Investigation of Some Microbiological and Chemical Parameters Associated with Spoilage of Cod Fish

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Abstract: The objective of this study was to look into the microbiological and chemical variations of cod fish. Samples were collected from raw material on a processing line and also from whole fish prior to mincing. Measurements on microbial load (Pseudomonas, Achromobacter, Flavobacterium, Coryneform and Micrococcus) were performed and also change in micro-organism and enzyme growth by temperature was measured. It is important to decrease the temperature to 0°C as soon as possible after catching. For fish in the tropical water area whereas the ambient temperature is around 25 – 30°C the rate of spoilage can be 25 times higher than when kept at 0°C.

Key words: Cod fish %Food microbiology %Chemical and mechanical damages

INTRODUCTION

Maintaining good quality of fish raw material for processing is very important issue. Therefore, the reasons for quality deterioration leading to spoilage need to be carefully determined. Just after death, fish can be soft for a few hours, then it becomes stiff. This phenomenon is called “rigor mortis”. The fish stays in the “rigor mortis” condition for a while, but then its flesh muscles become relaxed again. At that time the fish quality starts to decrease. The quality changes can easily be noticed and consist of changes in colour, odour, taste, appearance and texture and are therefore called sensory changes. One of the differences between fish appearance before and after rigor mortis is that the fish muscle is more elastic before rigor mortis. The time of pre-rigor mortis and rigor mortis varies according to species. It also depends on many things like temperature, handling, size and physical condition of the fish. Generally, it is preferred to extend the time before and during rigor mortis. There are some reasons for deterioration of quality and spoilage; they are autolysis, bacteria spoilage, rancidity and mechanical damage. Lowering the temperature by icing not only slows down the rigor mortis process, but also reduces the spoilage rate. Therefore maintaining low temperature during the handling and preservation process is very important [1 - 3].

The current article will throw light on some parameters associated with spoilage of fish.

Autolysis: The autolysis process relates to enzyme activities in fish (autolysis means self-digestion). Commonly the spoilage due to autolysis occurs first and is followed by spoilage due to bacteria and rancidity, but sometimes they overlap [4-6]. Unlike most fish, autolysis occurs very quickly in some shellfish like lobster and shrimp [7]. When the fish dies adenosine-triphosphate (ATP), which is the energy-rich organic compound in its muscle, will mostly be synthesized from glycogen, but also from creatine-phosphate (for finfish) and from arginine-phosphate (for cephalopods) under anaerobic conditions. The glycolysis (glycogen reduction process) still occurs continuously to create the end product of lactic acid. Because the end product of this process is lactic acid, the pH of the muscle will decrease. The ATP concentration gradually decreases and when it goes below 1 µmol/g in the muscle tissue the enzyme ATP-ase is activated. This leads to the stiffing of the muscle which will be constant (rigor mortis). The ATP is gradually degraded during time to some degraded products e.g. adenosine diphosphat, adenosine monophosphat, inosin monophosphat, inosin and hypoxanthin. Hypoxanthin is considered to cause the off-flavour in
Table 1: The relative change in abundance of different groups of bacteria in cod stored in ice

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>0 day (%)</th>
<th>5 days (%)</th>
<th>10 days (%)</th>
<th>15 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas</td>
<td>14</td>
<td>17</td>
<td>50</td>
<td>82</td>
</tr>
<tr>
<td>Achromobacter</td>
<td>33</td>
<td>49</td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Coryneform</td>
<td>41</td>
<td>33</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Micrococcus</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Spoiled fish. When the fish raw material is handled carelessly cells may be broken, which leads to the release of autolytic enzymes and this leads to the production of some spoilage substances. These substances create a very good environment for micro-organisms. Cathepsin, chymotrypsin, trypsin, carboxypeptidase, calpain, collagenase and TMAO-demethylase are all autolytic enzymes. Therefore, in order to maintain fish quality, enzyme activities should be prevented. Using low temperature is the most frequently used measure to limit enzyme activities [8-9].

**Bacteria:** Bacteria are capable of causing spoilage because of two important characteristics. First they are psychrotrophic and thus multiply at refrigeration temperatures. Secondly they attach various substances in the fish tissue to produce compounds associated with off-flavours and off odours. When the fish is alive the bacteria are found on the gill and skin and in the intestines, but can not attack the fish muscle. But, when the fish dies the bacteria can penetrate into the flesh muscle of the fish. When fish is preserved by icing the rate of bacterial penetration into the flesh muscle is much slower. Fish spoilage occurs when the enzyme of bacteria diffuses into the flesh muscle and the nutrition substances from the flesh muscle diffuse to the outside.

Spoilage will happen more rapidly for fish species with a thin skin layer. The number of bacteria in fish caught in temperate waters can develop even when in ice but the bacteria caught in tropical water grow slowly for one or two weeks in icing preservation [6].

There are many bacteria species present in spoiling fish, but there are only certain types that are considered to cause spoilage. The bacteria use their enzyme to change fish odour and flavour to sour, gassy, fruity and finally ammonia and faecal odour appear. Bacteria can still develop during icing as indicated by Hobbs [7] (Table 1).

Not all the growing bacteria are involved in the spoilage process. There are just a few bacteria species that become predominant and are mainly responsible for spoilage. For example in gutted cod, chilled by ice the specific spoilage organism (SSO) is *Shewanella putrefaciens* and in packaged cod fillet it is *Photobacterium phosphoreum* [3]. If the fish is preserved by icing or in lack of air the amount of *Pseudomonas* and *Shewanella putrefaciens* bacteria is not very high but *Photobacterium phosphoreum* bacteria becomes quite high. After a certain time in ice in aerobic conditions the *Pseudomonas* and *Shewanella putrefaciens* bacteria will become the predominant bacteria. In general in low temperature (0-5°C), *Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas* spp., and *Pseudomonas* spp. cause spoilage but in higher temperature (15-30°C) other species like *Vibrionaceae, Enterobacteriaceae* and the positive Gram bacteria cause spoilage [6]. The bacteria produce a high amount of volatile compounds. These are trimethylamine, volatile sulfur compounds, aldehydes, ketones, esters, hypoxanthine as well as other low molecular weight compounds. The bacteria *S. putrefaciens* and some *Vibrionaceae* produce H2 phosphoreum do not produce significant amounts of H2S. The volatile sulphur compounds have a very bad odour so even minimal quantities are considered to affect quality. The low temperature is very important in preservation of raw material. Especially in the range of 0-25°C the temperature strongly affects the bacteria activity (Figure 1). At 0°C the bacteria grow very slowly. The typical spoilage bacteria like *Shewanella putrefaciens* develop 10 times less in comparison with growing at the optimal temperature. Raising the keeping temperature thus increases the spoilage rate rapidly. Therefore,
it is important to decrease the temperature to 0°C as soon as possible after catching. For fish in the tropical water area where the ambient temperature is around 25 – 30°C the rate of spoilage can be 25 times higher than when kept at 0°C.

**Rancidity:** Fat oxidation usually occurs after autolysis and bacterial spoilage. The lipid concentration in fish can contribute to the spoilage process in fish. The fats in fish are mainly unsaturated fatty acids that are easily oxidized by oxygen from the atmosphere. High temperature or exposure to light can increase the oxidation rate. For fatty fish preserved in ice, spoilage due to rancidity is mainly caused by oxidation. This produces a bad and unpleasant odour as well as a rancid taste. Fat fish species like herring, mackerel and salmon are mostly affected by rancidity. The lean fish fat content is about 0.1-0.9% and the fat fish fat content is higher than 0.9% [14].

**Mechanical Damage:** If the fish is broken by harsh handling, it will be subject to mechanical or physical damage and become bruised and defected in outside appearance. But it is more important that some small cells will break leaving the enzymes free to react with other substances. Mechanical damage gives good conditions for some enzymatic activities. Fish kept in thick layers in a box with ice can cause high pressure between the ice and fish causing cells to break. All careless handling of fish raw material can result in bruised fish. This also opens channels for the micro-organisms to enter the fish flesh and enables quicker spoilage of the fish. In general, in order to maintain the fish raw material quality after catching, some measures for handling and preservation are needed to prevent all the quality change processes mentioned above [4].

**Analysis Methods for Quality Evaluation:** The methods of assessing freshness can be divided to two groups: sensory methods and non-sensory methods, where non-sensory methods include microbiological, chemical and physical analysis. Sensory assessment is a direct measure, but the nonsensory methods are indirect measurements. They should be used in combination [5]. The disadvantage of the sensory method is that it is subjective depending on the person who evaluates and people (panellists) have to be trained for fish sensory evaluation. The non-sensory methods are biological, chemical, physical. Their disadvantage is complexity because they require laboratory equipment [10].

**Sensory Method:** Sensory evaluation is a systematic assessment of the odour, flavour, appearance and texture of food. The Quality Index Method (QIM) is a seafood freshness quality control system that was developed by European fisheries research institutes. It is considered to be a rapid and reliable method for assessing freshness. QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 3 defect points. QIM is a practical rating system whereas the defect points are recorded. The sum of scores for all the characteristics is the overall sensory score. QIM gives scores of zero for very fresh fish, while increasingly larger totals result as the fish deteriorates. The description of evaluation of each parameter is written in a guideline. When the score is 18 or more the fish is considered spoilage [11].

**Microbiological Methods:** There are a lot of microbiological methods to determine fish bacteria e.g. plate count, direct microscopic count, ATP measuring, but the plate count is a traditional and common method with some different media like plate count agar or iron agar. The iron agar medium can be used in order to isolate spoilage bacteria that produce H2S and form black colonies on the agar media. Black and white colonies are observed and counted respectively. The black ones are referred to as spoilage bacteria, while the totals (black + white) are referred to as the total count which is a common method to determine the total content of bacteria in seafood. The iron agar method can sometimes detect higher bacteria amounts than plate count agar [12].

**Chemical Methods:** Chemical methods to measure freshness quality have been considered to be objective methods and therefore superior (less variable) to methods involving sensory evaluation. During post mortem storage microbiological spoilage causes the formation of volatile bases, which can be determined to measure indirectly the freshness quality of such seafood. There are a few substances that are usually determined to evaluate fish raw material freshness, e.g. total volatile basic nitrogen (TVB-N), trimethylamine (TMA), ammonia, biogenic amines, ethanol and indol. The TVB-N remains constant for the first days of storage or increases slowly but it rises fast later in the spoilage process. Therefore TVB-N is a very good indicator of spoilage in fish but it can be used for measuring later stages of deterioration [13, 14].
In conclusion, the main problems in handling of fish are poor chilling; and thus a lot of fish are already unacceptable at landing. All the activities should be carried out more quickly, but carefully and tenderly to avoid crushing the fish; keep the fish always in low temperature conditions (around 0°C) and in hygienic conditions.

REFERENCES