Detection of Bacterial Contamination of Some Traditional Frozen Fish in Some Local Markets in Tikrit City

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Abstract

This study was aimed to investigate bacterial contamination in frozen fish form samples collected from some local markets in several neighborhoods of Tikrit city. Thirty samples of frozen fish were collected for two types of fish common carp and fish fillet, laboratory bacterial isolation and diagnosis showed *Staph. aurues* samples constitute highest proportion of isolations which followed by *Aeromonaus hydrophyla*, *E.coli*, *Psudomonas. Spp* and *Salmonella* (7, 6, 5 and 3) isolations. Bacterial number of isolations has been calculated where it showed a variant in number of bacterial colonies, *Staph. aurues* which recorded the highest rate in colonies number. Chemical analyses to estimate the amount of protein and fat showed 9.43, 7.31% of protein and 3.9, 8.66 % fat , so as to moisture was high in both carp and elephant fish 80.5, 75.72% and ash 2.31, 7% in carp and fish fillet, respectively. The results of current study shows there are an inverse relationship between both used fish in study and the moisture in the fish.

Keywords: Fish, Markets, Bacterial, Frozen.

1. Introduction

The preservation of marine food by freezing is currently an important part of storage in most developed countries and it is considered as one of the most adequate ways to preserve and distribute products as it prevents perishable due to microbial. High rates of perishable frozen products is due to poor quality before freezing, dehydration and oxidation of fat that occurs during storage or misuse [1]. Eating frozen and imported fish is a major risk, especially when they are repeatedly frozen during the marketing phase, when the ice melts from the frozen fish which it provides a suitable media for the growth of microbes. Many bacteria are active after several days of freezing and are harmful and pathogenic, leading to the decomposition and rotten of fish meat that becomes unsuitable for human consumption [1]

Toxins are concentrated in fish tissue, especially known as the histamine fish poising, which is caused by un perfect freezing of fish, throughout insufficient temperatures and time, that it provides an opportunity of some bacterial species such as *E. coli* and *Klebeisella spp* that are basically normal flora within fish tissues. It contains the enzyme histidine carboxylase, which converts the histidine normally situated in tissues into histamine, when histamine level reaches between 20-50 mg / 100 mg it becomes within the limits of toxicity. Several cases of histamine poisoning were recorded when eating frozen fish [3-10].

2. Materials and Methods

2.1. Samples collecting

Two types of frozen fish were used in current study (carp fish and fish fillet), which is found in the local markets of Tikrit city within Salah al-Din province and stocked for long periods, 30 samples were used at the rate of 15 samples per species, under sterile and cooled conditions to collected samples are brought to the laboratory for testing and analyses for the experiment.
2.2 Preparation of media

Simple media for culturing such as solid nutrient agar, liquid and differential media such as MacConkey agar and manitol salt agar were used and amid the isolation of salmonella and the media were prepared according to the instructions of the equipped company.

2.3 Bacterial diagnosis

To detect bacterial contamination adopted the method mentioned in [6]. After bringing the samples to the laboratory they were prepared at a weight of 40 grams of each fish and from different parts of the body where 20 grams of them were placed in the center of the liquid media (nutrient broth). The other 20 grams was placed in the middle of a liquid media containing 225 ml of Lactose broth, then the decimal dilution was made for each sample of fish in nutrient broth 10⁻², 10⁻³, 10⁻⁴. The sample cultured in the lactose broth agar was incubate at a of 37°C m for 48 hours for salmonella isolating after its cultured on the special media.

2.4 Salmonella diagnosis

The method (6) was adopted by placing 20 g of fish in 225 ml of liquid medium lactose broth at a concentration of 1% and the naturalization process was carried out using a stomacher device and then incubated for 48 hours at a temperature of 37°C m. The bacterial culture was performed on the slant broth and tetraethionate with the addition of 100 ml of brilliant green dye, another sample cultured on XLD and Bismuth sulphate media.

2.5 Counting bacteria

The culturing and bacterial counting were performed according to method [11] using petri dishes in casting method by placing one ml of diluted solution from the sample processed in separate petri dishes containing 15 ml (Nutrient agar), (MacConkey agar), (Manitol salt agar) and (Salmonella agar), then shake the sample well by moving the dish quietly and leave to harden and incubate inverted at 37°C temperature for 24 hours. The formed colonies were calculated and the bacterial number extracted for each bacterial isolation detected per one gram of fish by multiplying the number of colonies in the inverted dilution used.

2.6 Chemical analysis

The methods described by the American Organization for Chemical Analysis [6] were followed to estimate the proportion of protein, fat, moisture and ash.

3. Results and Discussion

The results of this study showed different types of bacteria in table 1 of samples of frozen fish, so the bacterial isolations was Staph. aureus with 9 isolations, E. coli with 6 isolations, Aeromonas hydrophyla with 7 isolations, pseudomonas spp, with 5 isolations and Salmonella with 3 isolations. These isolations were in varying proportions according to type of fish which studied where it formed a bacterium was higher in the number of carp than in the fish fillet. Staph. aureus bacteria were the highest in carp, whereas, Psudomonas spp bacteria were the lowest in elephant fish. These results were similar to the study conducted [12].

| Bacteria             | Total number of isolations | Isolations in carp fish | Bacterial count | Isolations in fish fillet fish | Bacterial count |
|----------------------|----------------------------|-------------------------|----------------|------------------|----------------|
| S. aureus            | 9                          | 6                       | 6×10⁵          | 3                | 2×10⁷          |
| Aeromonas hydrophyla | 7                          | 5                       | 4×10⁴          | 2                | 2×10⁵          |
| Psudomonas spp.      | 5                          | 4                       | 2.5×10⁷        | 1                | 1×10⁵          |
| E. coli              | 6                          | 4                       | 3.5×10⁴        | 2                | 1×10⁵          |
| Salmonella sp.       | 3                          | 3                       | 2×10⁷          | -                | -              |

The same table also shows the rates of the different types of microbes in carp fish and fish fillet. It noted increase in the rate of bacteria S.aureus and Aeromonas hydrophyla, due to un suitable high temperatures in some central markets, they do not follow the method of storage and marketing fish using special cooling methods. Hence, these conditions help to grow
microbes, which contribute to the melting and warming of the outer surface, thus providing an opportunity for the growth of various microbes and causing perishable and unfit for human consumption.

Salmonella bacteria were isolated from three samples and diagnosed on the basis of its identification and chemical tests after the samples were cultured on the selenite as an enrichment media of Salmonella bacteria. Small and circular colonies with a dull yellow smooth edge were appeared unable on lactose fermentation and unable to ferment the sugar of zaylos when cultured on the XLD media [3]. All samples did not conform to Iraqi specifications [13].

| Kid of fish    | Protein% | Fat % | Moisture % | Ash % |
|---------------|----------|-------|------------|-------|
| Carp fish     | 9.43     | 3.9   | 80.5       | 8.66  |
| Fish fillet   | 7.31     | 2.31  | 75.72      | 7     |

Table 2 shows the percentages of chemical composition of fish species available in the study.

In study [16,17] showed that all fish under the conditions of slow freezing will lead to an expansion of the size of the snow crystals, which about melting will drift with a number of dissolved elements in the water and thus lead to a decrease in the nutritional value of those fish. And also [18] revealed that the process of dissolving meat is affected by atmospheric temperature or may be due to high fat content as the relationship between fat and moisture is reversible. The heat of the melting leads to a decrease in the humidity and protein and otherwise the proportion of both fat and ash increased due to the reverse relationship of both fat and moisture. The fat and ash ratio in the carp was 3.9, 8.66 respectively, and in the fish fillet it was 2.31, 7%, respectively.

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