjiang ("City of Seafood Gastronomy" in China). Each sample included 10 individuals which were picked out randomly.

2.2. DNA extraction, PCR amplification and electrophoresis

Total DNA was extracted from each individual of a sample (30 mg) by using the TIANamp Marine Animals DNA Kit (TIANGEN, China) in accordance with the manufacturer's instructions. DNA concentrations were measured using a U-1800 spectrophotometer (Hitachi, Japan). A pair of primers (16Sar: CGCTGTTATCAAAA-CAT and 16Sbr: CCGTCTGAACCTCAGATCACGT) reported by Palumbi (1996) was used for PCR amplification of each individual. The PCR amplification reactions were performed in a total volume of 50 μ L. Each reaction mixture contained 100 ng template DNA, 2 μ L each primer (10 μ mol/L), 5 μ L of 10 $\times$ Ex Taq buffer (20 mmol/L Mg^{2+} plus), 1 μ L dNTP mixture (10 mmol/L each, Sangon Biotech, China), and 0.5 μ L Ex Taq DNA polymerase (2 U/ μ L) (TaKaRa, Japan) as described in detail previously (Wen et al., 2011; 2012; 2015). PCR have been carried out in a T-100 thermo-cycler (Bio-Rad, USA). PCR cycles were an initial step of 2 min at 94 °C and 35 cycles of 30 s at 94 °C, 40 s at 52 °C, and 1 min at 72 °C, followed by a final extension at 72 °C for 10 min. The products of PCR amplification were analyzed by 1.2% agarose gel electrophoresis at 150 V for 50 min. The lengths of fragments were determined by comparing with the

100 bp DNA marker (TaKaRa, Japan).

2.3. DNA sequencing and BLAST for species identification

Amplified products were purified with AxyPrep™ DNA Gel Extraction Kit (Axygen, USA), then sequenced in both directions with Applied Biosystems 3730 Automatic Sequencer. The sequences were analyzed with the Chromas v2.23 software and aligned using Editseq software (DNASTAR Lasergene Version 7.1.0) and Jellyfish v1.4 software. The nucleotide sequences have been deposited in the Genbank database of the National Centre for Biotechnology Information (NCBI). After sequencing and removing the primer sequences, the species of all samples were identified by a BLAST analysis. The BLAST search available at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to assign a DNA sequence to a particular species. The correct assignment of individuals to species was performed by calculating the expected value of reference sequences' identity.

3. Results and discussion

3.1. PCR amplification and sequencing of 16S rRNA gene

With primer pairs of 16Sar and 16Sbr, predicted DNA fragments of mitochondrial 16S rRNA gene were successfully PCR-amplified from all 60 samples. The sizes of PCR products obtained from the samples are shown in Fig. 2. Though 10 random individuals of each sample were adopted for PCR, changes in the sizes of the amplification fragments did not detected, which manifested high-degree stabilization for the method. Mitochondrial DNA (mtDNA) is the preferred focus of PCR because it has a higher heat resistance and is more likely to be found in samples that have undergone processing, which is particularly important to identifying species of food that have been prepared for human consumption. There are also more copies of mtDNA within the mitochondria than nuclear DNA (nDNA) located in the cellular nucleus. Researchers are therefore more likely to recover mtDNA from forensic material because there are more copies of the mtDNA to sample (Clark, 2015). Therefore, the mitochondrial genes have been successfully amplified from some dried products, such as dried abalone (Chan et al., 2012), dried sea cucumber (Wen et al., 2010; 2011; 2012) and dried fish maw (Wen et al., 2015). After purification and bi-directional sequencing of the PCR products, clear and clean sequences were obtained. The sequences sequenced in this work have been deposited in the GenBank database under accession numbers KU504632–KU504636.

3.2. Species identification of commercial products

BLAST was initially developed to find regions of similarity between sequences, nowadays BLAST is a suitable technique used for the genetic identification of species (Armani et al., 2014; Galal-Khallaf et al., 2016; Kappel & Schröder, 2016; Khaksar et al., 2015; Quinto, Tinoco, & Hellberg, 2016). Specifically, it is worth highlighting that many species found in the NCBI database were not been properly assigned with the correct species. Considering that, only published sequences were chosen in this study (Feng, Li, Kong, & Zheng, 2011; Malkowsky & Klussmann-Kolb, 2012). In the present study, the 16S rRNA sequences generated from 34 samples (56.7%) were 500 nucleotide-long after alignments (cropped of primers sequences). BLAST analysis allowed unambiguous match (99.8–100% identity and 100% query cover with 0.0 E-value) with the GenBank references GU119969 and GU119970 corresponding to the scallop species *Argopecten irradians*. Other 26 samples (43.3%) with 583 bp-long sequences after alignment had best BLAST results

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