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# Species confirmation and evaluation of nutritive values of frozen fish products

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**Abstract**
Seafood mislabeling is a global issue following the increased worldwide seafood trade particularly of processed seafood products. Subsequently, there is a risk of unintentional substitution of low valued fish species for high valued fish in fishery products. Fish species can be identified by knowledgeable fishermen, wholesalers, restaurateurs and consumers while the specimen remains in its whole state. However, once products like fillets are prepared from the fish, species identification or confirmation becomes more difficult. Bioprospecting involves searching for, gathering and deducting genetic materials from samples that can be used in commercialized end products. Thus bioprospecting gives an idea about the genetic confirmation and nutritive content of the frozen fish food. Molecular techniques have proven to be effective in species identification and capable of bypassing the inherent problems of morphology-based identification methods. DNA barcoding represents an effective tool for fish authentication in convenience seafood. Biochemical techniques are used to estimate the nutritional content of the samples. The data generated from this study will be helpful in distinguishing among the frozen fillets of similar species in order to prevent fraudulent substitution with less valuable fish. The work demonstrates the comparison of the nutritive content of the fresh fishes and the frozen one.

**Keywords:** bioprospecting, DNA barcoding, nutritive content

## 1. Introduction

Bioprospecting is the field of science that includes searching, decoction of genetic material and screening of the biological diversity for commercially valuable genetic and biochemical resources [1]. It involves searching for, collecting and deriving genetic materials from samples which are used as commercialized agricultural, industrial or chemical processing end-products [2]. Recent food scares; malpractices by some food producers, religious reasons, food allergies have enormously strengthen public awareness regarding the composition of food products available in the market. Food debase is an age-old problem especially where there is a challenge between the physical availability of, and the market demand for, a food item. Food authentication is the process that substantiate that a food is in compliance with its label description. The risk and threat of food adulteration and mislabelling in the processed food have become a large concern and challenge for the food control authorities as well as consumers [3]. Over the last few years, there has been a tremendous growth in fish consumption due to changes in consumer attitudes towards health and nutrition. In India, during the last decade the seafood consumption has increased several folds and the seafood exports have increased significantly with earnings of 5 billion US dollars for the year 2013-14 [4]. The increase in the consumption pattern has resulted in high demand for different fish products such as frozen, ready-to-eat and ready-to-cook in both domestic and international markets. Fish species can be identified while the specimen remains in its whole state. Consumers are unable to recognize the external morphological features of fish when it is filleted or otherwise processed. Since many fish species are similar in taste and texture, several restaurant and other retailers substitute high market value fishes with low market value fishes purposely to make economic benefit [5]. However, as labels do not provide sufficient information about the true contents of a product, it is necessary to authenticate the components of processed food, thus protecting both consumers and producers from illegal substitutions. The authenticity and attestation of fish products is particularly important when fresh or frozen cuts of fish are encountered because misrepresentation of the actual product, whether through intentional or non-intentional mislabelling is known to occur [5]. Fish species identification in processed matrices, a DNA method is preferable because of stability at high temperature, presence in all tissue types, and greater variation with genetic code [6]. DNA barcoding demonstrates that mt COI (cytochrome oxidase1) used for barcoding contains enough variation that can accurately identify a large variety of animals to the species level [7].

Freezing is a frequently used preservation method to extend the shelf-life of fish [8]. In Canada increase in people's fish consumption reflects the very logical view that fish fat is high in polyunsaturated fatty acids, yet that fish is generally low in total fat [9]. This information can help to preserve the quality of fish especially during postharvest processing and storage of fish which otherwise could be affected by the level of moisture, protein and fat contents [10]. With this the present study was carried out with an objective of estimating the level of seafood mislabelling in Vadodara by DNA barcoding and checking the nutritional content of the Frozen Seafood products.

## 2. Materials and Methodology

### 2.1 Sample Collection

The seafood samples representing various frozen products of

fish were collected from super markets of different areas of Vadodara city. Samples were kept at -20°C until further processing and the details of the product (date of purchase, labelled species) on packet were considered.

## 2.2 DNA Extraction

Around 20mg of sample tissue was used for total genomic DNA extraction. Tissues were chopped using sterile blade and taken into 2ml micro-centrifuge tubes. 600µl of Lysis buffer (10% SDS {8ml} + 10X PBS {2ml}) was added and kept in water bath at 60˚C for 20 minutes. Centrifuged at 10000 rpm for 5 minutes. Supernatant was taken and equal volume of Isopropanol was added. Then tubes were kept in -20˚C for 1 hour. Then centrifuged at 10000 rpm for 5 minutes. Approx. 60-100 µl of 1.2 M NaCl was added to dissolve the pellet. 200µl of absolute Ethanol was added to it. Again centrifuged at 10000 rpm for 7-10 minutes. Pellets were taken and were dissolved in 25µl MilliQ water.

## 2.3 PCR Amplification

The partial fragment (~650 bp) of mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified from all the samples using different sets of primers FISH-BCL and FISH-BCH[11] and LCO 1490 and HCO 2198[12]. The PCR reaction was performed in a 20 µl volume with 50-100 ng templates DNA, 10 pmol of each specific primer, and 10µl of Quigen Standard Buffer. The PCR conditions were set for fish as 95° C for 5 min, 35 cycles of 95°C for 30 sec, 54°C for 30 sec and 72°C for 45 sec followed by 5 min at 72°C. The PCR products were visualized on 2% agarose gel. The A260/A280 ratios of the samples ranged from 50 to100 ng/ µl. Purification of COI gene amplified products was done using ExoSAP-IT® of affymatrix using reaction mixture of 10µl PCR product and 4µl ExoSAP-IT. Sequencing was carried out using BigDye® Terminator v 3.1 Cycle sequencing kit. Capillary electrophoresis of cycle sequenced products was performed on 3500 XL platform (Applied biosystems).

## 2.4 Data Analysis

Sequence analysis was done using sequencing analysis version 5.4 (Apllied Biosystems) and BioEdit, biological sequence alignment editor [13]. Consensus sequences generated after aligning gene sequences from forward and reverse primers. These sequences were subjected to Sequence match analysis using Basic Local Alignment Search Tool (BLAST) on NCBI.

## 2.5 Nutritional Assay

Biochemical analysis such as lipid content, protein content and carbohydrate content were analysed.

### 1. Total lipid content

Total lipids of the fish samples were extracted according to the method by Folch *et al.,* [14] and the lipid content was determined. The results were expressed as g lipid/100 g of the sample.

### 2. Total protein content

Samples (100 mg) for protein analysis were digested in 1 N NaOH for 24 h at room temperature and quantified according to Bradford [15] using bovine serum albumin as the standard. The results were expressed as g protein/ 100 g of the sample.

### 3. Total carbohydrate content

Carbohydrate concentrations were determined by a phenol-sulfuric acid colorimetric method [16], which measures absorption at 490 nm.

## 3. Results

In this study, 7 different fish products were collected from different super markets in Vadodara. All the products were raw and frozen. The products collected gave only a general description on the package but with less information on taxonomic details. Species identity of these 7 seafood samples was determined using DNA barcoding. The DNA was extracted from all the 7 samples (Fig. 1). The mitochondrial partial COI gene was amplified and sequenced from all the samples except few products (Fig.2). DNA sequences of 650-750bp were obtained for most of the products. The sequence similarity values are in the range of 98-100% and confirmed /authenticated the species identity of seafood products.

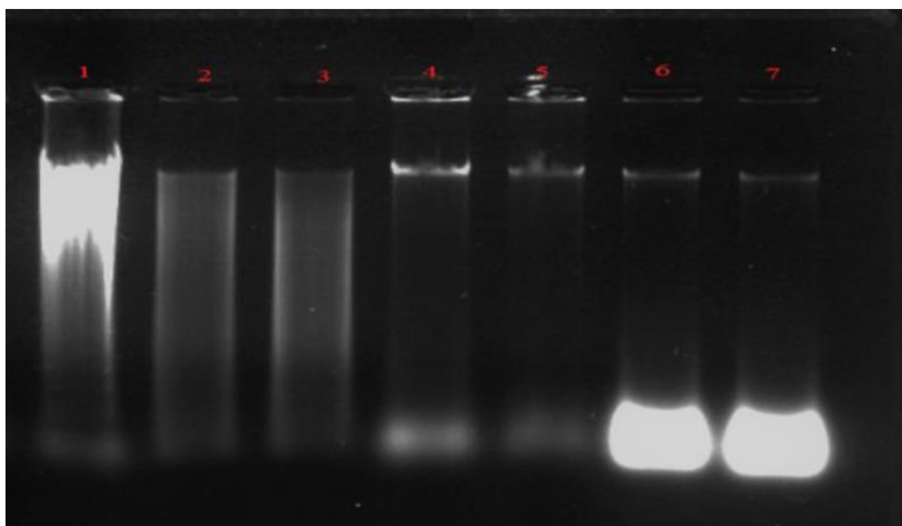


**Fig 1:** Gel Image of DNA Extraction (1) Atlantic salmon (2) Tilapia (3) Basa (4) Seer Fish (5) Silver Pomfret (6) Sardines (7) Indian Mackerel

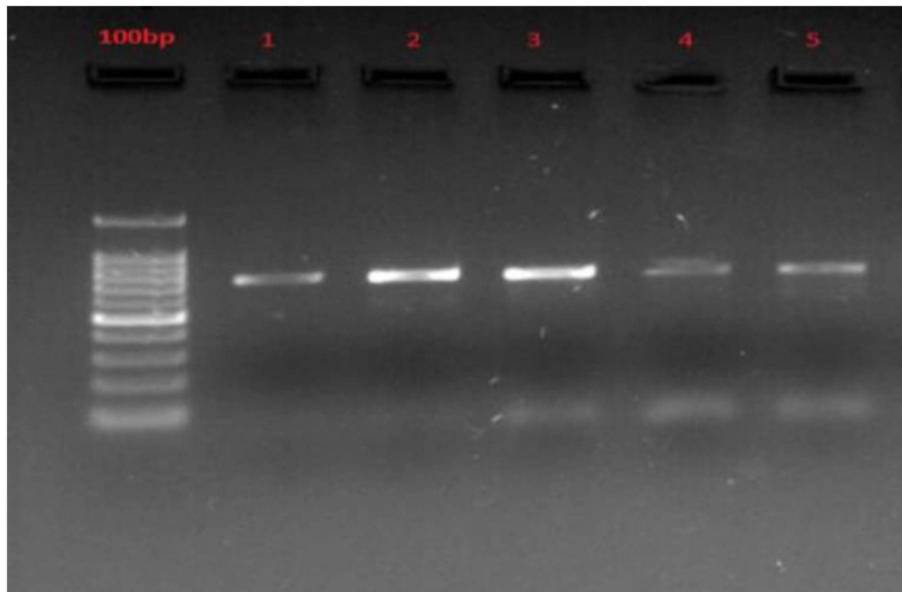


**Fig. 2** Gel Image of PCR Amplifications (1) Tilapia (2) Basa (3) Silver Pomfret (4) Sardine (5) Indian Mackerel

### 3.1 Sequence Analysis

These sequences were subjected to Sequence match analysis using Basic Local Alignment Search Tool (BLAST) on NCBI. Species identification results are mentioned in Table 1.

**Table 1:** Sequence analysis

| Sample No | Product sold as | Name provided on Packet | Species Identification (%) Similarity | Bold Accession No |
|---|---|---|---|---|
| 1 | Basa | Pangasius | Pangasinodon hypophthalmus (98%) | MSUAN003-17 |
| 2 | Sardine | Sardine | Sardinella longiceps (96%) | MSUAN004-17 |

### 3.2 Nutritional Assay

The nutrition Value of the fresh fishes was taken from the National Institute of Nutrition, Hyderabad [17]. The nutrition value provided on the packet of frozen fishes was compared to the value of fresh fishes with increasing time. The change in total lipid content (Fig. 3), protein (Fig. 4) and carbohydrates (Fig. 5) are taken in account.

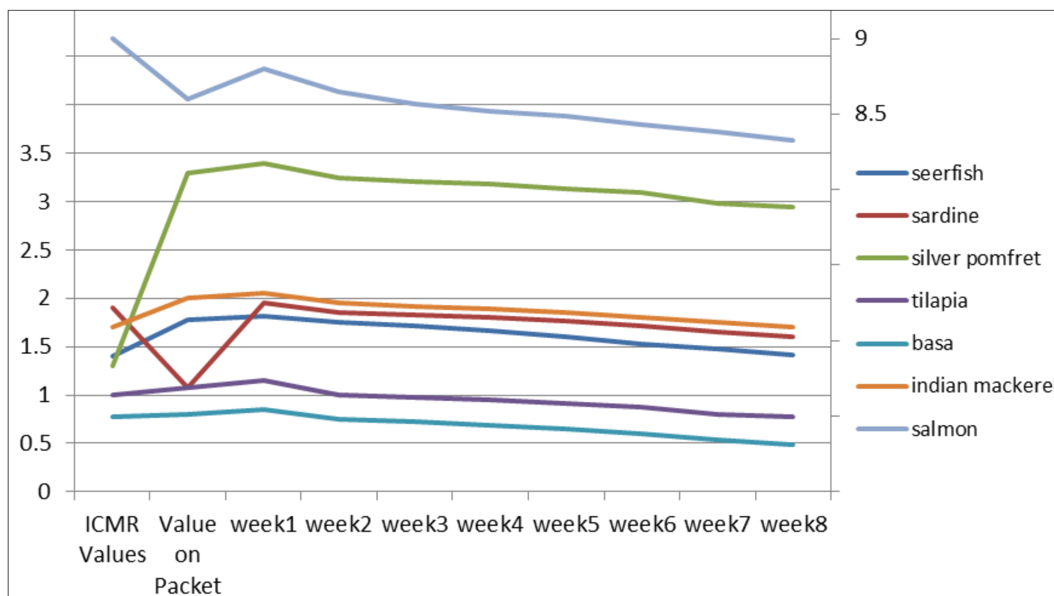


**Fig. 3** Graph showing Lipid content of the frozen fishes

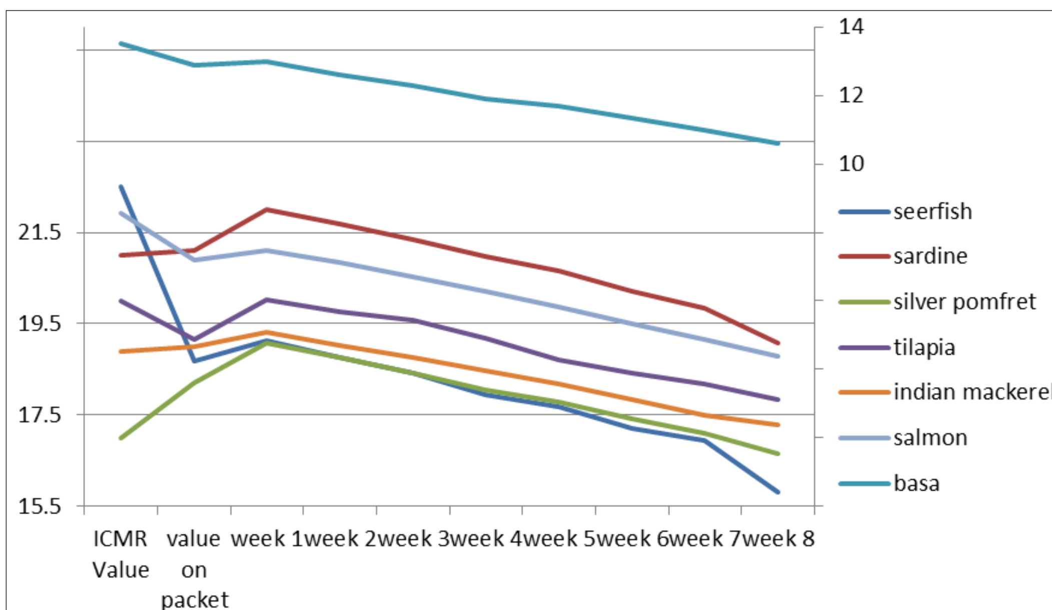


**Fig 4:** Graph showing Protein content of the frozen fishes

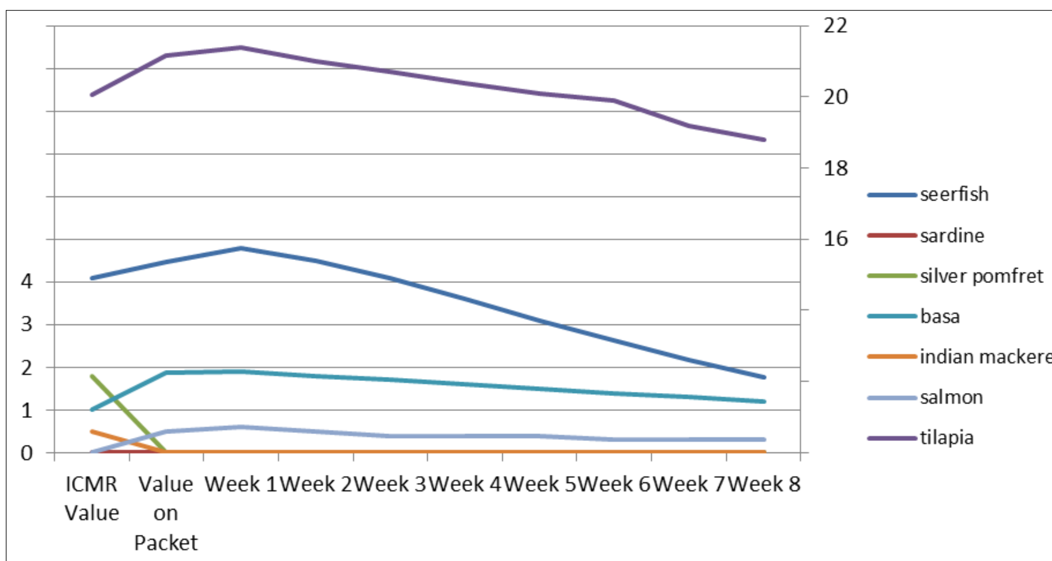


**Fig 5:** Graph showing Carbohydrate content of the frozen fishes

## 4. Discussions

The global trade and the increased demand for seafood products have encouraged the common practice of replacement of valuable species with species of lower value worldwide [18]. Species identification in food has increasingly acquired importance due to public health, economic and legal concerns [19]. The use of DNA barcoding to detect mislabelling of seafood products has revealed a number of substitutions [20]. Molecular identification techniques are needed to ensure proper labelling of marketed fish, to facilitate the detection of fraud, prevent negative effects on the fishing industry and, in particular, ensure consumer rights [21]. Species identification based on the level of COI sequence similarity with reference database sequences revealed that 22% seafood products were mislabelled in which 7% mislabelling is found in frozen seafood [22]. The level of seafood mislabelling is relatively higher in North America (25%) [20], Brazil (24%) [23], South Africa (31%) [24], Italy (32%) [25], but less in UK (5.66%) [26]. The high rate of incorrect labeling (48.5%), which, in 2 cases, was associated with health issues due to the presence of toxic fish species belonging to the Tetraodontidae family [27]. The price difference between species labelled on products and species identified by DNA barcoding revealed a difference of around $2-3/lb and it is in accordance of previous studies on seafood mislabelling, which also reported similar kind of economic deception in several countries [20, 25, 28]. The objective of this study is to explore the strategies available to detect the economically and criminally occurring mislabelling of food. The result obtained through barcoding of the samples shows no mislabelling. The name given on the product and the species identified through barcode show 95-99% similarity.

The consumption of fish containing valuable nutrients has recently increased [29, 30]. As the environment affects the fish in negative way, it must be consumed immediately after fishing

or must be stored under suitable conditions to reach consumers with its nutritional value preserved [31]. Longer shelf life and better quality can be made possible by using different processing techniques such as freezing [32]. The use of freezing for food preservation has rapidly developed; the fact that products, mainly fish, could be stored for considerable period and served after thawing, as fresh products are at the origin of its development as one of the most common methods of food preservation [33]. The preservation of food with reduced temperature and moisture levels appear to be less susceptible to microbiological deterioration and also often have enhanced chemical storage stability [34]. The frozen storage and subsequent defrosting of high fat content sardine caused changes in the structure and composition of the amino acids in the fish, affecting its nutritional quality [35]. Fish quality decreases during frozen storage as a result of increasing time and temperature of storage [36]. There is no significant difference in nutritional value of punti species *(Puntius sophore, Puntius sarana* and *Puntius gonionotus)* in frozen condition after twenty days [37].

In this present study there is also gradual change in nutritional quality with increasing time. The Total lipid content of Atlantic salmon, Tilapia, Basa, Seer fish, Silver Pomfret, Sardine, Indian mackerel provided on the packet was 8.60, 1.08, 0.80, 1.78, 3.30, 1.90, 2.0 gram/100g respectively, which is equivalent to the total lipid content of fresh one i.e. 9.0, 1.20, 1.0, 1.40, 3.10, 1.90, 1.70 gram/100g sample respectively. Within time period of 8 weeks there is only slight change in the total lipid content of frozen fishes i.e. 0.27, 0.31, 0.31, 0.36, 0.37, 0.30, 0.30 gram/100g sample. The protein content of Atlantic salmon, Tilapia, Basa, Seer fish, Silver Pomfret, Sardine, Indian mackerel provided on the packet was 20.90, 19.15, 12.88, 18.68, 18.20, 21.0, 19.0 gram/100g sample respectively, which is equivalent to the protein content of fresh samples i.e 22.0, 20.0, 13.15, 22.50, 17.0, 22.0, 18.9 gram/100g respectively. After 8 weeks there is only slight change in the protein content of these frozen fishes i.e 2.11, 1.31, 2.27, 2.86, 1.54, 1.91, 1.72 gram/100g sample. Silver Pomfret, Sardine, Indian Mackerel do not contain carbohydrate. The Total carbohydrate content of Atlantic salmon, Tilapia, Basa, Seer fish, Silver provided on the packet was 0.5, 21.17, 1.87, 4.48 gram/100g respectively, which is equivalent to the total carbohydrate content of fresh samples, i.e 0.0, 22.0, 2.0, 5.0 gram/100g respectively. After 8 weeks there is slight change in its content i.e. 0.2, 2.37, 0.67, 2.71 gram/100g.

## 5. Conclusions

We have not found any mislabelling in present work however, we do not rule out possibility of mislabelling. For such confirmation more variety of products required to be tested. We also conclude that one may consume the fishes after preservation. But we should try to consume the fish in fresh condition as early as possible as quality degradation is certainly there if kept for long period even as persevered material.

## 6. Acknowledgement

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