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Seafood Microorganisms and Seafood Safety

HSING-CHEN CHEN

Department of Marine Food Science, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung 202, Taiwan, R.O.C.

ABSTRACT

The predominant microflora in fresh seafood including finfish and shellfish comprises of *Acinetobacter, Aerobacter, Aeromonas, Alcaligenes, Alteromonas, Bacillus, Clostridium, Corynebacterium, Flavobacterium, Micrococcus, Moraxella, Proteus, Pseudomonas,* and *Vibrio.* These major seafood bacteria are affected quantitatively by the water quality, fishing method and storage condition. Consumption of mishandled seafood can result in food-borne illnesses due to the proliferation of indigenous pathogens like *Vibrio, Clostridium botulinum* type E and *Aeromonas, and* the contamination by extraneous pathogens including *Salmonella, Shigella, Listeria monocytogenes, Campylobacter jejuni, Staphylococcus, Escherichia coli,* Hepatitis A, and Norwalk virus. To ensure food safety, raw fish slices must be thoroughly clean in order to eliminate contamination of pathogens, since wasabi, a condiment paste, cannot efficiently inhibit microbial growth. Refrigeration at 5°C reduces the growth of the mesophiles on seafood, while freezing below-20°C depresses the enzymatic activity of psychrophiles. Microorganisms in dried, salted, smoked, and fermented seafoods are inhibited by low water activity and the preservatives. Minced seafood products should be adequately refrigerated, although they are classified as prepared food. Canned seafood should be commercially sterilized.

Key words : Seafood microorganisms, food safety.

INTRODUCTION

Seafoods are an important dietary protein source for humans. Since their flesh is composed of soft tissues with high moisture and free amino acid content as well as water extractable nitrogenous compounds, seafoods are digestable and nutritious. However, under condition of mishandling, microorganisms can easily proliferate on seafoods.

In 1993, the total landing of seafood in Taiwan was 1,423,971 metric tons (MT), including 1,075,785 MT of finfish, 336,936 MT of shellfish, and 11,250 MT of miscellaneous products(1). People in Taiwan enjoy fresh, processed and even raw seafood. However, outbreaks of seafood-borne illnesses occasionally occur in Taiwan. In 1993, there were 5 outbreaks which comprised 6.5% of the total occurrence of food-borne diseases(2).

This review describes microorganisms which

Correspondence to : Hsing-Chen Chen　　*Accepted for Publication : Aug. 16, 1995*

are found to exist intrinsically and extrinsically on seafoods and their effects on food safety. Microbiological quality of finfish, shellfish, and processed products is discussed.

MICROORGANISMS IN SEAFOOD

The predominant microflora found in fresh finfish and shellfish are shown in Table 1. There are some diversities in microorganisms between these two groups of seafoods.

Factors Affecting the Microbial Diversity in Finfish

Variations of microflora on finfish are influenced mainly by their eating habits and living environments. In same water area, different species of fish may harbour different microflora, because of their ingestion of different food. Since seawater sustains a diversity of microorganisms, in particular *Moraxella, Corynebacterium, Acinetobacter, Vibrio, Flavobacterium, Pseudomonas, and Photobacterium*[17,18,19], the microbial flora found on seafood are primarily affected by the seawater and geography. Cold marine water fish, for instance, carry mainly psychrophilic gram-negative bacteria such as *Moraxella, Acinetobacter, Pseudomonas, Flavobacterium,* and *Vibrio*[3,4], while warm marine water fish harbor numerous gram-positive, mesophilic bacteria such as *Corynebacterium, Bacillus,* and sometimes enteric bacteria[3,4].

The bacterial counts of fish also vary with different methods of capture. Trawled fish usually carry bacterial loads 10 to 100-fold higher than those of lined fish, because fish are dragged for long periods of time along the sea bottom prior to landing[3].

Putrefactive Capacity of Microorganisms

Microorganisms capable of producing hydrolytic enzymes (e.g. proteases, lipases, and DNase) degrade seafood more easily. Some species of *Pseudomonas* and *Alteromonas* exhibit strong spoilage activity while *Moraxella, Acinetobacter,* and *Alcaligenes* are moderately active.

Table 1. The predominant microorganisms found in fresh finfishes and shellfishes

Seafood	Microorganism	Reference
Finfish	*Acinetobacter, Aeromonas, Alcaligenes, Alteromonas, Bacillus, Corynebacterium, Flavobacterium, Micrococcus, Moraxella, Proteus, Pseudomonas, and Vibrio*	3,4,5
Shellfish	*Acinetobacter, Aerobacter Aeromonas, Alcaligenes, Arthrobacter, Bacillus, Candida, Clostridium,* coliforms, *Corynebacterium, Flavobacterium ,Lactobacillus, Micrococcus, Moraxella, Moraxella-Acinetobacter, Proteus, Pseudomonas, Rhodotorula, Sarcina, Staphylococcus, Torulopsis, Trichospora,* and *Vibrio*	6,7,8,9,10,11, 12,13,14,15,16

Aerobacter, Lactobacillus, Flavobacterium, Micrococcus, Bacillus, and *Staphylococcus* show a low spoilage activity and only then under specific conditions[20]. Seafoods with different compositions exhibit different tendency in microbial degradation. Using the growth of *Pseudomonas* as an index in five species of seafoods at 35°C, crab is the most susceptible to microbial spoilage, followed by mackerel, cuttle fish, sword shrimp and pomfret, in descending order[21].

Distribution of Microorganisms in Body of Finfish

Microbial levels in different parts of the fish also vary: skin, 10^2-10^7 CFU/cm^2, intestinal fluid, 10^3-18^8 CFU/ml; and gill tissue, 10^3-10^6 CFU/g[22,23]. Microflora can also vary with the species of fish. The predominant bacteria in the intestinal tract of carp, for instance, include *Aeromonas hydrophila, Bacteroides* type A, *Citrobacter freundi, Pseudomonas,* and *Micrococcus,* while those in tilapia are mainly composed of *Bacteroides* type A and B, *Plesiomonas shigelloides* and *Aeromonas hydrophila*[24]. In addition, the same fish reared in seawater or fresh water can harbor different bacterial flora in the intestine. The dominant genera in the intestinal content of salmon reared in fresh water, for instance, include *Aeromonas* and *Enterobacteriaceae;* while *Vibrio* predominates in salmon reared in seawater[25].

Microbial Flora in Shellfish

Shellfish comprises two groups, crustaceans (crabs, shrimp, lobster, crawfish, etc.) and mollusks (bivalves, squids, snails, etc.). The predominant bacterial floras in fresh hard-shell shrimp (*Metapenaeopsis barbatus*) harvested from the Taiwan Strait are *Acinetobacter* (33%), coliforms (11%), *Photobacterium* (9%), *Pseudomonas* (9%), and *Vibrio*(7%)[10]. On the other hand, the natural microbial floras of freshly caught Georgia coast shrimp are *Acinotobacter, Enterobacter* and *Flavobacterium*[26]. Shrimp unloaded from the trawler have an average bacterial count of 6.0×10^5/g and market shrimp, 3.2×10^6/g. Bacterial counts used for shrimp quality indicator are 1.3×10^6/g (acceptable); 1.1×10^7/g (fair); and 1.9×10^7/g (poor)[27].

In Taiwan the most popular edible crab is the serrated crab (*Scylla serrata*). However, there is little documentation on the distribution of microflora in the crabs in Taiwan. In the United States, the hemolymph of about 20% healthy blue crabs from Chincoteague Bay is found sterile according to tests carried out on 290 freshly caught crabs[28]. Higher bacterial floras are found in the blue crabs from the Columbia River, U.S.A, the waters close to human habitation than that found in the tanner crabs from the Bering Sea, an area far from human habitation[29]. The gills of crabs are heavily contaminated by bacteria ($10^3\sim10^7$/g), when compared to $1\times10\sim4\times10^2$/g in muscle tissue[29].

Being filter feeders, bivalves pass a large volume of water through gills to obtain oxygen and food. Particulate matter, including microorganisms, are trapped on the gills, then transferred to the mouth, and finally digested. Since bivalves sometimes rear of live in estuarine areas where waters are contaminated with sewage, pathogens are occasionally found in them[30].

SEAFOOD SAFETY

Degradation of seafood after harvest begins with enzyme reaction; the intrinsic enzymes in tissue decompose macromolecules such as proteins, glycogen and nucleic acids into small molecular substances which are available for microbial growth. The proliferation of microorganisms results in further decomposition, and the subsequent production of simple derivatives of tissue substances and of metabolites such as trimethyl-amine, fatty acids, aldehydes, ketones, ammonia, carbon dioxide, etc. (Fig.1). The rate of degradation is temperature dependent. The oxidation of lipids in flesh occurs even at low temperatures that the activity of microorganisms is almost completely inhibited. Some of these

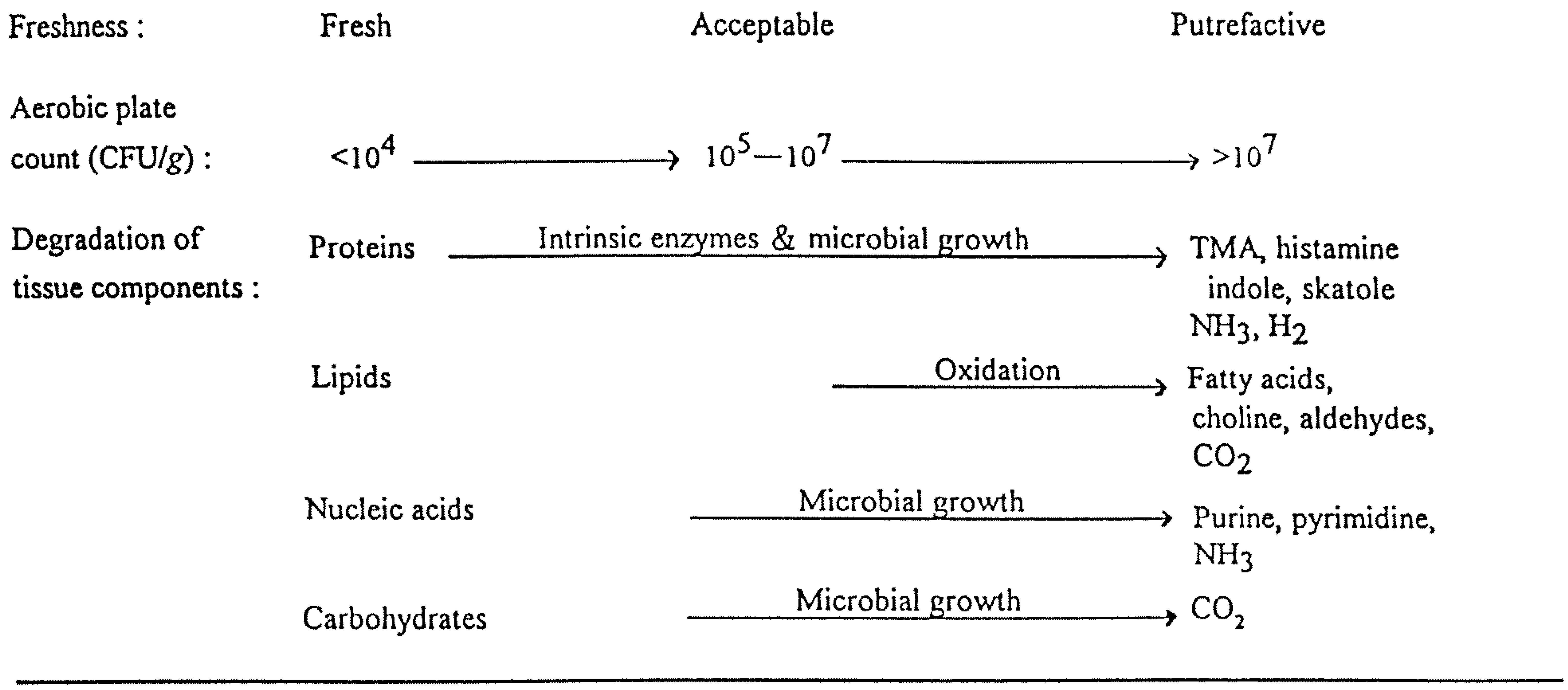

Fig. 1. Correlation of freshness, microbial spoilage, and chemical degradation of seafood after harvest.

products have an off-flavor and/or are toxic. These substances in food, after ingestion, will sometimes cause illness. In addition, the consumption of seafood contaminated with pathogens and/or the toxins produced by them is hazardous. Accordingly, sanitation and food safety of raw and processed seafoods are reviewed.

Raw Seafood

In certain areas of the world, some fresh seafoods are eaten raw, such as raw fish slices (sashimi) and raw oysters. Since seafood, especially shellfish, may contain a variety of pathogens including both indigenous and the extraneous species, the ingestion of raw seafood imposes a threat to consumer health. Some indigenous pathogens found include *Vibrio*, *Clostridium botulinum* type E, *Aeromonas*, and poisonous phytoplankton, while extraneous pathogens include *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, Hepatitis A, and Norwalk virus. Food-borne illness resulting from eating raw seafood occurs sometimes caused by *Vibrio parahaemolyticus* and *V. vulnificus* (Table 2).

In Taiwan, tuna, oil fish, sword fish, cuttle fish, and purplish amberjack (*Sericola dumerili*) are generally used as the material for raw fish slices. Chen and Chai[52]examined the sanitation quality of 100 raw sliced fish samples from 9 supermarkets and retail stalls. They found the sanitation quality of raw fish slices in supermarkets is better than that in retail stalls. Based on the levels of aerobic plate coun (APC), the 9 sampled locations could be separated into two groups (I and П). The averages of APC, total coliforms (TC), and volatile base nitrogen (VBN) of raw fish slices in groups I and П are $2.6\times10^5$and 3.5×10^6CFU/g, 24 and 90 MPN/g, and 10.6 and 11.2 mg/100g, respectively. They suggested that the upper acceptable limits of sanitation levels of APC, TC, and VBN in raw fish slices were 5×10^5 CFU/g, 50 MPN/g, and 15 mg/100g, respectively. They found that the cloth used for cleaning cutting blocks, knives, and fingers is the source of recontamination of raw fish slices. In Taiwan and Japan, the slices are conventionally eaten with wasabi paste both for flavor and for the purpose of reducing microbial load on the slices. However, wasabi can not only promote the growth of some bacterial strains[52], but also induce mutation of *Salmonella* sp[53].

Under the National Shellfish Sanitation Pr-

Talbe 2. Pathogens of seafood-borne illnesses excluding cases of paralytic shellfish poisoning

Pathogen	Major Vehicle seafood	Reference
Aeromonas hydrophila	Shillfish (oyster, clam)	31
Campylobacter sp.	Processed seafood	32
Clostridium botulinum type E	Semi-preserved seafood	33,34
Escherichia coli	Processed seafood	32
Listeria monocytogenes	Raw fish, shellfish	35
Plesiomonas shigelloides	Cuttle fish, raw oyster, salted mackerel	36
Shigella spp.	Raw fish, shellfish	32
Salmonella spp.	Processed seafood	37
Staphylococcus spp.	Processed seafood	38
Vibrio cholerae O1	Raw or under cooked shellfish	39,40
V.cholerae non O1	SAA[a]	41
V. fluvialis	Shellfish (oyster, clam, shrimp, and crawfish)	42
V. furnissii	SAA	42
V. hollisae	SAA	42
V. mimicus	SAA	42
V. parahaemolyticus	Raw seafood	42,43
V. vulnificus	Raw or under cooked mollusks	44,45,46
Hepatits A	shellfish	47,48,49
Norwalk virus	Shellfish	50

[a] same as above.

ogram (NSSP) of the United States, all waters where shellfish are harvested must meet certain standards. Growing waters are then classified as: approved zone—shellfish may be harvested and sold for human consumption, and the total coliform count in the water should not exceed 70 MPN/100ml; conditionally approved zone—basically clean but known to suffer from predictable periods of contamination, when they are closed; restricted zone—suffering from a certain degree of pollution, but shellfish may be taken from these areas and relayed or depurated in clean area so that they are safe to market; conditionally restricted zone—as with conditionally approved zone, this covers foreseeable fluctuations in water quality; prohibited zone—permanently closed to shell fishing either because they are too heavily polluted with sewage or with marine biotoxins or because they have not been surveyed (51).

Fecal coliforms are generally used as indicators for shellfish and shellfish growing water quality. There are two microbiological guidelines applied to determine the acceptability of shellfish meat after harvest. At wholesale market level, bivalves should have a standard plate count (35°C) of less than 5.0×10^5/g and a MPN of fecal coliform of less than 230/100g(30).

Refrigerated and Frozen Seafood

Keeping seafood at low temperature is a common method to reduce quality degradation. Exposure to low temperature can lead the leakage of cellular materials(57,58,59,60) and degradation of RNA(61,62), resulting in the injury and death of microorganisms(54,55,56). However, injury to the microorganisms by freezing can be repaired to a certain extent during thawing(63). Refrigeration at 5°C stops the growth of the mesophiles, and when the temperature is further lowered, psychrophiles and psychrotrophiles are eliminated. Nonspore-forming gram-negative *Pseudomonas* spp. are cold sensitive, while gram-positive *Micrococcus, Lactobacillus and Streptococcus* are more resistant[37,38].

Frozen seafood should be stored at or below -20°C. During frozen storage, the number of inactive cells formed depends on the length of storage time, In addition, pretreatment (e.g. bleeding, ice grazing) can affect the quality of seafood during frozen storage. In frozen peeled shrimp, for instance, decomposition can be exacerbated due to deteriorated raw materials, inadequate processing conditions and delayed peeling at room temperature without adequate icing[64].

Certain pathogenic microorganisms are resistant to freezing temperatures[66]. Some *V.Parahaemolyticus* cells inoculated into oysters, sole fillets and crabmeat can persist at -15 or -30°C with a higher survival ability at -30°C, although there is a sharp reduction in viability during freezing[66]. *L. monocytogenes* has a higher tendency in injury and death at -18°C rather than -198°C[67].

Dried, Salted, and Smoked Seafood

Dehydration is an simple way to store seafood under low water activity (Aw) which inhibits microbial growth. The process is conducted simply by drying seafood under the sun, hot air or in the wind with or without preservatives to produce dried, salted or smoked seafood.

In Taiwan, popular dried seafoods include dried squid and dried sea-eel. Since no preservatives are used during processing, the products should be kept dry to inhibit the growth of xerophiles.

Preservation of fish using salt (sodium chloride) is a rather primitive method. However, the process is easy and economical, salt-preserving technique is handed down and further developed. Salted mackerel, salted round herring, salted Chinese herring, and salted jelly fish are examples of salted seafood processed in Taiwan.

A low concentration of salt in food will contribute to a good taste, while a high concentration will produce distinctive flavors in fish. Sodium compounds may have technological or functional objectives. They may extract water or salt-soluble proteins. These proteins form a matrix which binds fat, water, lean meat and other ingredients upon heat coagulation, resulting in products of acceptable yield, quality and overall identity[68].

Although a high concentration of salt inhibits the growth of most microorganisms, halophiles (e.g. *Halobacterium, Halococcus,* and *Rhodotorula*) and the halotolerant organisms (e.g. *Staphylococcus*) can grow or survive in the presence of salt at concentrations above 0.2 M [69]. In seafood preserved by salt, some of the above organisms can produce red pigments, such as bacteriorubein[69]. Some fungi are xerophiles, *Penicillium* and *Aspergillus* are the major molds found in dried roun herring[70].

Since excess salt-intake is harmful to human health, low salt-containing seafoods are currently advocated. Accordingly, in order to preserve low salt-containing seafood products, refrigeration should be employed during storage.

Wood smoke contains antimicrobial agents, namely acids, alcohols, aldehydes, ketones, phenols, and carbonyl compounds[37]. The preservative effect is a result of the combination of drying and the deposition of the chemicals. Recently, preservatives other than those products from the thermal decomposition of wood have been applied. *L. monocytogenes* in cold-process (smoked) salmon is inhibited by sodium nitrite and sodium lactate[71,72].

Fermented Seafood

Salt-fermented seafood products such as shrimp sauce, fish sauce, oyster sauce, cuttle fish gey (in pieces), oyster gey, snail gey and anchovy gey are produced in Taiwan. Likewise, other countries produce salt fermented seafood specialties: sikhae (in Korea), Pedah (Indonesia), Hoi-dorng (Thailand), Tambadiang (Sri Lanka), and Matjes-herring (Denmark). Pastis (Philippines), Budu (Malaysia), and Nampla (Thailand) are instances of seafood sauce[73].

The sanitation quality of fermented seafood in Taiwan has been examined[74]. Among 31 samples of seafood gey and 19 samples of seafood sauce, the VBN contents of seafood gey and seafood sauce are 30-154 and 148-640 mg/100 g, respectively. Total coliforms were absent and neither *V. parahaemolyticus*, nor *Salmonella were* detected in any of the samples; while *Staphylococcus aureus* was found in 2 sauce samples. *Arthrobacter* and *Bacillus* are the predominant genera isolated from these products.

The salt-fermented seafoods in Thailand are mainly contaminated with *Lactobacillus plantarium, Sta. epidermidis, Micrococcus* sp., *Pediococcus halophilus*. In those from Korea, on the other hand, *Pediococus, Sarcina, Halobacterium, Micrococcus*, and yeast predominate[73].

The manufacture of fermented seafood products is traditionally processed without heating. Since they have not been sterilized by heat, the products are subject to spoilage by microorganisms with different qualities from batch to batch. In order to reduce these defects, the processing of seafood sauce has been modified[74, 75]. For the manufacture of shrimp sauce, for instance, shrimp heads and shells are homogenized with phosphate-buffered saline into a paste which is then autoclaved to inhibit all of the enzymes and microorganisms prior to seeding with microbial starter. The flavor of the sauce can be modified by the cultivation of starter[76].

Minced Seafood Products

Minced seafood products are processed by mixing surimi with salt, starch, and flavoring. Surimi is a paste of washed ground fish, shrimp, or squid. Raw seafood, ingredients, and improper handling can contribute to microorganism contamination in minced seafood products. Because of insufficient heat treatment, degradation of the products occurs within 24 hr when stored at room temperature (25°C), resulting in sliminess, discoloration, softening and even molding if stored longer.

Lizard fish is commonly used as raw material for minced seafood products in Taiwan. The fish is contaminated with proteolytic bacteria, such as *Alcaligenes, Flavobacterium, Pseudomonas*, and *Proteus*, and also with starch hydrolyzers, such as *Vibrio, Bacillus*, and *Sarcina*[21]. Starch is capable of harboring many species of *Bacillus* which are isolated from minced seafood products[77]. These strains include *B. licheniformis, B. megaterium, B. Pumilus*, and *B. polymyxa*[78]. Chu et al. [79]examined 16 samples of minced seafood products sold in supermarkets in 7 cities in Taiwan. They found that the APC of 13 samples was higher than 5×10^4/g(an upper limit for prepared food), and 11 samples were contaminated by coliforms. To inactivate non-spore-forming bacteria, adequate thermal processing is required. This must elevate the coldest temperature of the minced seafood products to 65°C for at least 3 min.

Canned Seafood

Mackerel and milk fish are commonly processed into canned fish in Taiwan. Since most canned seafoods are commercially sterilized with the 12D process to destroy all pathogens and other microorganisms. Food-borne outbreaks caused by consuming canned seafood rarely occur in Taiwan. The spoilage of canned seafood can result from the survival of heat-resistant spore-formers (e.g *B. stearothermophilus, B. coagulans, C. thermosaccharolyticum* and *Desulfotomaculum*) or from contamination by mixed cultures from leakage[37].

CONCLUSIONS

Finfish and shellfish are contaminated by microorganisms from water and sediments, gills, body surfaces, and intestines prior to harvest. Because of recontamination subsequent to harvest, seafoods are further spoiled by other microorganisms. Since immunity will be lost upon death of finfish and shellfish, seafoods will be degraded by autolysis and subsequently by the deterioration of microorganisms and chemical reactions. Putrefactive bacteria play an important role in the deterioration of seafood. Storage of seafood at low temperatures can prolong the shelf-life of products. However, freezing cannot sterilize microorganisms in seafood. Frozen storage can only slow down the reduction in freshness of seafood but cannot upgrade the quality of frozen products nor maintain the stable quality for unlimited length of storage. Seafood cannot be eaten raw, provided they are sterile or without contamination by pathogens.

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水產食品微生物與水產食品安全

陳幸臣

摘　要

新鮮水產食品(包括魚類與介類)的優勢菌相，主要受到棲息的水域、漁獲法及貯藏條件而異。這些菌相一般包括*Acinetobacter, Aerobacter, Aeromonas, Alcaligenes, Alteromonas, Bacillus, Clostridium, Corynebacterium, Flavobacterium, Micrococcus, Moraxella, Proteus, Pseudomonas*及*Vibrio*等。漁獲後由於常在病原菌，如*Vibrio, Clostridium botulinum* E型菌及*Aeromonas*的繁殖及外來病原菌如*Salmonella, Shigella, Listeria monocytogenes, Campylobacter jejuni, Staphylococcus, Escherichia coli,* Hepatitis A,及Norwalk病毒的污染，攝食處理不當的海鮮，常會引起食物中毒。爲了保障食品安全，生魚片本身必須很清潔，因爲伴食的山葵泥幾乎無抑菌效果。冷藏在5°C可降低水產食品上好溫菌的生長，冷凍於−20°C以下可抑制好冷菌酵素活性。在水產乾製品、鹽藏品、燻製品及發酵品上的微生物會因降低水活性及添加防腐劑的作用而被抑制。雖然煉製品爲調理食品，但亦應冷藏，罐藏品應該達到商業殺菌的程度才安全。