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Review Article

THE INVESTIGATION OF THE IMPACT OF DIFFERENT FACTORS ON DNA ISOLATION FROM FOOD PRODUCTS: A VERSATILE EXAMPLE; SEAFOOD

Elif Tuğçe Aksun Tümerkan [1,a,*], Özgür Emiroğlu [2,b]

[1] *Department of Technical Research and Quality Control, General Directorate of State Hydraulic Works, Ankara, Turkey*
[2] *Eskişehir Osmangazi University, Faculty of Arts and Sciences, Department of Biology, Eskişehir, Turkey*

**Corresponding Author:*
E-mail: eliftugceaksun@dsi.gov.tr

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a: ORCID 0000-0003-1993-0569, b: ORCID 0000-0002-4433-4286

ABSTRACT. Food fraudulent activities are emerging issues for consumer with threatening public health. Mislabeling, species substation are main fraudulent actions in food industry. Due to highly profitable rate of seafood, this industry has also facing with these fraudulent activities. Different analyses have improved for reliable, fast and cheap species identification. While some protein based methods such as Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis ((SDS- PAGE) and Isoelectric focusing (IEF), DNA based techniques are more reliable for species identification. Quality of DNA is the main requirements for success amplification of DNA and therefore trustworthy species classification. This review focuses on the evaluation of different factors on quality of DNA from processed and non-processed seafood. Different processing techniques, extraction methods and target gene properties are discussed. While DNA extraction from fresh fish, non-processed seafood is easy, and purification rate of DNA is high, different processing techniques, food matrices when stored with different indigents and treatment with salt and acid cause to degradation of DNA. High thermal process and pressure treatment main reason of non-success DNA extraction. Different Extraction methods have also effect on quality of DNA based on used chemicals or commercial kits. Targeted gene and fragments properties are other important factors on quality of DNA. Depends on raw material, processing techniques and extraction methods, characteristic of targeted gene differ.

Keywords: *DNA isolation, DNA extraction, genetic research, processing methods, seafood*

INTRODUCTION

Seafood is essential animal protein source, accounting for more than 17% of the global population's intake of total animal protein in 2013 [1]. Over the past decades, seafood production has an increasing trend at an average rate of 3.2% per year. Due to biological composition and being highly perishable, fish and selfish, different processing techniques applied for improving nutritional value and extending shelf life of these products and creating service alternatives to consumer. Due to the annual seafood consumption has doubled in the past decades reaching approximately 20 kg per capita globally, seafood is accepted the most traded commodities [2, 3]. Similar to other food industry, different food fraudulent activities such as species substation and mislabeling have become serious problems for producer, seafood industry and consumers, recently. In the other food fraudulent actions, seafood is in the list of top 10 food products that are generally subject to species substitution and mislabeling due to increased international trade of seafood [4,

5]. While fraudulent actions of seafood has been most likely seems cause to just unfair economic income, it has also caused to life-threatening serious health risks such as allergies, toxicity and other health problem especially for pregnant women and young children [6].

Whereas, the external morphological characteristics such as body shape, kind of scale and fin position or its number are sufficient to identification of unprocessed or fresh fish species, these classifications do not meet the requirement for species identification of processed fish or seafood products [7]. To overcome this challenge, several molecular approaches, especially DNA based or protein based analysis have developed for avoid possible fraud in the seafood industry. Some research highlighted that DNA-based methods more appropriate than protein based analysis for processed fish [8, 9]. The success of these reliable methods depends on processed raw material, processing techniques and steps, packaging properties and storage conditions. This review focuses on the clarifying the effect of different factors on isolated DNA quality from various processed and non-processed seafood.

MATERIALS AND METHODS

This review has been developed with the aim of evaluating the effect of different factors on the isolated DNA quality from processed and non-processed seafood and generating an updated information on DNA isolation of seafood and its effects on food fraudulent research.

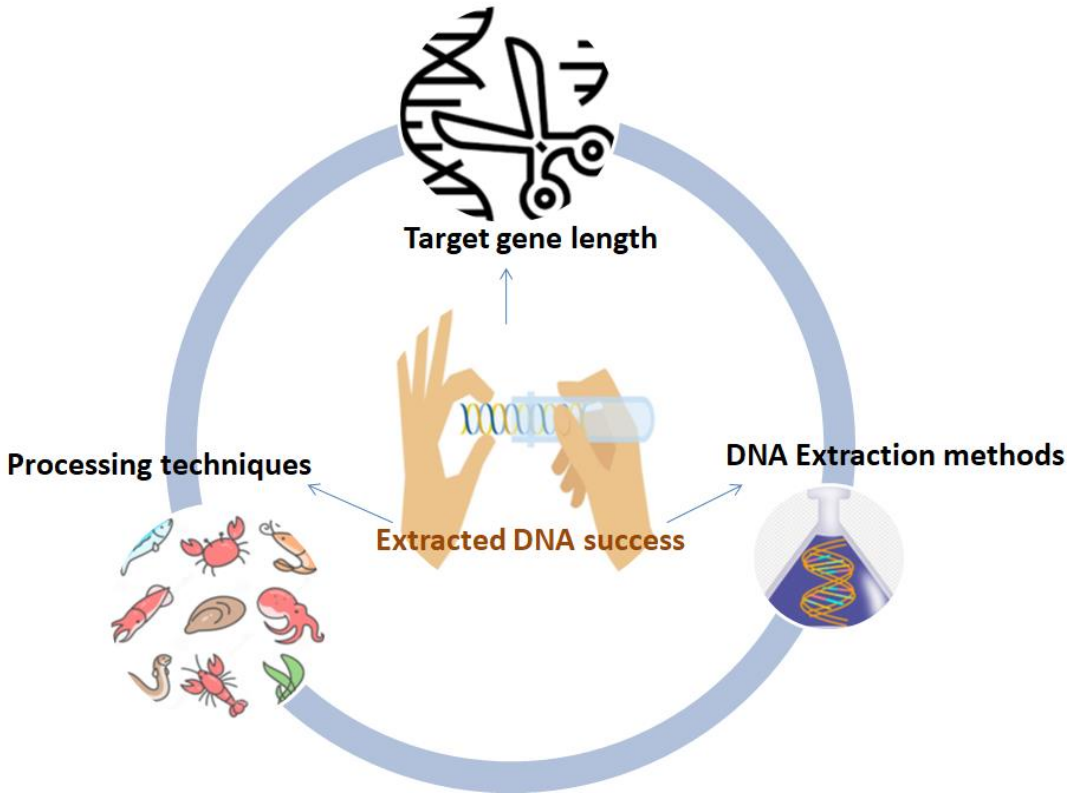

Fig. 1. *Summarization of different factors on DNA extraction quality from fresh and processed seafood*

RESULTS AND DISCUSSION

Table 1 shows relevant research on the DNA isolation from different processing techniques applied various seafood. Figure 1 show that summarization of wide range of influences on DNA isolation process from seafood.

Influence of raw material and processing techniques

While molecular analysis widely use to identification of fish species that clarifies the phylogenetic variations among species using standardized gene fragment, this method has been used for monitoring traceability and food safety over the last decade [10, 11]. To successful genetic research, approved PCR amplification is the main requirement and extracted DNA purity and quality have great importance [12]. While the yield of purified DNA from raw material relatively easily, extraction of a well quality of DNA from processed seafood is complex and has some challenges due to thermal processes, high pressure and variation in acidity during processing. The quality of extracted DNA from fresh, non-processed or non-physically damaged seafood is vary depends on species properties and extraction methods. Different processing techniques such as high-heat treatment, salting, or smoking cause to degradation of DNA [13, 14]. The DNA purity of processed seafood does not affected by heat or pressure treatment and pH variations, it also influenced by filling media in the case of processed fish or shellfish served in a packaging material. For instance, filling media plays key role for DNA quality of canned fish, usage of spies, vinegar or different oil indigents effect directly DNA qualification and quantification. Other thermal process such as smoking and boiling, frying or drying also degraded DNA of fish and fish product [15]. Salting and treatment with acid are the another damaging processes for achieving high quality of DNA from seafood. As regard as processing methods, the contacted materials such as oil or filing medium are in packaged seafood products. For instance; the type of can-filling medium and the differences in thermal conductivity and acidity of other components in the can are the main factors affecting the canning process and the quality of tuna and consequently the degradation of DNA from tuna. Sunflower oil and olive oil are the most commonly used oils in canning industry with some benefits; while usage of sunflower oil as filling medium leads to more palatable tuna with relatively lower price, utilization of olive oil retards the oxidation of canned tuna and offers the better colorimetric properties [16]. Recently, utilization of different sauces and spices as filling medium has become popular in order to meet the consumers demand. While several spices have used as flavoring and coloring agents in food products, these seasonings can also used in fraudulent actions such as masking the rancid taste of food [17]. The DNA extraction method is another key factor that affects the quality of DNA. Different chemical, enzymatic and lysing protocols have been utilized over recent years [18]. There are many commercial kits that have been used in addition to chemical-based methods but these approaches have been rarely compared [19]. These factors cause to muscle protein denaturation, which make difficult to reaching to DNA during extraction methods.

Effect of DNA extraction methods on purity of DNA

The DNA extraction method is another key factor that affects the quality of DNA. Different chemical, enzymatic and lysing protocols have been utilized over recent years [18]. There are many commercial kits that have been used in addition to chemical-based methods but these approaches have been rarely compared [19]. Quality of DNA from processed seafood also influenced by extraction methods. Numerous protocols for DNA

extraction from fresh fish and processed seafood have been applied in order to achieving better quality of DNA [20]. While chemical based protocols such as phenol–chloroform-mixture methods, CTAB method or salt binding methods are used for DNA extraction, commercial kits such as Qiagen, Chelex, Roche and Wizard have widely used for the same aim. Depends on processed seafood or raw material conditions various extraction methods utilized for success DNA isolation. While chemical based man-made extraction methods are comparatively cheaper than commercial kits, but these methods take more time than kits [21, 22]. Multiple factors during processing using spices or acids and thermal treatment caused to more degradation of DNA and it make difficult to purification of DNA. In these case some extraction procedure used together. While the purity, yield or concentration of DNA have attracted the greatest interest by researchers, having an optimal 260/230 ratio is more important for success PCR [23]. The DNA yield vary depending on organic compound contamination and the effectiveness of different extraction methods on the removing of organic contaminants.

Targeted gene properties and fragment lengths

DNA-based methods have considered as the promising approaches for the species identification efficiently which can be carried out successfully without any initial information about sample [24]. While different molecular genetic markers have been utilized for identification of species, the mitochondrial genome (mtDNA) such as cytochrome oxidase (COI) cytochrome b (cyt-b) and 12s ribosomal RNA (12S rRNA) have been successfully used for species identification in fish species even in cryptic species [25]. Recent advances in barcoding and sequences systems allow to will enable DNA sequencing to be readily applied to the analysis of heavily processed and multiple species in processed seafood products [26]. The achievement of DNA based analysis mainly depends on the quality of purified DNA and amplification process. While the DNA-barcoding with mitochondrial genes have more than 500-600 bp, has failed in some processed food products due to DNA degradation in sequences of less than 300-400 bp [27, 28] and DNA mini-barcoding designed universal primers focusing on a shorter DNA sequence has promising approach to used even in very close species and in cases of highly processed food [29]. Shokralla et al.,[26] indicated while DNA barcoding identified the fish species with 20.5% rate, this rate achieved up to 93% when the mini-barcoding used in canned seafood product.

Different gene used in DNA based species classifying or food fraudulent analysis. While mitochondrial genome (mtDNA) has been generally applied in genetic research with some benefit such as containing higher number of copy in extracted samples, quicker assessment than that of genomic DNA caused by higher base substitution degree [5]. The Cytochrome oxidase subunit I (COI) and cytochrome b (Cyt b) genes facilitate to reliable classification and determination of differentiation between species from the same families [30]. Target gene bas pair length effect on successful amplification. For example, the identification of highly processed seafood such as canned or salted fish, various mitochondrial markers differ from 100 up to 300 base pairs (bp), have been used successfully with cytochrome b and 16S rRNA [29]. COI and Cyt b genes give chance to identification of genetically close species such as different tuna sub-species in food fraudulent research. Cyt b gene has resistance to against high temperature, and high mutation, which are important advantages [31]. Fragment length is also important parameter for well amplification and therefore reliable species identification. COI gene is one of the most targeted gene marker and longer barcoding (approximately 650-bp)

and comparatively shorter barcoding (with lesss than 350-bp) have offered identification of species both from fresh fish and highly processed seafood products[32].

Table 1. *Relevant recent research on quality of DNA from seafood products*

Seafood product	Targeted Gene/bp length	Main findings	References
Cuttlefish, products, octopus products, squid products	Cytochrome c oxidase I (COI) and 16s ribosomal RNA gene (16SrRNA)/ 526 to 658 bp for COI and 503 to 513 bp for 16SrRNA	Seafood matrices and used gene are important for DNA isolation DNA	Wen et al.,[33]
Smoked, canned, minced, boiled tuna	cytochrome b gene	Different processing methods, in terms of temperature and influence on DNA yield	Piskata, et al., [34]
Cooked, peeled and unpeeled or frozen shrimp	Cytochrome b gene/ 315 bp	Length of DNA fragments and processing methods extraction procedures on DNA yield.	Besbes and Sadok al.,[35]
Canned Tuna with different media	cytochrome *b*/100, 150, 200, 250 and 300 bp	The highest DNA quality observed in tuna with oil, whereas tuna packaged with vinegar caused to the lowest DNA quality. Length of DNA fragments effected by DNA quality	Chapela et al.,[21]
Canned sardine and anchovy	Mitochondrial cytochrome b gene / 358 bp	Canning and usage of salt and spice during processing of fish damage the DNA quality of fish	Besbes et al.,[35]
Sushi	16S rRNA and Cytochrome C oxidase subunit I (COI) gene/ 655-658bp	COI gene was successes than 16S rRNA gene	Armani *et al*.,[29]
Sharks	coxI gene, 550 bp	Pre-processing methods as freezing or chilled storage influence on DNA quality	Barbuto *et al*., [36]
Fillet of Alaska Pollack, Red Cod, Atlantic Salmon, Nile Perch and Tilapia	Cytochrome oxidase I gene / 650 bp	DNA purity vary depends on processing and extraction procedure	Changizi et al.,[37]
Fresh, Frozen, Roasted of Salmon and Carp	COI gene /650 bp	Processing techniques and extraction methods widely effect on DNA yield	Xiong et al.,[32]

CONCLUSION

The current review of criticism of different factors on DNA isolation from several seafood products as an example from food matrices. This review has shown that not only the different extraction methods, but also the targeted gene and its length have impact on the isolated DNA quality and quantity. Additionally, different food matrices contacted the main food material such as spice and oil change the DNA isolation from food matrices. This review also looked at the differences between processed and un-processed seafood products affected by several processing methods including high pressure and thermal treatment. Since, the DNA quality characteristics, including yield, purity and amplificability of isolated DNA ate the main factors on the following molecular research on the food items, these properties need to be deeply considered. While the comparison of different research on the DNA isolation from food items which driven by several factors, the findings of this review will contribute to supporting the better understanding of the importance of DNA quality and quantity from seafood. Detection of fraudulent actions in different food industry can be possible within meeting the isolated DNA requirements, especially for fisheries products. There are still some gaps about isolation of DNA from processed food which is the main problem for the detection of fraudulent in food industry. Further research subjecting the food traceability and seafood processing industry would allow to filing these gaps.

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