CATALASE ACTIVITY AS AN INDEX OF MICROBIAL LOAD AND END-POINT COOKING TEMPERATURE OF FISH

FAHAD M.R. BIN JASASS and DANIEL Y.C. FUNG

Department of Animal Sciences and Industry
Kansas State University
Manhattan, KS 66506-1600

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ABSTRACT

Psychrotrrophs that are responsible for spoilage of many aerobically cold-stored foods are strongly catalase positive. This study was conducted to ascertain the feasibility of using the Pasteur pipette catalase test developed by Fung (1985) to estimate the microbial population in raw fish during aerobic cold storage and to determine the end-point cooking temperature of fish. Fish samples (cod fillets, catfish, halibut fillets, rainbow trout, and salmon fillets in three replications each) were stored at 7°C and monitored at 0, 6, 12, 24, 48, and 72 h and up to 7 days. Fish surface, fish juice, and fish meat samples were obtained by conventional microbiological methods and were serially diluted in 0.1% of peptone water to a final 1:100 dilution. To perform the catalase test 0.1 mL of the 1:10 or 1:100 dilution of the sample was placed into the wider portion of the heat-sealed Pasteur pipette followed by 0.1 mL of 3% H₂O₂. The liquid mixture then was shaken into the heat-sealed capillary portion. The pipette was then inverted and after 5 min, the lengths of the gas column and the liquid column were measured in mm; the gas column was expressed as a percentage of the total, which was considered as the percentage catalase activity (% CA). Viable cell counts (VCCs) of these samples also were made. As VCCs increased on fish surface, in fish juice, and in fish meat samples, concomitant increases occurred in % CA. The lowest level detect by this method was about 10⁴ CFU/cm², mL, or g. Catalase activities of 0.0-0.5% on fish surface, <0.3% in fish juice, and <1% in fish meat, corresponded to low total counts of 10²-3 CFU/cm², 10⁵ CFU/mL, and 10⁴-1 CFU/g, respectively. Catalase activities of 0.6-1.5% on fish surface, 4.3-8.2% in fish juice, and 2.3-3% in fish meat, corresponded to high total counts of 10⁴ CFU/cm², 10⁶ CFU/mL, and 10⁵-6 CFU/g, respectively. Catalase activities of 1.7-2.5% on fish surface, 8.3-12.4% in fish juice, and 5-6% in fish meat, indicated that fish was unacceptable for consumption. Fish samples were cooked at temperatures of 40°C, 50°C, 55°C, 60°C,

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65°C, 70°C, and 75°C to determine the end-point temperature. Each sample was heated at each temperature for: 36 s, 1 min, 2 min, 3 min, and 5 min in a temperature-controlled water bath. Samples that had reached an internal temperature of 65°C showed no catalase activity in the test tube method or very little activity in the capillary tube method and contained no live bacteria, indicating that fish was safe for consumption.

INTRODUCTION

Microorganisms in Food

The microflora in food are related to the raw material and the environment and some are a result of handling and processing (Jay 1992). These microorganisms can be beneficial or harmful from the public health standpoint (Fung 1986). The beneficial microorganisms cause fermentation to produce various foods such as wine, cheese, beer, vinegar, bread, and soybean products. Also, they can be eaten as mushrooms, yeasts, and single-cell proteins. Pathogenic microorganisms such as Escherichia coli 0157:H7, Clostridium botulinum, Salmonella spp., Staphylococcus spp., and Vibrio spp. and many others can be carried in food and, if consumed by susceptible persons, can induce infection, intoxication symptoms, and sometimes death.

Microorganisms can cause considerable spoilage in our food supplies. When oxygen tensions, moisture, acidic or basic conditions (pH), temperature, and nutrients are suitable, microorganisms grow in foods, metabolize food components, and produce undesirable substances such as organic acid, slime, odoriferous compounds, and pigments (Fung 1985). Rapid methods for detection of spoilage as well as pathogen were reviewed by Dziezak (1987) and Fung (1994).

No standard method exists for defining food spoilage. Fung et al. (1980) established a numerical guideline for fresh meat. With 10⁵ to 10⁷ CFU/cm² or g is considered to have a low bacteria count. When meat reaches 10⁷ to 10⁸ CFU/cm² or g, it is considered to have an intermediate count. When meat reaches 10⁸ to 10⁹ CFU/cm² or g, it is considered to have a high count. When meat reaches 10⁹ CFU/cm² or g, it is considered to have very high count. Meat will have odor when it reaches 5 × 10⁷ CFU/cm² or g. Slime will occur when meat reaches 10⁶ to 10⁹ CFU/cm² or g. The International Commission on Microbiological Specifications for Foods established guidelines for microbial limits in several foods (Foster et al. 1977). A maximum total count of < 1.0 × 10⁶ CFU/g, which is considered a good quantity, was recommended for raw frozen fish; however, > 1.0 × 10⁷ CFU/g is considered unacceptable (Foster et al. 1977).
Microorganisms in Fish

Classification of Fish. Fish are classified into three groups: Cephalaspidomorphi (jawless fish) such as lampreys and slime-eels; Chondrichthyes (cartilaginous fish) such as shark and rays; and Osteichthyes, which contains an enormous variety of species such as lungfish and all bony fish (Huss 1988). More than 21,500 different species of sea creatures occur in the world, and some of them are known as edible fish and shellfish. According to Shahidi and Botta (1994) 100 million metric tons of fish and marine invertebrates have been caught in the world every year. Processed food utilize 20%, while 30% are consumed, and the rest are rejected as waste.

Fish also can be classified as vertebrate finfish or invertebrate shellfish. The term vertebrate refers to fish that have bony skeletons including vertebrae. Fish with vertebrate are classified further on the basis of their fat content as lean or fat (Freeland-Graves and Peckham 1987). Lean fish have less than 5% fat in their edible flesh. Cod, haddock, halibut, whiting, perch, rockfish, flounder, and sole meet the qualification of low-fat fish. The percentage of fat in marine fish is dependent on the kinds and species. Four groupings relative to fat content are lean (< 2% fat): cod, haddock, pollock; low fat (2-4% fat): sole, halibut, redfish; medium fat (4-8% fat): most wild salmon; and high fat (8-20% fat): herring, mackerel, and many farmed salmon (Shahidi and Botta 1994). Figure 1 shows the classifications of fish. Rainbow, trout, salmon, canned Norwegian sardines, canned tuna in oil, and smelt are considered medium fat because they contain 5-15% fat (Freeland-Graves and Peckham 1987).

Total Count of Microorganisms in Fish. The bacterial floras in fish are influenced by the surrounding water and temperature. Fish caught in cold European water held many psychrophilic bacteria. These bacteria can grow at refrigeration temperature, and their activities shorten the shelf-life of fish in cold storage (Vaz-Pires et al. 1994). Huss (1988) studied microorganisms in the intestines and surface (skin and gill) of live fish and freshly caught fish. The initial flora of fish is dependent on the contamination level of water, handling, and area of catch (Huss 1988). Fish are covered with slime, which helps them to move easily in the water. This slime covering the fish skin contains many bacterial genera such as Pseudomonas, Acinetobacter, Moraxella, Alcaligenes, Micrococcus, Flavobacterium, Corynebacterium, Sarcina, Serratia, Vibrio, and Bacillus (Frazier and Westhoff 1988). The numbers of microorganisms range from 10^2 to 10^4 CFU/cm^2 on the skin, from 10^3 to 10^5 CFU/g in the gills, and from 10^4 to 10^6 CFU/g in intestines (Huss 1988; Kraft 1992; Ward and Hackney 1991). However, fish from clean cold water contain a lower number of microorganisms than fish from polluted areas or warm tropical water (Huss 1988). Tropical fish mainly harbor mesophilic bacteria such as Micrococcus, Bacillus, and coryneforms (Huss 1988;

Fish harvested from deep, clean, and cold water have fewer bacteria than those from shallow and muddy water. The microbial flora of freshly caught fish includes aerobic or facultatively anaerobic, psychrotrophic, and Gram-negative rods of genera *Pseudomonas*, *Alteromonas* (*Shewanella*), *Morasella*, *Acinetobacter*, *Flavobacterium*, *Cytophage*, and *Vibrio* (Huss 1988). Fish from fresh water carry all the flora found in saltwater fish plus some genera of *Aeromonas*, *Lactobacillus*, *Brevibacterium*, *Alcaligenes*, and *Streptococcus* (Frazier and Westhoff 1988).

During cold storage in ice, the microbial flora shifted to *Pseudomonas* (Shewan 1971). At the end of fish storage life (14 days), *Pseudomonas* and *Alteromonas*
(Shewanella) comprised approximately 80% of the total flora (Hall 1992; Shewan 1971). Shewanella putrefaciens, Pseudomonas, and Moraxella produce undesirable fruity odors and musty odors (Hall 1992).

**Contamination of Fish and Public Health Hazard.** Quality control in the food industry refers to all the procedures taken to ensure that the food can meet high standards. The target of quality control in the fish industry is to minimize contamination by microorganisms (Huss 1988).

*Pseudomonas, Flavobacterium, Cytophage, Acinetobacter, Moraxella, Aeromonas, Corynebacterium, Streptococcus, Klebsiella, Alcaligenes, Bacillus,* and *Micrococcus* are found commonly in water and may contaminate food (Birnbaum 1979). Pathogens such as *Salmonella, Vibrio parahaemolyticus,* and *E. coli* can be introduced to fish through contaminated water and cause public health hazards (Chichester and Graham 1973; Huss 1988).

Shelf-life increases to 15-20 days when fish are placed in ice, which retards microbial activities by lowering the temperature (Huss et al. 1992). However, contaminated ice can be another source of pathogens and microorganisms in fish. The ice that is used to preserve fish must be of a good bacteriological quality (Frazier and Westhoff 1988).

After being caught and handled, fish are exposed to additional contamination on board fishing vessels. Unhygienic practices, especially unwashed hands, clothes, equipment, decks, and storage facilities, can contaminate fish (Chichester and Graham 1973). To avoid contamination of fish, boats, decks, bins, containers, and equipment must be cleaned and sanitized (Frazier and Westhoff 1988). Evaluation of bacteriological quality of seafood was studied by Gram (1987, 1992).

**Foodborne Illness from Seafood.** The subject of microbial food poisoning was reviewed by Eley (1996). Potential pathogens such as *E. coli, S. aureus,* and *C. perfringens* have been isolated from seafood. Outbreaks of food poisoning have been related to mishandling and contamination of foods in food service areas or at home (Jay 1992). Pathogens found on seafood include *Vibrio* spp., *Klebsiella* spp., *Staphylococcus* spp., *Aeromonas* spp., *Listeria monocytogenes,* and viruses (Huss et al. 1992; Hobbs 1983). Refrigeration alone cannot prevent pathogens from growing in food. In addition, temperature abuse, improper storage temperature, and prolonged thawing can contribute to outbreaks of foodborne illness (Kraft 1992).

Fish that are caught from unpolluted water are free from most pathogens, except the possible occurrences of *Clostridium botulinum* and *Vibrio parahaemolyticus* (Huss 1988). *Clostridium botulinum* type E was found in some fish products, but only rarely. This organism can grow at 3°C and produce toxin in beef under anaerobic conditions (Kraft 1992). Other organisms that can grow at low temperatures and produce toxins are *Listeria monocytogenes,* *Yersinia*
enterocolitica, Escherichia coli, and Aeromonas hydrophilia (Kraft 1992). Because Salmonella, Staphylococcus aureus, Bacillus cereus, and Vibrio parahaemolyticus can grow at 5-12°C (Kraft 1992), refrigeration alone cannot prevent a potential health hazard from these pathogens.

Table 1 summarizes data from Centers for Disease Control (CDC) showing that seafood was responsible for 10.5% of all outbreaks and 3.6% of all cases of foodborne illness from 1978 to 1987 (Shahidi and Botta 1994). More recent CDC (1996) data indicated the occurrence of 146 outbreaks and 1372 cases of foodborne diseases from fish and 34 outbreaks and 542 cases from shellfish from 1988-1992.

<table>
<thead>
<tr>
<th>Category</th>
<th>Outbreaks</th>
<th>Cases</th>
<th>Outbreaks</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shellfish</td>
<td>13</td>
<td>137</td>
<td>40</td>
<td>476</td>
</tr>
<tr>
<td></td>
<td>118</td>
<td>3271</td>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>Fish</td>
<td>336</td>
<td>1548</td>
<td>29</td>
<td>227</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>203</td>
<td>3</td>
<td>61</td>
</tr>
<tr>
<td>Total</td>
<td>349</td>
<td>1685</td>
<td>69</td>
<td>703</td>
</tr>
<tr>
<td></td>
<td>134</td>
<td>3474</td>
<td>4</td>
<td>118</td>
</tr>
</tbody>
</table>

Source: Shahidi and Botta 1994

Spoilage of Fish

**Spoilage of Fish by Microorganisms.** Psychrotrophs are the bacteria that most commonly cause spoilage of meat, poultry, eggs, dairy products, and fish during cold storage. At the first stage of spoilage, the bacteria grow prodigiously on the surface (Kraft 1992). The growth of microorganisms depends on the intrinsic (pH, moisture content, oxidation-reduction potential, nutrient content, antimicrobial constituents, and biological structures) and extrinsic (temperature, relative humidity of environment, and presence and concentration of gases in the environment) parameters of foods (Jay 1992). Intrinsic parameters are less subject to manipulation, whereas extrinsic parameters are influenced greatly by food processing, storage, and transportation.
Microorganisms invade the vascular tissue of fish muscle or penetrate the skin (Huss 1988). Microorganisms also can penetrate the fish meat via collagen fibers during storage at temperature above 8°C (Huss 1988). Bacteria grow rapidly on the skin of dead fish and produce slime on the surface. However, bacterial penetration of muscle tissue occurs more slowly, and breakdown of the muscle structure causes spoilage. Bacteria can penetrate processed fish parts such as fillets more rapidly than an intact whole fish (Hall 1992).

The spoilage rate of fish depends on the species and type, the initial microbial flora, the location of the catch, the content of the catch, processing methods, and method of storage (Banwart 1979). Fish and seafood products typically are kept at low temperature (<10°C) and under aerobic conditions. Psychrotrophic bacteria are able to grow at commercially refrigeration temperatures. Psychrotrophs can be divided into two groups. The first group can grow at 0°C or less, with an optimal temperature of 25-30°C and a maximum temperature of 30-35°C. The second group grows at 7°C or less, regardless of optimal and maximal temperatures of the fish (Kraft 1992). Because fish are perishable, they must be chilled or stored at 0-4°C to decrease the actions of microorganisms and enzymes.

Psychrotrophs comprise many kinds of bacteria that are Gram-negative and Gram-positive; aerobes, anaerobes, or facultative organisms; spore formers and nonspore formers; motile and nonmotile; rods, cocci, or vibrio (Kraft 1992). Table 2 lists many bacteria that exhibit psychrotrophic characteristics (Jay 1992).

_Pseudomonas_, the most numerous psychrotroph, is a Gram-negative motile nonsporogenous rod that can utilize noncarbohydrate carbon compounds for energy and simple nitrogenous foods. Some species have proteolytic and lipolytic activities, grow rapidly, use oxidative products to produce slime on the surface, and grow and multiply at 3°C-10°C. Some species such as _P. fluorescens_ produce pigment in food and are resistant to many disinfectants and sanitizers that are used in the food industry (Frazier and Westhoff 1988; Banwart 1979).

_Aeromonas putrefaciens_ (known as _Shewanella putrefaciens_) is a major cause of fish spoilage at 0°C (Jørgensen and Huss 1989). It is capable of utilizing trimethylamine oxide (TMAO) and producing H₂S from cysteine. _Shewanella putrefaciens_ is Gram-negative, catalase positive, ornithine decarboxylase positive, and salt tolerant at 6%NaCl. The generation times for this organism are 8.4-17.9 h and 39-92 min at 0°C and 25°C, respectively. _Shewanella putrefaciens_ is the main spoiler in whole cod stored in ice and in chilled, vacuum-packed, cod fillets (Dalgaard _et al._ 1993). Its extensive production of H₂S cause an off-odor (Dalgaard 1995).

Shelf lives of cod were 11-12 days with storage at 0°C; 6-8 days at 0.5°C; 5-6 days at 3°C; 2-3 days at 8°C; and 20-30 h at 10°C (Huss 1988). Many bacteria that spoil fish are capable of growth at -3°C and show good growth at between 0°C-10°C (Jay 1992). Bacterial spoilage in fish can occur between 0°C-4°C; however, a temperature of -10°C will prevent spoilage (Shewan 1971). At -18°C, off-odor and
off-flavor did not appear in fish that was stored vacuum-packed for 126 days; however, at \(-7^\circ\text{C}\), the off-flavor and off-odor increased because of autolytic catabolism (Fletcher and Statham 1988). Vacuum packaging and modified atmosphere packaging can increase the shelf-life in beef, pork, and poultry by 1 month (Dalgaard 1995). In a modified atmosphere, Gram-negative aerobic bacteria are inhibited by the absence of \(O_2\) and the high concentration of \(CO_2\) (Dalgaard et al. 1993; Jay 1992). Fish fillets assessed by sensory analysis had shelf lives of 11 days when stored at \(-2^\circ\text{C}\); 7 days at 0.6°C; and 3 days at 5°C (Einarsson 1994).

Bacteria in cod stored in ice had a lag period of approximately 2-3 days, after which the log phase increase occurred until the 10th day when the total count was \(10^6\) CFU/cm² of skin or g in muscle (Shewan 1971). After 0.6 days storage in ice, cod had no marked spoilage. Even after 7-11 days, it showed no odor. After 11-14 days, it showed some sourness and a slightly sweet or fruity odor. After more than 14 days of storage, \(H_2S\) and other sulphide odors, fecal odors, and strong ammoniacal odors were evident (Shewan 1971).

**Biochemical Spoilage**

Table 3 shows the proximate composition of fillets from various fish species. Because fish undergo rapid autolysis by enzymes, they are more perishable than other meats (Frazier and Westhoff 1988). They contain many unsaturated fats, which are more susceptible to oxidation than saturated fats (Fennema 1996). Lipid oxidation in seafood, especially those products that contain a high percentage of lipids, decreases quality.

The first index of spoilage of fish is the presence of odors at the gills. Odors can be fishy, slate, musty, rancid, sour, ammoniacal, yeasty, fruity, sweet, acid, or putrid (Banwart 1979). Slime in fish consists of mucopolysaccharide components, free amino acids, TMAO, and piperidine derivatives (Jay 1992). As fish spoils, the trimethylamine (TMA), ammonia, histamine, hydrogen sulfide, and indole increase (Jay 1992).

Flavor and texture determine the sensory quality of fish (Shahidi and Botta 1994). The sensory quality of fish is reduced during storage by physicochemical changes that result in spoilage (Hall 1992). Autoxidation in fresh and frozen fish produce undesirable flavors. The quality of fish also will decrease when it is stored in ice over 6-7 days because of autolytic changes in flavor; however, the fish are still acceptable (Hall 1992). *Pseudomonas* spp. are capable of reducing the TMAO to TMA in fish, which causes an off-flavor and off-odor (Malle et al. 1986). The production of TMA can be caused by multiple bacterial dehydrogenases (Malle et al. 1986).

Skin color of fish varies among species too. Bright and shiny skin is a sign of good quality fish. After the fish dies, the skin color becomes dull and loses brightness. The eyes also are good indicators of fish quality. The eye on newly
TABLE 2.
SOME PSYCHROTROPHIC AND MESOPHILIC SPOILAGE GENERA AND PATHOGENIC BACTERIA FROM FOODS AT LOW TEMPERATURE

<table>
<thead>
<tr>
<th>Spoilage Types</th>
<th>Min Growth Temperature (°C)</th>
<th>Catalase Reaction</th>
<th>Pathogens</th>
<th>Min Growth Temperature (°C)</th>
<th>Catalase Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter</td>
<td>4</td>
<td>+</td>
<td>Aeromonas</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Aeromonas</td>
<td>5</td>
<td>+</td>
<td>Bacillus</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>A. caligenes</td>
<td>NS</td>
<td>+</td>
<td>Campylobacter</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>Alveimonas</td>
<td>NS</td>
<td>+</td>
<td>Citrobacter</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>Arrobacter</td>
<td>5</td>
<td>+</td>
<td>Citrobacter</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>Bacillus</td>
<td>NS</td>
<td>+</td>
<td>Escherichia</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>Brevibacterium</td>
<td>5</td>
<td>+</td>
<td>Klebsiella</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>Brochothrix</td>
<td>NS</td>
<td>+</td>
<td>Listeria</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Chromobacterium</td>
<td>2</td>
<td>+</td>
<td>Salmonella</td>
<td>5-10</td>
<td>+</td>
</tr>
<tr>
<td>Clostridium</td>
<td>NS</td>
<td>-</td>
<td>Sophyococcus</td>
<td>5-10</td>
<td>+</td>
</tr>
<tr>
<td>Escherichia</td>
<td>NS</td>
<td>+</td>
<td>Vibrio</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Flavobacterium</td>
<td>5</td>
<td>+</td>
<td>Yersinia</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>2</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucosnostoc</td>
<td>5</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbacterium</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Microoccus</td>
<td>NS</td>
<td>+</td>
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<td></td>
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</tr>
<tr>
<td>Moraxella</td>
<td>2</td>
<td>+</td>
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<tr>
<td>Pseudomonas</td>
<td>4</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serratia</td>
<td>4.5</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>NS</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthomonas</td>
<td>5</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS=Not Specific, + = Positive, - = Negative
Sources: Eley 1996; Kraft 1992; Juy 1992
caught fish is convex, the pupil is black, and the cornea is translucent. As time after death increases, the eye flattens and sometimes become concave, the pupil turns gray and sometimes even creamy brown, and the cornea becomes opaque and discolored (Dore 1991).

Discoloration can occur in fish because of microbial growth. For example, pink color in salted fish is due to the growth of Halobacterium or Halococcus. Pink color in oysters is related to yeast (Rhodotorula). Yellow and red colors in squid are related to Pseudomonas putida and Serratia marcescens, respectively. Table 4 shows defects of seafood caused by microbes.

Use of Catalase Test for Rapid Estimation of Bacteria in Fish

Function of Catalase Enzyme. In 1818, Thenard discovered that hydrogen peroxide in animal tissue was decomposed to water and molecular oxygen. Loew in 1901 named the enzyme that caused this catalase (Wang 1985). Catalase is a constituent enzyme of all animal organs, erythrocytes, all plants, and all aerobic microorganisms. The function of catalase is to protect the cell from toxic hydrogen peroxide. The mechanism for the decomposition of hydrogen peroxide by catalase was reported by Chance (1948).

Hydrogen peroxide is destroyed in the cell by either catalase or peroxidase. Equations 1 and 2 show that superoxide dismutase converts the superoxide radical to hydrogen peroxide and molecular oxygen. Then the catalase breaks down hydrogen peroxide into water and oxygen (Davis et al. 1990). At a low

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TABLE 3
PROXIMATE COMPOSITION OF THE FILLETS OF VARIOUS FISH SPECIES

<table>
<thead>
<tr>
<th>Species</th>
<th>Scientific Name</th>
<th>Water %</th>
<th>Lipids%</th>
<th>Protein%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue whiting</td>
<td>Micromesistius poutassous</td>
<td>79-80</td>
<td>1.9-3.0</td>
<td>13.8-15.9</td>
</tr>
<tr>
<td>Cod</td>
<td>Gadus morhua</td>
<td>78-83</td>
<td>0.1-0.9</td>
<td>15.0-19.0</td>
</tr>
<tr>
<td>Eel</td>
<td>Anguilla</td>
<td>60-71</td>
<td>8.0-31.0</td>
<td>14.4</td>
</tr>
<tr>
<td>Herring</td>
<td>Clupea harengus</td>
<td>60-80</td>
<td>0.4-22.0</td>
<td>16.0-19.0</td>
</tr>
<tr>
<td>Plaice</td>
<td>Pleuronectes platessa</td>
<td>81</td>
<td>1.1-3.6</td>
<td>15.7-17.8</td>
</tr>
<tr>
<td>Salmon</td>
<td>Salmo salar</td>
<td>67-77</td>
<td>0.3-14</td>
<td>21.5</td>
</tr>
<tr>
<td>Trout</td>
<td>Salmo trutta</td>
<td>70-79</td>
<td>1.2-10.8</td>
<td>18.3-19.1</td>
</tr>
<tr>
<td>Tuna</td>
<td>Thunnus sp.</td>
<td>71</td>
<td>4.1</td>
<td>25.2</td>
</tr>
</tbody>
</table>

Sources: Huss 1988
concentration of hydrogen peroxide in the cell, catalase works as peroxidase, which makes the hydrogen peroxide react with glutathione to produce water and glutathione disulfide (Davis et al. 1990; Scott and Eagleson 1988). Equations 3 and 4 explain the decomposition of hydrogen peroxide by peroxidase.

\[
\begin{align*}
\text{Superoxide dismutase} & \quad 2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \uparrow \\
\text{Catalase} & \quad \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \uparrow \\
\text{Glutathione peroxidase} & \quad \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSSG} + 2\text{HO}_2 \\
\text{Glutathione reductase} & \quad \text{GSSG} + \text{NADPH} + \text{H}^- \rightarrow 2\text{GSH} + \text{NADP}^+ \\
\end{align*}
\]

Note
NADPH: Nicotinamide adenine dinucleotide phosphate, reduced form
NADP+: Nicotinamide adenine dinucleotide phosphate, oxidized form

Hydrogen peroxide can react with catalase to produce two different compounds. Compound I has a green color that consists of a ferric iron peroxide complex with ionic bonds (Chance 1948). Under suitable conditions, compound I is converted to compound II, which consists of ferric iron peroxides with covalent bonds and has a light red color (Chance 1948). The red color will change to dark red when compound II reacts with hydrogen peroxide (Boyer et al. 1959). Equations 5, 6, and 7 explain the decomposition of hydrogen peroxide by catalase.

\[
\begin{align*}
\text{Cat (OH)\textsubscript{2}} + \text{H}_2\text{O}_2 & \rightarrow \text{Cat (OH)}\text{, (OOH) green (I)} \rightarrow \text{Cat (OH)}\text{, (OOH)}^- \text{ light red (II)} \\
\text{Cat (OH)\textsubscript{2}} \rightarrow \text{light red (II)} & \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{Cat (OH)}\text{, (OOH)}^- \text{ dark red} \\
\text{Cat (OH)}\text{, (OOH)}^- + \text{H}_2\text{O}_2 & \rightarrow \text{Cat (OH)}\text{, (OOH)}^- + \text{H}_2\text{O} + \text{O}_2 \\
\end{align*}
\]

Chemical Structure and the Effects of Temperature and pH on Catalase. Enzymes are protein molecules synthesized by living cells for catalysis reactions in cellular metabolism. They are vital for biological reactions. Each enzyme consists of more than 100 amino acid residues (Styren 1995). Catalase consists of a ferric heme protein containing four atoms of iron per molecule. The molecular weight for catalase is 220,000 Daltons. Catalase has very high activity; e.g. one molecule of catalase can decompose 44,000 molecules of peroxide per second (White et al. 1954). Catalase is inhibited by cyanide, phenols, azide, H$_2$O$_2$, alkali,
TABLE 4.
SOME DEFECTS OF SEAFOOD CAUSED BY MICROBES

<table>
<thead>
<tr>
<th>Product</th>
<th>Defect</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Fish</td>
<td>Off-odor</td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Alteromonas</em></td>
</tr>
<tr>
<td></td>
<td>Fruity odor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td></td>
<td>Ammoniacal odor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Alteromonas</em></td>
</tr>
<tr>
<td></td>
<td>II, S odor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Alteromonas</em></td>
</tr>
<tr>
<td>Salted fish</td>
<td>Pink color</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Halobacterium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Halococcus</em></td>
</tr>
<tr>
<td></td>
<td>Dun (red growth) color</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hemispora stellata</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Sporosarcina epizoom</em></td>
</tr>
<tr>
<td></td>
<td>Cheesy, putrefactive odor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Red halophilic bacteria</em></td>
</tr>
<tr>
<td>Crayfish</td>
<td>Sweet to foul odor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lactobacillus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Coryneforms</em></td>
</tr>
<tr>
<td>Oysters</td>
<td>Pink color</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Yeast (Rhodotorula)</em></td>
</tr>
<tr>
<td>Shrimp</td>
<td>Off-odor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em></td>
</tr>
<tr>
<td>Squid</td>
<td>Yellow discoloration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas putida</em></td>
</tr>
<tr>
<td></td>
<td>Red discoloration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Serratia narcescens</em></td>
</tr>
</tbody>
</table>

Sources: Banwart 1979

and urea. High concentrations of hydrogen peroxide reduce the enzyme's catalytic ability.

The rate of an enzymatic reaction will increase with increasing concentrations of the enzyme until it reach a maximum of reaction (Conn and Stumpf 1976; Stryer 1995). Maximum velocity means that all catalase sites are filled, and the reaction rate will not increase. Also, the rate of enzyme reaction is affected by temperature and pH. The reaction rate increases with increasing temperature until 45°C, the
reaction rate decreases above 45°C, and the functional properties of enzymes are
destroyed by enzyme protein denaturation above 55°C (Conn and Stumpf 1976).
At pH 3 to 10, catalase enzyme is stable, but at lower than 3 or higher than 10, the
function of catalase enzyme will decrease (Wong 1995).

Microorganisms that Produce Catalase. Bacteria are classified into two
groups according to catalase reactions. Bacteria are considered catalase positive
when bubbles are generated on the surface of colonies after application of 3%
hydrogen peroxide. Catalase-negative bacteria cannot generate bubbles after
application of 3% of hydrogen peroxide. Catalase is found in all aerobic bacteria
and some facultative bacteria; however, superoxide dismutase is found only in
some aerobic bacteria (Conn and Stumpf 1976). Therefore, catalase activity can
be used in estimating bacterial contamination of food products before and after
processing and in determining the cooking endpoint temperature of some foods.

Micrococcus luteus has the strongest catalase-producing ability. Staphylococcus aureus and Pseudomonas fluorescens are second strongest.
Escherichia coli and Bacillus cereus are next, and Salmonella spp. have the least
catalase activity (Wang and Fung 1986). Boisneau et al. (1990) isolated and
identified Pseudomonas spp. and Micrococcus spp. as the two most important
genera for the spoilage of refrigerated cod fillets. Both genera are very good
catalase producers.

Herbert and Pinsent (1947) reported that 100 g of pure dry Micrococcus
lysodeikticus contains 2 g of catalase. The weight of the bacteria was \(2.5 \times 10^{-11}\)
g, which included 20,000 enzyme molecules that could decompose an amount of
hydrogen peroxide equal to 47 times their weight. Aspergillus niger, Penicillium
vitalie, and Micrococcus lysodeikticus are used commercially to produce catalase
the enzyme is used in food processing when hydrogen peroxide is undesirable in
food products, also in cake baking, irradiation of food, and hydrogen peroxide
sterilization (Frazier and Westhoff 1983).

Methods of Determining Catalase Activity. Catalase activity has been
measured by titrimetric, spectrophotometric, and manometric methods; the
Catalasemeter with the Paper Disc System; and test tube and capillary tube
methods. Measuring the activity of catalase has been proposed previously for
a bacterial quality assessment of food (Charbonneau et al. 1975).

Titrimetric Method. A titrimetric method was developed by Von Euler and
Josephson in 1926 (Wang and Fung 1986). A known concentration of hydrogen
peroxide was mixed with phosphate buffer, and the mixture cooled to 0°C. Catalase
solution then was added to the solution. At 0, 3, 6, 9, and 12 min, 5 mL of the
mixture was removed. The reaction was stopped by adding 5 mL of 2 N sulphuric
acid. Catalase activity was calculated by the following formula;
Kat. F. Katalase fahigkeil (catalase activity) = \( K/W \)
\( K = (2.303/t) \log \frac{X_0}{X_t} \min^{-1} \) at 0C
K: catalase activity
\( t \): time
\( X_0 \): titration value in 0 time
\( X_t \): titration value in \( t \) time
W: W gram, dry weight of catalase
This is the best method to test pure or partially purified catalase.

**Spectrophotometric Method.** A spectrophotometric method was introduced by Chance and Herbert in 1950. The spectrophotometer measures catalase activity by reduction of light absorption at 230 to 250 nm. The decrease of light transmission is due to decomposition of hydrogen peroxide by catalase. The procedure consists of measuring the absorbance at 0, 10, 20, 30, 50, and 70 s for a mixture of 3 mL 1:500 hydrogen peroxide and 1 \( \mu \)L catalase solution (Wang and Fung 1986).

**Manometric Method.** The manometric method was introduced by George (1948) to measure the amount of oxygen evolved during the reaction between the catalase and hydrogen peroxide. This test can determine the velocity of the reaction.

**Catalaseometer with Paper Disc System.** The Catalaseometer with the Paper Disc System was developed by Bio-Engineering Group Ltd. (New Haven, CT). This instrument measures the flotation time of a paper disc containing catalase. A test tube filled with 5 mL \( \text{H}_2\text{O}_2 \) solution (3% v/v in water; ethylenediaminetetraacetic acid \( 10^{-6} \) M) is placed into the instrument, and a paper disc is impregnated with sample and then immediately dropped into the test tube (Boismenu et al. 1991). The paper disc drops to the bottom of the test tube by gravity. As the paper disc passes through a light beam, it triggers a timer to start timing the test. If the sample contains catalase, it will react with \( \text{H}_2\text{O}_2 \), and the gas bubbles formed will cause the disc to float. The time between when the paper disc activates the start signal as it drops into the tube and when the paper disc returns to the surface and stops the timer is the flotation time. The flotation time is inversely proportional to the amount of catalase activity in the paper disc, i.e. a short flotation time indicates high catalase activity, and a long flotation time indicates low catalase activity. The lowest level of detection with this method is \( 10^4 \) CFU/g.

**Test Tube Method.** A test tube method has been used to determine the rate of reaction between catalase and hydrogen peroxide. The procedure consists of putting the sample (solid, liquid) in the sterile test tube and then adding hydrogen peroxide. Result is recorded as strong, moderate, low, or no catalase activity in
samples. This method was used for measuring catalase activity in meat (Kustyawati 1991).

**Capillary Tube Method.** Fung (1985) designed a simple capillary tube method using a heat-sealed Pasteur pipette. Catalase and hydrogen peroxide are allowed to react in the inverted Pasteur pipette with the heat-sealed end facing upward. If catalase activity is high enough, gas will be generated in the capillary tube and trapped at the top. The amount of gas can be expressed as percentage of the total liquid column. The sensitivity of this method is about $10^4$ CFU/mL of catalase-producing culture. Details of this method will be discussed in the materials and methods section.

**Use of Catalase Activity for Evaluating End-point Cooking Temperature of Fish.** Ang et al. (1994) studied the end-point temperature of further processed poultry and used catalase activity to determine doneness. They found that when the product reached 71.5°C, no catalase activity was observed. This is a simple way to check doneness of meat products.

Cooking refers to all methods of heating such as boiling, broiling, grilling, baking, deep-frying, poaching, pan-frying, and steaming. Fish or seafood can be cooked by any one of these methods. Broiling or grilling the fish involves cooking quickly at higher temperatures 375°F (190°C). Fish are put over heat until the color changes to opaque. Baking is considered a dry method, where fish is put in the oven at 350°F (180°C) until the color becomes opaque. Deep fat frying is the most popular method for cooking fish. Fish is put in oil and cooked at 350°F (180°C) until the color changes to golden or brown. Poaching involves cooking fish in liquid, which holds in the flavor. The temperature of the liquid should be between 160-180°F (71-82°C). The American Home Economics Association (1993) discussed cooking temperature and reported that “an internal temperature of 145°F (60°C) insures the fish is safe to eat”.

The objective of cooking fish is to develop desirable flavor, aroma, appearance, and texture; soften connective tissue; and at the same time inactivate spoilage and harmful microorganisms and enzymes. Improper cooking temperature and time can reduce nutritional value and destroy vitamins of fish. Catalase activity of heat-treated seafood can provide information about its doneness as well as its safety for consumption.

**MATERIALS AND METHODS**

**Fish Samples Preparation**

**Sources.** All experiments were repeated three time. For each replication, new batches of cod fillets (from Pacific Ocean), catfish (from Mississippi), halibut fillets (from Alaska), rainbow trout (from Idaho), and salmon fillets (from
Washington) were purchased. Each batch consisted of 10 samples of cod fillets, halibut fillets, and salmon fillets or 10 whole trout and catfish. Each fish or fillet was put in a sterile plastic bag at the local retail store. The sample (4-5 lb.), well surrounded with crushed ice, was brought to the laboratory within 20-30 min of purchase. In the laboratory, the bags were given numbers and immediately refrigerated at 7C. For each replication of the experiment, one individual catfish and trout and one fillet of cod, halibut, and salmon were selected at random at 0 h, 6 h, 12 h, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, and 7 days of storage at 7C.

Fish Surface Samples. To obtain surface bacterial counts and catalase activity, a sterile template (4 cm x 4 cm) was put on the surface of the sample, and a cotton swab (Sterile Dacron Polyester Applicator, Hardwood Products Company, Gulford, ME) was used to swab three times in three directions. The swab was placed in a 10 mL tube of 0.1% sterile peptone water, the end of the swab was broken off, and the tube was capped and shaken five times. Figure 2 shows preparation of the surface sample for the measurement of the catalase activity and viable cell count.

Fish Juice Samples. To determine the bacterial count and catalase activity of fish juice for each sample, 80 g of fish was weighed aseptically. The sample was put in a sterile stomacher bag and rolled 20 times by a rolling pin. One mL of juice was taken from the bag filter and transferred to 9 mL of 0.1% sterile peptone water. At 4 days of storage, 0.1 mL of juice was taken from the bag filter and transferred to 9.9 mL of 0.1% sterile peptone water; at that time very little juice was available. Figure 3 shows preparation of the juice sample for measurement of catalase activity and viable cell count. The amount of juice extract was considered extract release volume (ERV) and was used to compare viable cell count and catalase activity similar to study of ERV for ground beef by Jay (1966).

Fish Meat Samples. To determine the bacterial count and catalase activity, 11 g of fish meat was weighed aseptically and put in a sterile stomacher bag followed by 99 mL of 0.1% sterile peptone water. The stomacher bag was put in the Stomacher (Tekmar, Cincinnati, OH), which massaged the meat with two paddles for 2 min to dislodge the microorganisms into the solution. One mL of solution was taken from the bag filter and transferred to 9 mL of 0.1% sterile peptone water. Figure 4 shows preparation of the meat sample for the measurement of catalase activity and viable cell count.

Heat Inactivation Study

Sample Preparation for Inactivation of Catalase During Cooking of Fish. Five tubes of 20 g each of catfish, cod, halibut, rainbow trout, and salmon were
Place sterile template on the fish surface

Sterile Template 4cm x 4cm

Swab three times in three direction, then place swab back into 10 ml of 0.1% sterile peptone water, break off the end of the swab, cap and shake the tube

Prepare serial dilutions of peptone water

FIG. 2. PREPARATION OF THE SURFACE SAMPLE FOR THE MEASUREMENT OF CATALASE ACTIVITY AND VISIBLE CELL COUNT

heated at each constant temperature of 40C, 50C, 55C, 60C, 65C, 70C, and 75C for 36 s, 1 min, 2 min, 3 min, and 5 min after come up time (usually 2 min). These tubes were placed in the Precision HOL 8 circulation water bath (Precision Scientific Group, Chicago, IL.). A microprocessor thermometer type JKT and a thermocouple model HH23 inserted in the meat center (Omega Engineering, Inc., Stamford, CT) were used to measure internal temperature of fish during heating.
Juice

Rolled 80 grams of fish in stomacher bag 20 with a rolling pin, pushing the meat toward the bag filter

Transfer 1 or 0.1 ml of juice from the stomacher bag filter to 9 or 9.9 ml of 0.1% sterile peptone water

Prepare serial dilutions of peptone water

![Diagram of serial dilutions](image)

**FIG. 3. PREPARATIONS OF THE JUICE SAMPLE FOR THE MEASUREMENT OF CATALASE ACTIVITY AND VIVABLE CELL COUNT**

When the meat reached the desired temperature, the timer was activated. The meat was allowed to continue cooking for a designated time interval (0, 36 s, 1 min, 2 min, 3 min, and 5 min). Then the tubes were moved to an ice bath for 15 min (Kustyawati 1991). After that, the catalase activity was measured by the test tube and capillary tube methods. Figure 5 shows preparation for inactivation of catalase during heating and measurement of catalase activity.
CATALASE ACTIVITY

Meat

By aseptic technique, weigh 11 g of meat and transfer it to a stomacher bag followed by 99 ml of 0.1% sterile peptone water.

Put stomacher bag in the Stomacher for 2 minutes to homogenize the sample.

Prepare serial dilutions of peptone water.

Transfer 0.1 ml of dilution 1:100 into the capillary tube followed by 0.1 ml of 3% hydrogen peroxide. Then shake the liquid into the capillary portion of the system, and invert the sealed Pasteur pipette so that gas development can be observed.

After 5 minutes, measure the amount of bubbles with the micrometer.

Enumerate total count by using the Spiral plate system.

Incubate plates at 32°C and count after 48h.

Prepare serial dilutions of peptone water for measurement of catalase activity by the capillary tube method and viable cell count.

FIG. 4. PREPARATION OF THE MEAT SAMPLE FOR THE MEASUREMENT OF CATALASE ACTIVITY AND VIVABLE CELL COUNT
Meat

By aseptic technique, weigh 20 g of meat and transfer to sterile test tube

Heat tubes of meat at 40°C, 50°C, 55°C, 60°C, 65°C, 70°C, and 75°C for 36 sec, 1 min, 2 min, 3 min, and 5 min

Cool tubes in ice bath for 15 minutes

Measure catalase activity by test tube method

Put 2 grams of heated meat in sterile test tube followed by 4 mL of 3% hydrogen peroxide; record the result after 2 minutes.

FIG. 5. PREPARATION FOR INACTIVATION OF CATALASE ACTIVITY DURING HEATING FISH AND MEASUREMENT OF CATALASE ACTIVITY BY TEST TUBE METHOD AND CAPILLARY TUBE METHOD

Catalase Test

Capillary Test Tube. To perform the catalase test on the fish surface and heated fish meat, 0.1 mL of the 1:10 dilution was used. For fish juice or raw fish meat, 0.1 mL of the 1:100 dilution was used. After 0.1 mL of the sample was placed into the wider portion of the heat-sealed Pasteur pipette, 0.1 mL of 3% H₂O₂
was added. The liquid mixture then was shaken into the heat-sealed capillary portion similar to shaking mercury in a thermometer. The pipette then was inverted. After 5 min, the lengths of the gas column and the liquid column were measured in mm (0.1 mm); the gas column was expressed as a percentage of the total and was considered the percentage catalase activity (% CA). Figure 6 shows the capillary tubes and measurement of the CA.
Test Tube Method. To determine the catalase activity with the test tube method, 2 grams of heated meat was transferred to a sterile test tube followed by 4 mL of 3% hydrogen peroxide (Kusyawati 1991). After 2 min, catalase activity was measured by using a semiquantitative scale illustrated in Fig. 7.

Viable Cell Count Methods

Spiral Plating. All viable cell count data for raw fish were obtained by the spiral plate method. After serial dilutions of 1:100, 1:1000, and 1:10000 were prepared (Fig. 2, 3, and 4), 0.05 mL of sample was distributed (by spiral plate) onto the surface of rotating 10-cm agar plates beginning at the center and moving outward. The plates were incubated at 32°C for 48 h and counted manually. Manual counting was performed by placing plates on the spiral grid system and counting the number of colonies in various sections, then dividing the number of colonies by the volume of sample deposited in that particular section and multiplying by the dilution factor. This number was expressed as CFU/cm², mL, or g.

Record a after 2 minutes the results was

- = No catalase activity, + = Slight catalase activity, ++ = Moderate catalase activity, +++ = Strong catalase activity, ++++ = Very strong catalase activity

FIG. 7. TEST TUBE SEMIQUANTITATIVE SCALE
(Kusyawanti 1991)
Standard Plate Count Method. Cooking data were obtained by the standard plate count method. The standard plate count also was used to determine the viable cell count in cooked meat. To perform the standard plate count, 1 mL of dilution medium (Fig. 5) was transferred to sterile petri dishes and sterile plate count agar was added. After plates solidified, they were incubated at 32°C for 48 h. Visible colonies were counted by a Quebec counter and multiplied by the dilution factor. This number was expressed as CFU/g.

Statistical Analysis

To evaluate variability of the analysis, r-square, correlation coefficient, F-value, and p-value were determined with SAS (1985). The regression was applied to the following variables: viable cell counts on surface, viable cell counts in juice, viable cell counts in meat, % of catalase activity on surface, % of catalase activity in juice, % of catalase activity in meat, and time.

The general linear model was used to analyze the data for cooking fish. The independent variables were: temperature with eight levels (control, 40, 50, 55, 60, 65, 70, and 75°C), and time with five treatments (control, 36 s, 1min, 2min, 3min, and 5min). The dependent variable was % of catalase activity in heated meat.

RESULTS AND DISCUSSION

The central theme of this research was that as fish spoils, concomitant increases of catalase activities occur on the surface of fish, in fish juice, and in fish meat. These activities result from a combination of bacterial catalase and nonbacterial catalase-like enzymes in fish. Because aerobically cold-stored fish is spoiled by psychrotrophs and most psychrotrophs such as Pseudomonas, Microoccus, and Staphylococcus are highly catalase positive, an increase of catalase activity indicates an increased chance of spoilage of the fish product. Catalase activity is very simple to measure and provides real-time data to predict spoilage potential of fish products.

Catalase Activity and Viable Cell Count of the Fish Surface

Figure 8 shows the relationships between viable cell counts and % catalase activities of the surface of cod fillets. Similar curves were obtained for catfish, halibut fillets, rainbow trout, and salmon fillets (data are available but not shown). They show a positive trend of increases in catalase activities and total counts on surfaces of all fish during 7 days of refrigeration at 7°C. Figure 9 presents the scatter diagram showing correlation coefficients for viable cell counts versus catalase activities of r = 0.90 for cod fillets. Correlation coefficients for catfish, halibut fillets, rainbow trout, and salmon fillets was r = 0.88, r = 0.94, r = 0.76, and r = 0.91, respectively. The data indicate that the microbial loads and catalase
activities increased concomitantly on the fish surfaces during cold storage.

On day 0, surfaces of cod fillets, catfish, halibut fillets, rainbow trout, and salmon fillets had initial total counts of $3.3 \times 10^3$, $9.3 \times 10^3$, $1.0 \times 10^4$, $2.3 \times 10^4$, and $4.4 \times 10^4$ CFU's/cm$^2$, respectively, and showed 0.00%, 0.32%, 0.42%, 0.14%, and 0.12% catalase activity, respectively. According to Gillespie and Macrae (1975), the total count in freshly caught fish ranged from $4 \times 10^3$ to $8 \times 10^4$ CFU's/cm$^2$. Banwart (1989) mentioned that the total counts in freshly caught fish skin ranged between $10^2$ and $10^4$ CFU's/cm$^2$ depending on species and environment. Thus, the initial bacterial counts on the fish surfaces in this study

![Graph showing viable cell count and catalase activity over time](image)

**FIG. 8. PERCENTAGE CATALASE ACTIVITY AND VIABLE CELL COUNT ON COD FILLET SURFACE DURING STORAGE FOR 7 DAYS AT 7°C**
were normal. Also, low catalase activities indicated that the fish samples were fresh and acceptable.

As storage times increased (from 0 h to 7 days), catalase activities and viable cell counts (VCC) on the surface increased accordingly. At 3 days of incubation, the catalase activities increased to 1.10% for cod fillets, to 2.79% for catfish, to 2.86% for halibut fillets, to 0.75% for rainbow trout, and to 1.35% for salmon fillets. At this stage, the bacterial loads were \(2.3 \times 10^5\), \(3 \times 10^4\), \(1.2 \times 10^4\), \(9.8 \times 10^5\), and \(6.8 \times 10^6\) CFU/cm\(^2\) for cod fillets, catfish, halibut fillets, rainbow trout, and salmon fillets, respectively. On last day of incubation, total bacterial counts had reached \(10^7\) CFU/cm\(^2\) for all the samples, and catalase activities had increased to 3.66-5.86%.

FIG 9. CORRELATION OF PERCENTAGE CATALASE ACTIVITY AND VIVABLE CELL COUNT ON COD FILLET SURFACE
The spoiled surfaces of cod fillets, catfish, halibut fillets, rainbow trout, and salmon fillets as determined by odor, had total counts of $2 \times 10^6$, $2.4 \times 10^7$, $1.1 \times 10^7$, $6.6 \times 10^6$, and $5.1 \times 10^5$ CFU/cm$^2$, respectively. These figures correspond to catalase activities of 1.1%, 2.7%, 2.4%, 1.3%, and 0.8%, respectively. After 3 days, the total count had reached $10^6$ CFU/cm$^2$, and an off-odor was noticed. According to Banwart (1989), fish are spoiled when the total counts on the skin reach $10^6$ CFU/cm$^2$. Slime appeared on the surface after 5 days of storage at 7°C, and the total count was $10^6$ CFU/cm$^2$.

Catalase activity as measured by the capillary tube method was able to estimate the microbial population on the surface of fish with a lowest detection level of $1 \times 10^4$ CFU/cm$^2$. Table 5 illustrates the estimation of total count by measuring the catalase activity on the fish surface. Thus, when the % CA is 0 to 0.5, the fish can be considered as fresh and acceptable. When % CA reaches 0.5-1.5%, the fish is nominally acceptable. A % CA of 1.8 and higher indicates that fish is not acceptable.

**Table 5.**

<table>
<thead>
<tr>
<th>% Catalase Activity</th>
<th>Total Count</th>
<th>Bacterial Range</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$10^5$ CFU/cm$^2$</td>
<td>Low</td>
<td>0</td>
</tr>
<tr>
<td>0.1-0.5</td>
<td>$10^4$ CFU/cm$^2$</td>
<td>Moderate</td>
<td>0</td>
</tr>
<tr>
<td>0.6-1.5</td>
<td>$10^6$ CFU/cm$^2$</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>1.8-2.5</td>
<td>$10^7$ CFU/cm$^2$</td>
<td>Very high</td>
<td>2</td>
</tr>
<tr>
<td>2.6-5.9</td>
<td>$10^8$ CFU/cm$^2$</td>
<td>Slime</td>
<td>3</td>
</tr>
</tbody>
</table>

0 = No off-odor, 1 = Very little off-odor, 2 = Strong off-odor, 3 = Very strong off-odor

Sources: Gram et al. 1987

**Viable Cell Count and Catalase Activity of Fish Juice**

Figure 10 shows the relationships between viable cell counts and catalase activities of juice from cod fillets. Similar curves were obtained for catfish, halibut fillets, rainbow trout, and salmon fillets (data are available but not shown). They show a positive trend of increases in catalase activities and total counts from
fish juice for up to 3 or 4 days of refrigeration at 7°C. Figure 11 shows the scatter plots and the correlation coefficients for viable cell counts versus catalase activities of \( r = 0.96 \) for cod fillets. Correlation coefficients for catfish, halibut fillets, rainbow trout, and salmon fillets was \( r = 0.86, \ r = 0.85, \ r = 0.98, \) and \( r = 0.87. \) Microbial loads and catalase activities increased concomitantly in fish juice during the first 3 or 4 days of storage.

On day 0, juices of cod fillet, catfish, halibut fillet, rainbow trout, and salmon fillet had an initial total counts of \( 3.1 \times 10^7, 3.9 \times 10^7, 4.3 \times 10^7, 8.7 \times 10^7, \) and \( 3 \times 10^7 \text{ CFU/mL}, \) respectively, and the catalase activities were 4.0%, 4.3%, 3.1%, 1.6%, and 6.3%, respectively.

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**FIG. 10. PERCENTAGE OF CATALASE ACTIVITY AND VYABLE CELL COUNT IN COD FILLET JUICE DURING STORAGE FOR 7 DAYS AT 7°C**
As storage time increased (from 0 h to 7 days), catalase activities and viable cell counts of the fish juice increased accordingly. At 3 days of incubation, catalase activities increased to 12.2% for cod fillets, to 11.0% for catfish, to 13.6% for halibut fillets, to 7.1% for trout, and to 15.8% for salmon fillets. Total bacterial counts were $1.4 \times 10^8$, $3.7 \times 10^8$, $2.1 \times 10^8$, $1.1 \times 10^8$, and $8.4 \times 10^7$, respectively. On the last day of incubation, the total count had reached $10^{8.9}$ CFU/mL for all the samples, and catalase activities had increased to 7.6-14.4%.

**FIG. 11. CORRELATION OF PERCENTAGE CATALASE ACTIVITY AND Viable CELL COUNT IN COD FILLET JUICE**
The reason for a decline of % CA in fish juice after 4 days was not clear but probably was a shift from catalase-positive bacterial population to catalase-negative population. According to Boismenu et al. (1991), *Micrococcus* (catalase positive) was replaced by streptococci (catalase negative) on fish samples after 3 days of incubation at 4C. Another reason may have been replacement of strong catalase-producing bacteria weak catalase-producing bacteria. Wang and Fung (1986) observed that different bacterial species produce different amounts of catalase activities. Other enzymes in fish tissue such as lipoygenase and cyclooxygenase may affect catalase activity.

### TABLE 6.
PERCENTAGE OF CATALASE ACTIVITY AND ESTIMATION OF TOTAL COUNT IN FISH JUICES

<table>
<thead>
<tr>
<th>% Catalase Activity</th>
<th>Total Count CFU/mL</th>
<th>Bacterial Range</th>
<th>Odor</th>
<th>ERV mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4.3 %</td>
<td>&lt;10^5</td>
<td>Low</td>
<td>-</td>
<td>4.5</td>
</tr>
<tr>
<td>4.3-6.3 %</td>
<td>10^6</td>
<td>Moderate</td>
<td>-</td>
<td>4.3-3.3</td>
</tr>
<tr>
<td>6.4-8.2</td>
<td>10^6</td>
<td>High</td>
<td>1</td>
<td>3.9-2.1</td>
</tr>
<tr>
<td>8.3-12.4</td>
<td>10^7</td>
<td>Very high</td>
<td>2</td>
<td>2.0-1.3</td>
</tr>
<tr>
<td>12-4-10.7</td>
<td>10^8</td>
<td>Slime</td>
<td>3</td>
<td>1.2-0.8</td>
</tr>
</tbody>
</table>

0 = No off-odor, 1 = Very little off-odor, 2 = Strong off-odor, 3 = Very strong off-odor
Sources: Gram et al. 1987

Spoiled juices of cod fillets, catfish, halibut fillets, rainbow trout, and salmon fillets, determined by odor, had total bacteria counts of 1 × 10^8, 2.5 × 10^8, 1.4 × 10^8, 6.6 × 10^7, and 6.6 × 10^7 CFU/mL, which corresponded to catalase activities of 12.2%, 11.1%, 13.6%, 7.1%, and 15.8%. The total counts at 7 days of storage reached 10^8-10^9 CFU/mL in all samples. An off-odor can be detected when the total count reaches 10^8 CFU/mL (Jørgensen and Huss 1989). Catalase activities also increased significantly. The lowest total count obtained in juice was 3-8 × 10^6 CFU/mL, which corresponded to catalase activities of 4.3-6.3%. Table 6 shows percentages of catalase activities and estimations of total counts in fish juice.

**The Extract-Release Volume (ERV)**

In the course of this study, we observed that the amount of juice extracted from 80 g of fish meat decreased as storage time increased. Data on ERV from beef
reported by Jay 1966, indicated a similar phenomenon. Beef released larger amount of juice when the microbial counts are low compared to meat which has higher total counts. After the beef was spoiled, it released low amounts of juice or none.

Figure 12 shows the relationships of ERV from 80 g of cod fillets. Similar curves were obtained for catfish meat, halibut fillets, rainbow trout, and salmon fillets (data not shown), with viable cell counts and catalase activities. As storage time increased (from 0 h to 7 days), catalase activities and viable cell counts in the meat increased, but volume of juice extracted from 80 g of meat decreased. From 0 to 3 days of incubation, the volume of extracted juice decreased from 3 mL to

![Graph showing relationships of extract-release volume (mL) of 80 g of cod fillet meat with percentage catalase activity and viable cell count.](image-url)
0.6 mL for cod fillets, 4.3 mL to 2 mL for catfish, 9.2 mL to 2 mL for halibut fillets, 3.2 mL to 1.5 mL for rainbow trout, and 3.0 mL to 1.9 mL for salmon fillets. On the last day of incubation, a low volume of juice (0.5-1.2 mL) was extracted from all samples of fish meat. A high ERV from fish meat indicated that the samples were fresh and had low microbial loads. Thus, ERV of fish can predict quality (Table 6).

**Viable Cell Count and Catalase Activity of Fish Meat**

Figure 13 shows the relationships between viable cell counts and catalase activities of meat from cod fillets. Similar curves were obtained from catfish,

![Graph showing the relationship between time and catalase activity.](image)

*FIG. 13. PERCENTAGE OF CATALASE ACTIVITY AND VIABLE CELL COUNT IN COD FILLET MEAT DURING STORAGE FOR 7 DAYS AT 7C*
halibut fillets, rainbow trout, and salmon fillets (data are available but not shown). They show a positive trend of increases in catalase activities and total counts on fish meat during 7 days of refrigeration at 7°C except for salmon fillets, which showed a decline after 4 days. Figure 14 presents scatter plots showing correlation coefficient for viable cell counts versus catalase activities of $r = 0.92$ for cod fillets. Correlation coefficients for catfish, halibut fillets, rainbow trout, and salmon fillets was $r = 0.93$, $r = 0.92$, $r = 0.94$, and $r = 0.85$, respectively.
At 0 time, viable cell counts on meat of cod fillets, catfish, halibut fillets, rainbow trout, and salmon fillets were $2.8 \times 10^4$, $2.8 \times 10^5$, $6.4 \times 10^4$, $1.1 \times 10^5$, and $7.2 \times 10^4$ CFU/g and catalase activities were 1.3, 2.6, 1.7, 1.2, and 4.1%, respectively. According to the International Commission on Microbiological Specification for Food (Foster et al. 1977), fish will be acceptable when the total count is less than $10^6$ CFU/g and will be rejected when the total count is higher than $10^9$ CFU/g. Thus, the initial counts on the fish meat in this study were normal. Also, low catalase activities indicated that the fish samples were fresh and acceptable.

As storage time increased (from 0 h to 7 days), catalase activities and viable cell counts on the fish meat generally increased. At 3 days of incubation, catalase activities increased to 6.6% for cod fillets, to 6.9% for catfish, to 5.2% for halibut fillets, to 4.2% for rainbow trout, and to 10.8% for rainbow trout. On the last day of incubation the total count had reached $10^6$-10$^9$ CFU/g for all the samples, and catalase activities had increased to 4.3-8.6%.

The spoiled meat of cod fillets, catfish, halibut fillets, salmon fillets, and rainbow trout, determined by odor, had total counts of $1.3 \times 10^7$, $3.4 \times 10^5$, $1.1 \times 10^7$, $1.2 \times 10^6$, and $2.2 \times 10^5$ CFU/g, respectively. These figures correspond to 6.6, 6.9, 6.2, 4.1, 10.8% catalase activities, respectively. After 3 days, off odor was noticed, and the total count had reached $10^6$-10$^7$ CFU/g. A slime on the meat was present after 5 days of storage at 7°C, and the total count was $10^9$ CFU/g. Table 7 shows percentages of catalase activities and the equivalent viable cell counts in fish meat.

**Table 7.**

<table>
<thead>
<tr>
<th>% Catalase Activity</th>
<th>Total Count</th>
<th>Bacterial Range</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2%</td>
<td>$10^4$ CFU/g</td>
<td>Low</td>
<td>0</td>
</tr>
<tr>
<td>2.3-3.6%</td>
<td>$10^5$ CFU/g</td>
<td>Moderate</td>
<td>0</td>
</tr>
<tr>
<td>3.7-5%</td>
<td>$10^6$ CFU/g</td>
<td>High</td>
<td>1</td>
</tr>
<tr>
<td>5-6%</td>
<td>$10^7$ CFU/g</td>
<td>Very high</td>
<td>2</td>
</tr>
<tr>
<td>6.0-7%</td>
<td>$10^8$ CFU/g</td>
<td>Slime</td>
<td>3</td>
</tr>
</tbody>
</table>

0 = No off-odors, 1 = Very little off-odor, 2 = Strong off-odors, 3 = Very strong off-odors

Sources: Gram et al. 1987

**End-Point Cooking Temperature in Fish**

Figure 15 shows heated inactivation of viable cell counts in cod fillets. Similar curves were obtained from catfish, halibut fillets, trout fillets, and salmon fillets (data not shown). The initial microbial populations in these fish were $3.9 \times 10^5$, 
$2.2 \times 10^6$, $2.7 \times 10^5$, $5.6 \times 10^4$, and $1.1 \times 10^4$ CFU/g, respectively. At 40°C, total counts were reduced by 1 log CFU/g for all samples and treatment times (36 s, 1, 2, 3, and 5 min). At 60°C, total counts were reduced by 3 logs for all samples and times. No viable cells were recovered when fish meats were heated at 65°C for 36 s, 1, 2, 3, or 5 min. According to Townsend and Blankenship (1988), the Food Safety Inspection Service (FSIS) recommended suitable temperatures and times for cooking meat. For example, the minimal internal temperature for beef is 54.4°C and should be held for 121 min, or until the internal beef temperature reaches 62.8°C; internal temperatures should be 71.1°C for poultry; and 68.3°C for smoked poultry.

Figure 16 shows heat inactivation of catalase activities in cod fillets. Similar curves were obtained for catfish, halibut fillets, trout fillets, and salmon fillets (data not shown). Catalase activities, using 1:10 dilution samples, were: 22% for cod, 32% for catfish, 25% for halibut, 33% for trout, and 27% for salmon. At 40°C, catalase activities were reduced by about half. The percentages of catalase activities remaining decreased as the temperature increased. At 60°C and above, no

![Graph](image_url)

**FIG. 15. TOTAL VIABLE CELL COUNTS IN COD FILLETS HEATED AT DIFFERENT TEMPERATURES FOR DIFFERENT TIMES**
FIG. 16. PERCENTAGES OF CATALASE ACTIVITY (MEASURED BY CAPILLARY TUBE) IN COD FILLETS HEATED AT DIFFERENT TEMPERATURES FOR DIFFERENT TIMES.

TABLE 9.
PERCENTAGE OF CATALASE ACTIVITY IN FISH COOKED AT DIFFERENT TEMPERATURES

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Catalase Activity by Capillary Tube Method</th>
<th>Catalase Activity by Test Tube Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>+++</td>
</tr>
<tr>
<td>40°C</td>
<td>11.3-17.9</td>
<td>+++</td>
</tr>
<tr>
<td>50°C</td>
<td>6.3-12.9</td>
<td>+++</td>
</tr>
<tr>
<td>55°C</td>
<td>2.4-9.9</td>
<td>++</td>
</tr>
<tr>
<td>60°C</td>
<td>1.0-4</td>
<td>-</td>
</tr>
<tr>
<td>65°C</td>
<td>0.4-0.8</td>
<td>-</td>
</tr>
<tr>
<td>70°C</td>
<td>0.0-0.4</td>
<td>-</td>
</tr>
<tr>
<td>75°C</td>
<td>0.0</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Indicate no catalase activity; ++ = Indicate slight catalase activity; +++ = Indicate moderate catalase activity; ++++ = Indicate strong catalase activity; +++++ = Indicate very strong catalase activity; Sources: Kastlawati 1991.
catalase activities were observed with the test tube method, but a low percent (0.8-1\%) of catalase activity was observed with the capillary tube method. When the internal fish temperature reached 65C, minor catalase activities (0.4-0.8\%) was observed in the capillary tube method. Very little catalase (0.0-0.4) remained when the meat was cooked to 70C.

Heating seafood for 36 s, 1, 2, 3, and 5 min did not produce statistical differences in reducing bacterial counts. The data demonstrate that after fillets of catfish, cod, halibut, salmon, and trout were heated at 65C for 36 s, they were safe to eat and had very low catalase activity. Table 8 shows the percentages of catalase activities in fish cooked at different temperatures.

CONCLUSIONS

(1) - The simple capillary tube catalase method using a Pasteur pipette can predict the microbial quality of raw fish by measuring % CA on the fish surface, in fish juice, and in fish meat.

(2) - Fish is considered good when the surface has 0-0.5 %CA, the juice has <4.3% CA, and the meat has <2% CA, which correspond to total counts of 10^6 CFU/cm², 10^4 CFU/mL, and 10^5 CFU/g, respectively.

(3) - Fish is considered marginally good when the fish surface has 0.6-1.5%CA, the juice has 4.3-8.2% CA, and the meat has 2.3-5% CA, which correspond to total counts of 10^7 CFU / cm², 10^6 CFU/mL, and 10^6 CFU/g, respectively.

(4) - Fish is considered marginally spoiled when the surface has 1.7-2.5% CA, the juice has 8.3-12.4%CA, and the meat has 5-6%CA, which correspond to total counts of 10^7 CFU/cm², 10^7 CFU/mL, and 10^7 CFU/g, respectively.

(5) - The capillary tube catalase method and the test tube method can predict the degree of doneness of cooked fish.

(6) - When no catalase activity or very little catalase activity remains after cooking, the fish is safe for consumption, because catalase is inactivated at an internal end-point temperature of 65C-71C.

REFERENCES


GRAM, L., TROLLE, G. and HUSS, H.H. 1987. Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. Int. J. Food Microbiol. 4, 65-72.


