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Research Article

The Influence of Processing on the DNA Integrity in Several Raw Materials of Marine Foods

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Abstract: With the development of molecular biotechnology, methods for identification of marine food are developed from protein to DNA. The experimental materials are striped bass, Sturgeon and mandarin fish. They were processed with boiling, salting and microwave-heating methods. DNA-based research has been conducted in almost every corner in food material identification. This study is mainly about the identification methods of distinguishing processed aquatic products and the influence of processing on the DNA integrity in the raw materials of marine foods.

Keywords: DNA integrity, marine foods, processing

INTRODUCTION

The nutritional value of marine food is high, in recent years the international Shanghai ocean food consumption has gradually increased. Some merchants in the pursuit of high profits, then the adulteration in marine food processing in the fraud, with low sea food instead of expensive sea food for sale, also greatly harm the interests of consumers, on the other hand also caused the unfair commercial competition (Carrera *et al*., 2000). Development of DNA detection technology makes DNA in food detection possible for species identification of genetically modified food identification of food raw materials, detection of bacteria and viruses in food, food traceability of raw materials etc (Moretti *et al*., 2003). In recent years, the DNA extraction method for deep processing of plant products have already obtained a certain progress, however, aquatic products DNA extraction at home and abroad is still in the research stage. Aiming at all kinds of false adulterated phenomenon of current aquatic products processing industry, a pressing matter of the moment is to develop efficient, stable authentication of aquatic products. Extraction of deep processing of aquatic products DNA is crucial for species identification of aquatic products has significance (Yancy *et al*., 2008). In recent years, due to the destructive effect of genetically modified food inspection need food processing methods on DNA in food attracted the attention of people. But the research object is currently focused on corn, rice and other mature transgenic plants research reports on the sea food is little (Hird *et al*., 2005). Marine biological change composition different from terrestrial organisms such as the moisture content difference of salt content was higher in the food composition of the will which DNA affects in the processing (Rehbein *et al*., 2002). So it is necessary to conduct in-depth study of marine food. This paper chooses *Oncorhynchus keta*, *Thunnus obesus* and *Scomberomorus niphonius* as the experimental object. Select the boiling temperature, curing of high concentration of minerals, raw materials are provided in order to help detect sea food DNA and preliminary study of its impact on DNA chain length and purity of DNA microwave radiation extraction process (Kochzius *et al*., 2008).

Marine food species identification used in DNA sequences. The use of DNA technology to identify marine processed foods are basically based on PCR amplification, DNA material and therefore, the integrity of the target DNA fragment of genomic mutation rate and sequence length traits are important determinants. But the sea food is usually heated, high pressure, radiation treatment, DNA breakage occurs in the process, for the extraction of DNA reduced, so select the appropriate analysis of gene is a key factor in achieving the marine food species identification. Usually in the deep processing of marine food through identification, gene fragments chosen should at 300 bp or less. Currently in the marine food species identification in selected DNA types are:

- **Mitochondrial DNA:** Cyclic structure of mtDNA it has better heat resistance and a relatively high abundance, so that mtDNA in the marine species identification in processed foods generally be preferred.
- **Core genes:** Nuclear DNA sequences for the identification of the main 5S rRNA gene, p53 gene, ITS2 loci (The nuclear ribosomal internal

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transcribed spacer 2 locus), 18S rRNA gene, α-actin gene and the major histocompatibility complex II β1 gene (Cozzohno and Murray, 2004).

For fresh and frozen sea food such as roughing, due to DNA degradation is relatively small, you can use the traditional method of DNA extraction or minor adjustments according to the situation; for deep processing of marine foods such as canned food, according to the process used in the ingredients Select the appropriate DNA extraction methods, in order to guarantee access to good quality DNA (Downey *et al*., 2000).

The other is based on molecular biology methods DNA techniques, the use of DNA identification techniques for the species with higher specificity, sensitivity and reliability than protein technology, the main reasons are:

- DNA better than the heat resistance protein, although the DNA molecule present in the degradation process, but can still be extracted a small DNA fragment.
- Due to the degeneracy of the genetic code and non-coding region, DNA can provide more information.
- DNA technology Effect of the type, age and other factors and is not tissue.

Therefore, the use of DNA technology to identify and processing of seafood has more advantages than traditional techniques. Currently, methods based on molecular biology techniques in the identification of marine DNA processed food species has become a hot research.

MATERIALS AND METHODS

Materials: The striped bass was purchased from Qingdao Mackay Lok supermarket, Sturgeon and mandarin fish purchased from Qingdao Yongwangdongtai Limited by Share Ltd, to buy the supermarket all kinds of raw materials after cold preservation and immediately transferred to the laboratory for processing, other reagents are made of pure reagent.

High temperature cooking, microwave heating and curing processing:

High temperature processing: The 1.0 g sample into boiling water 10 min.

Microwave: Microwave oven to high fire for 1000 W, the food raw materials are cut into 23 g pieces into a microwave oven center position 1.5 min.

Pickling process: Sturgeon and mandarin fish meat cut into 23 g pieces into a Petri dish after sterilization with distilled water in the water surface was not have joined the 0.5 g solid sodium chloride and fully mixing such as sodium chloride dissolved all can supplement the solid sodium chloride and sodium chloride is no longer dissolved cover Petri dish cover and the use of plastic wrap completely wrapped in 7 day at room temperature.

DNA extraction: The group cut after full grinding grinding process with 0.2 mL1 * TEBuffer 1.5 mL centrifuge tube in tube separately in lysis buffer 60 mmol/L Tris HCl pH pre configured = 8.025 mmol/L EDTA to reduce the viscosity and the protection of DNA into the molecular integrity of 50±5 mg samples fully after grinding, 1.5 mmol/L NaCl1% SDS direct lysis buffer. Fresh and preserved striped bass except fresh and preserved striped bass is added into the sample does not contain SDS lysis buffer to prevent its under high salt concentration precipitation in the boiling water bath enzyme inactivation after 3 min is added into SDS sample into 2 μL 10 mg/mL RNase in a water bath at 37°C for 20 min to remove the RNA add in the sample 300 L phenol chloroform isoamyl alcohol is 25:24:1 full vibrating mixing 7800 r/min at 4°C centrifugal 2 min from supernatant on 1.5 mL centrifuge tube to repeat the above operation to full removal of proteins into supernatant concentrated sodium acetate solution at the concentration of 0.3 mmol/L and adding the total volume of liquid 2.5 times ethanol mixing at -20°C static 30 min samples at 4°C for 12000 r/min centrifugal 15 min remove supernatant centrifugal tube with 200 L 70% ethanol cleaning precipitation at 4°C 12000 rmin centrifugal 5 min supernatant was removed and then 37°C dry residual ethanol and the addition of 50 L1×TEBuff The dissolution of Er DNA each treatment was performed 3 times parallel test (Chapela *et al*., 2002).

UV visible absorption and electrophoresis analysis: Determination of DNA concentration and A260A280 value using 1% agarose gel electrophoresis DYCP31DN Beijing six one detection of DNA molecular fragment less than 100 V electrophoresis of 45 60 min with EB stain in dark conditions BioRadUniversalHoodII staining of 20 min by gel imaging system analysis of the results of electrophoresis using Nanodrop 2000 American Thermo.

RESULTS AND DISCUSSION

The establishment of material selection and test method: Using DNA technology to identification of marine food processing basically are amplified by PCR as the foundation, so the DNA raw materials, the target DNA fragments of integrity, genomic mutation rate and the length of the sequence were important determinants (Lowenstein *et al*., 2009). But the sea in food processing is usually heated, pressure, radiation treatment, DNA fracture, reducing the availability of DNA extraction, so

the choice of appropriate analysis of gene is the key factor to realize marine food species identification. The general was identified in the deep processing of sea food; gene selection should be below 300 bp. The current selection in the marine food species identification of DNA types are:

- **The mitochondrial DNA:** The cyclic structure of mtDNA has better heat resistance and relatively high abundance, the mtDNA in the marine food processing in general as the preferred species identification. Among them, the most widely used is the Cyt B gene, has been used to identify *Engraulidae*, *Gadous*, *Pleuronectiformes*, *Anguilla* and *Scombroidei* etc. In addition, tRNA sequences of mitochondrial Cyt B region and its adjacent (tRNAGlu-Cyt b), 12S rRNA gene, 16S gene, rRNA regulatory region (D-loop), cytochrome oxidase subunit gene (Cytochrome, oxidasesubunit III, COX III) and COX III and ATP as e between the lateral area (ATCO) have been used for species identification of marine food.
- **Nuclear gene:** For nuclear DNA sequence identification are the 5S rRNA gene, ITS2 gene, P53 sites (The nuclear ribosomal internal transcribed spacer 2 locus), 18S rRNA gene, a-actin gene and the major histocompatibility complex II beta 1 gene. Such as: 5S rRNA gene has been used for identification of mackerel, COD, salmon (*Salmonidae*) and shark (*Selachii*) larvae or eggs frozen or canned food. For fresh and frozen, rough sea food, due to DNA degradation is relatively small, DNA extraction using traditional methods or according to slight adjustment; for deep processing of marine foods such as canned food, can be used according to the process of batching, choose a suitable DNA extraction method in order to secure better quality DNA. In a word, related sequences to identify marine food species generally choose mtDNA as the object of study, followed by nuclear gene sequences. In addition, microsatellite label has been used as a study on the development of large marine species system, study the identification is still not widely used in marine food species (Chapela *et al*., 2007). Polymorphism, repetitive DNA sequences based on multi gene family is a kind of available resources. Such as the actin gene family, has been used in identification of many vertebrate species. The gene coding sequence, length and the number of Chi Ko sequence difference is expected to be used in marine food species identification of the actin gene family (Clarke *et al*., 2006).

This study analyzes three kinds of typical strong high value sea food as experiment object. Bass is rich in protein, vitamin A, B vitamins, calcium, magnesium, zinc, selenium and other nutritional elements; has the kidney, the spleen and stomach, phlegm cough. It also can treat quickened produce less milk and other disease,

Table 1: The concentration of DNA in raw material

Raw materials	Concentration	A260/A280
Striped bass	405±35	1.70-1.76
Sturgeon	380±40	1.84-1.89
Mandarin fish	482±46	1.75-1.86

Table 2: Division of agents for ISMR

Raw materials	Concentration	A260/A280
Striped bass	672±42	1.76-1.79
Sturgeon	256±37	1.74-1.80
Mandarin fish	568±40	1.65-1.73

mothers and women eat bass is a kind of filling body and does not cause nutritional food, nutrition surplus caused obesity is fitness blood, spleen and replenishing qi and yi smoked in the ankang body, bass is steamed to keep more nutritional value. According to the Chinese academy of sciences of Ocean detection: muscle contains ten many kinds of essential Minolta acids, fat contain 12.5% "DHA" and "EPA") (also called brain gold, to soften the heart head blood-vessel, promote brain development, improve intelligence, preventing senile dementia has good effect; Cartilage and bone marrow have anti-cancer agent, can be completely eaten, known as "the shark fins, sturgeon bone" (Carrera *et al*., 2000).

After processing need to extract the pure DNA to analyze so author adopted SDS cracking phenol-chloroform method to remove protein and alcohol precipitation method for analysis of other impurities samples. When the extraction process of DNA damage taken a heat treatment to inactivate DNase nucleic acid enzymes such as measures to improve the existing extraction method. Due to general processing method will not result in a fall off the bases such as serious damage, 11 and had the greatest influence DNA integrity of DNA test results 15 to 16 agarose gel electrophoresis was chosen for this analysis of the length of the extracted DNA and ultra micro spectrophotometer Nanodrop determination of the concentration and purity of DNA (Moran and Garcia-Vazquez, 2006).

The purity of DNA using A260/A280 this ratio to the DNA of the appraisal of pure numerical general right left at 1.8. When A260/A280 ratio indicates the high purity within the range of 1.7 to 2.0. When mixed with protein in general this value is less than 1.7 and when mixed with RNA ratio may be greater than 2.0.

The Table 1 shows the untreated bass, mandarin fish, sturgeon samples extracted to obtain the DNA of the thick degree between 350-500 NGL A260/A280 mostly between 1.7-1.9, show the purity of DNA obtained high quality conform to the requirements of the experiments.

The influence of cooking for genomic DNA of material: Extracted after high temperature cooked food raw material organization DNA concentration obvious changes have taken place are shown in Table 2. With the DNA of raw materials Obtained DNA concentrations than salmon rose nearly double parallelism.

Table 3: Division of agents for ISMR

Raw materials	Concentration	A260/A280
Striped bass	234±42	1.66-1.72
Sturgeon	262±37	1.60-1.70
Mandarin fish	248±40	1.65-1.69

Table 4: Division of agents for ISMR

Raw materials	Concentration	A260/A280
Striped bass	281±42	2.06-2.19
Sturgeon	486±57	2.04-2.18
Mandarin fish	568±101	2.28-2.31

Compared with the DNA of the raw material striped bass and mandarin fish obtained DNA concentration rose nearly double and has good parallelism, but Sturgeon extraction DNA concentration decreased. This may be due to high temperature heating within the food ingredients on enzyme activity of DNA molecular chain structure caused by irreversible effects. For example histones with thermal denaturation of the DNA during DNA come together with the protein during extraction into the organic phase together, at last the resulting low yield of DNA.

In the Table 2 the A260/A280 values of striped bass and mandarin fish remain consistent with DNA extracted between 1.70-1.85 purity, it meet requirements of raw material, which still has the largest A260/A280. We can see in the Table 2 the Sturgeon value changed small and it less parallelism much compared to the other three kinds of fish.

Effect of the genomic DNA of the raw material is heated by microwave: Microwave heating is vibration friction heat and heat the food utilization of water molecules in the food. The different water content of food is quite different in degree of microwave heating. Marine food raw materials as one of the high water content of food, easy to be heated to cause the changes of nucleic acid. Three species of marine food raw materials in the damage after microwave heating is far less than the boiling, probably because of the shorter heating time is short and the high temperature condition in time.

Table 3 shows that the microwave heating of the resulting DNA concentration of salmon extract significantly decreased the difference there is between about 20% in the experimental group in parallel. Big-eye tuna, mackerel and blue dots talons shrimp parallelism has absorbed value increased slightly lower at 260 nm UV samples-visible absorption spectrum of the curve more smooth, but with standard DNA spectral peak positions And curves. Morphological differences are mainly large blue shift show DNA purity decreased. A260/A280 values were greater than 2.00 also reflects lower DNA purity microwave treatment which may be caused by eggs. White matter and other complex structures and structural RNA binding or RNA-DNA extraction process make it difficult to be RNase decomposed mixed samples.

Microwave heating is to use the vibration of water molecules in food to generate heat and friction heating of food, water content of different foods makes different degree of microwave heating process differ. As one of the marine food ingredients high water content foods easily be heated to cause changes in four kinds of nucleic acid material damage marine food heated in the microwave to boil at far less than microwave treatment may be due to a short time and in a high temperature state in which the time is shorter (Santaclara *et al*., 2006).

Effect of curing the raw material of the genomic DNA: Table 4 shows that after processing three kinds of pickled fish food raw extract obtained DNA concentrations were significantly decreased and the parallelism between the parallel groups more consistent. A260/A280 DNA purity reflected in performance between 1.60-1.70 indicate the purity of extracted DNA may be mixed with a small amount of reduced protein and other impurities. On the other hand wavelength scanning spectrum shows a smooth curve and relatively accurate peak position showed no obvious due to reduced DNA purity marinated shrimp processing Talon little use here is not their experiment.

In the presence of high salt concentration easily denatured inactivated protease, preserved at the beginning of the activity may be due to DNase and gradually lose part of the DNA causing decomposition of the sample when the high concentration DNA and DNase inactivation completely destroyed even if it is no longer save still a lot of complete genomic DNA present.

The first thing of the detection of DNA is DNA extraction and complete extraction of shear grinding operation but also on the possibility of DNA damage. Forbes found that dry grinding wheat DNA degradation does not make them fresh grain operation but was significantly affected the integrity of the DNA which. Thus the impact of this proposed mainly from the damaging effects of the nucleic acid enzyme. Results of this study also showed that aquatic foods DNase thus higher DNase activity of some species of sturgeon in the extraction process, paying particular attention to suppress severe DNA damage system in which the role of nucleases. In this study, pre-heating 5 min is a better way to solve the marine food DNA extraction problem. Also, consider adding nuclease inhibitors. Such as adding a small amount of metal ion chelators (10 mmol/L EDTA or sodium) citrate can inactivate DNase basic 24, joined SDS and other detergents also allows most of the protein denaturation inactivation. Different species of biological raw materials vary greatly in the presence of nucleases and DNA and other aspects of the status of the experiment needs to be effective to use a method to select.

International DNA technique for the diagnosis of marine food origin of species has been a lot of research, which provides the technology for marine food adulteration false decision support, some of these methods is practical, they have been or may be used as the standard method for identification of marine food. However, research on identification of species of marine food sources also faces many difficulties. First of all, it is estimated the people of the world consumption of seafood has more than 20000, identification method is the use of protein and DNA polymorphism on fingerprint analysis technology, often based on existing species specific and many species have similar fingerprints, or individuals of the same species due to intraspecific variation and display different chromatogram fingerprints, which makes the identification of complicated; secondly, degeneration and DNA degradation protein marine food processing, the analysis method of processed seafood is particularly difficult; third, marine food processing usually adding vegetable oil, various additives and seasonings, effective extraction of these substances will affect DNA, on the other hand, may play a role in the amplification of PCR inhibitors, process, therefore, the species identification prior to extraction, for DNA optimization, in order to ensure a sufficient amount of DNA extracted and reduce or eliminate PCR inhibitors, these conditions make DNA technology application in the food industry has become relatively difficult.

For the destruction of nucleic acid enzymes process DNA cannot be ignored, this DNA degradation by DNase caused during storage of fresh food is very likely to occur 25. Although DNA degradation phenomenon has occurred in this study but the impact of three kinds of processing methods are not the same temperature cooking process in which DNA degradation caused by the most serious of this general warming of the heating process will take some time due to the role of DNase provided the conditions. Another high-temperature melting itself may occur generate additional stress cooking process combined effect of several factors causing serious degradation of DNA, although the literature indicates that heat can damage the nucleic acid of hydrogen. But unless there are between single-stranded nucleic acid enzymes bases cracking hydrogen it does not make the phosphodiester bond easier to break the DNA backbone, while the same is due to the heating effect of microwave treatment faster than traditional methods of heat transfer and uniform heating. Nuclease can be a faster deactivation of the DNA which may also still part of the microwave treatment, after the degradation of genomic DNA does not exist. Marinated processing aquatic products processing using the more it differs in that it mainly caused by the heating process in food enzymes denatured by high ionic strength to inhibit the activity of DNase, DNA degradation and therefore the curing process occurs mainly in the initial processing and DNase Once successfully inhibited DNA also will no longer affect the long-term preservation. High ionic strength can also improve the stability of DNA duplexes and reduce some of the risk of causing a high ion binding to DNA decomposition of magnesium ions to reduce DNA damage through competition.

CONCLUSION

At present, the method of DNA technique for the diagnosis of marine food species also has many challenges, such as repair of highly processed and mixtures of DNA products; establish more simple, fast and inexpensive analytical method can also identified and the mixed sample identification and quantitative analysis of species variety in food. Therefore, gene chip, chip laboratory, quantitative real-time PCR technique and species specific primers multiplex PCR technology may play a role in the future of marine food source species identification. In addition, a variety of seafood species gene database information collected, impact on marine food processing identification will be more and more. This study shows that, marine food processing method and DNA extraction methods will affect the quality of DNA. Consider these factors to process qualitative and quantitative detection in marine food in DNA. Also worth noting is, influence the process of DNA is inevitable. Have therefore must choose the appropriate primer to avoid false negative results in the actual detection.

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