Histamine Formation and Bacterial Spoilage of Albacore Harvested off the U.S. Northwest Coast

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ABSTRACT

Iced and previously frozen albacore were monitored for histamine formation and bacterial growth during storage at 0–37°C. The optimum temperature for histamine formation in albacore tuna (Thunnus alalunga) was 25°C, and whole fish were more susceptible to histamine formation than dressed fish at that temperature. Storage at 25°C resulted in the highest histamine level, 60.4 mg/100g in whole fish stored for 7 days. When albacore were frozen prior to storage, reduced amount of histamine was found at 7.14 mg/100 g after 7 day storage at 25°C, only after decomposition became obvious. No histamine was found in any of the albacore samples stored in ice for 18 days.

Key Words: albacore, bacterial spoilage, histamine, storage

INTRODUCTION

SCOMBROID POISONING RESULTS FROM INGESTION OF FOODS containing high levels of histamine and is one of the three most frequently reported illnesses associated with seafood consumption in the U.S. (Bean and Griffin, 1990; FDA, 1994). Among seafoods, it is mainly associated with scombroid fish species, such as, tuna, bonito and mackerel, containing high levels of free histidine in muscle (Taylor, 1986). Histidine can be converted to histamine during decomposition by histamine-producing bacteria possessing histidine decarboxylase (Rawles et al., 1996). Various types of fish implicated in scombroid poisoning have been found to contain high levels of histamine. Sailfish meat involved in a scombroid poisoning case had 168 mg histamine/100g muscle (Hwang et al., 1995). Histamine levels up to 919 mg/100g were found in samples of tuna sashimi implicated in scombroid fish poisoning (Taylor and Lieber, 1979). The histamine content of marlin implicated in a poisoning incident ranged between 93.5 and 276 mg/100g; while that of fresh marlin did not cause symptoms of poisoning, and was undetectable (<5 ppm) (Morrow et al., 1991). The histamine content of hot-smoked mackerel samples that had been implicated in a scombrototoxic incident was 270 mg/100g, whereas that of fresh hot-smoked mackerel was 25 ppm (Clifford et al., 1989). The histamine content of canned tuna implicated in poisoning was 116 mg/100 g muscle, while that of wholesome canned tuna was 2.74 mg/100g muscle (Kim and Bjeldanes, 1979).

Histamine formation is most often induced by high temperature abuse of fish postharvest, and the accumulated level is affected by the combination of time and temperature. Kahawai showed the highest rate of histamine formation and accumulation at 25°C, 330 mg/100g muscle within 2 days, followed by 30°C (Fletcher et al., 1995). Mahi-mahi showed the highest histamine accumulation at 32°C, reaching 250 mg/100g of fish in 24h, when incubated at 0–32°C (Baranowski et al., 1990). Oil-preserved anchovies stored at 20°C showed an increased level of histamine, 80 mg/100g, in 2 mo storage, reaching a peak at 300 mg/100g at 7 mo (Rodríguez-Jerez et al., 1994). At lower temperatures, 0–10°C, histamine was formed but at the reduced level mainly by psychrophilic and psychrotrophic histamine-producing bacteria (Baldrati et al., 1980; Frank and Yoshinaga, 1984; Ryser et al., 1984; Mori et al., 1988; Baranowski et al., 1990).

Albacore tuna (Thunnus alalunga), which belongs to Scombrids family, has good eating quality and has been a highly valued fish. There has been a large effort to harvest albacore off the Northwest coast of the U.S. (Craven et al., 1995). It is known to have a relatively long shelf-life and contains negligible quantities of histamine immediately after catch. However, improper handling of albacore can cause histamine formation due to the high levels of free histidine in its muscle, >1000 mg/100g (1%, w/w) (Fletcher et al., 1995). The FDA (1996) established the hazards analysis and critical control point (HACCP) program and set up guidelines for histamine at 5 mg/100g for scombroid fish species, which was tenfold less than the previous guideline (Craven et al., 1995). Our objective was to study product safety of albacore by inducing histamine production under controlled storage conditions. Effects of frozen storage and evisceration of fish were also studied.

MATERIALS & METHODS

Samples and preparation

Iced albacore samples were purchased from a commercial processor in Newport, OR and transferred in ice to the Oregon State University-Seafood Lab overnight. Although the history of fish could not be verified, in that fishing season (1996), Albacore were troll-caught about 100–200 miles off the Oregon coast. Fishermen were advised to chill the fish in slush ice immediately after catch and maintain it in ice on board. The weight of the albacore ranged between 12 and 14 kg each (avg 13.2 kg). Upon arrival at the OSU-Seafood Lab, half of the fish were dressed by eviscerating guts and removing gills. Fish were rinsed with water and used as dressed fish.

Frozen albacore were obtained from a chartered boat. They had been blast-frozen on board immediately after catch and kept frozen at −30°C until used (≈4 mo). The fish were thawed at room temperature (15°C) overnight and used as whole fish.

Sample storage

The iced albacore were divided into two groups of whole and dressed fish and both groups were stored at 0, 25, 30, and 37°C. Samples were taken from each group, storage temperature and sampling period. The initial sampling site was the nuchal region (nape) directly behind the head above the lateral line, and the sampling site moved along the lateral line to the anterior. Muscles were removed aseptically from fish and analyzed for aerobic plate count (APC) and histamine content. Samples were taken every 12h for 30 and 37°C storage. For 25°C, samples were taken every 24h. At 0°C, samples were analyzed after 1 wk, and every 24h thereafter.

Frozen albacore were thawed overnight at ambient temperature (15°C). The previously frozen fish were stored at 25 and 30°C, at which high levels of histamine were found with iced fish. Samples

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were taken every 24h as described and analyzed for APC and histamine content.

**Bacterial enumeration**

APC was determined in duplicate by the standard method (FDA, 1992). Muscle samples (10g) were aseptically removed from fish and blended with 90 mL of saline solution (0.85%) followed by serial dilution in the same solution. Each diluted sample (1 mL) was dispensed and poured with Plate Count Agar (Difco Co., Detroit, MI) supplemented with 0.5% NaCl. The APC plates were incubated at 35°C for 2 days except for those stored at 0°C or previously frozen. For iced albacore stored at 0°C, the plates were incubated at 15°C for 5 days, and for previously frozen albacore, they were incubated at 25°C for 3 days.

**Histamine analysis**

Histamine was analyzed in duplicate by the standard fluorometric method (AOAC, 1995). Muscle (10g) was homogenized in 50 mL of methanol for 2 min and heated in a water bath at 60°C for 15 min. After cooling to 25°C, the volume was adjusted to 100 mL with methanol and filtered through Whatman #1 paper. The methanol filtrate was collected and loaded onto an ion exchange column (200 × 7 mm) with Dowex 1-X8 (Sigma Chemical Co., St. Louis, MO), which was converted to hydroxide form by 2N NaOH. The column eluant was analyzed for histamine, and the fluorescence intensity was determined using a spectrophotofluorometer (Aminco Bowman, Silver Spring, MD) at excitation wavelength of 350 nm and emission wavelength of 444 nm.

**RESULTS & DISCUSSION**

**Changes in bacterial counts of iced albacore**

The condition of iced albacore used for storage was excellent as judged by color, odor and firmness of muscle. The muscle was translucent white. The initial APC of the iced albacore muscle was below the colony counting range (25–250 CFU/g). Although intact muscle was sterile, APC of albacore started to increase rapidly during storage at 30 and 37°C (Fig. 1). After 12h, APC was 10³ CFU/g and increased to 10⁶–10⁷ CFU/g after 48h in both whole and dressed fish. Off-odor was evident at 24h, and the muscle turned opaque-white as when it is cooked, due to protein denaturation. The APC reached almost 10⁸ CFU/g after 36h, and fish were completely decomposed by day 2 as when it is stored at 25°C.

**Histamine in iced albacore**

Histamine analysis

Histamine in iced albacore was slow compared to the APC increase. During storage up to 3 days at 30 and 37°C, histamine levels were below 5 mg/100g, although fishes were completely decomposed by day 2 (Fig. 4). Fish stored whole at 30°C showed the highest level of histamine, 39.1 mg/100g, at day 3. At 25°C, histamine level was negligible (<5 mg/100g) until day 4, but a rapid increase was observed thereafter (Fig. 5). Histamine levels in fish were higher at 25°C than at 30 or 37°C. Whole fish stored at 25°C showed the highest histamine level, 60.4 mg/100g, at day 7. We found that 25°C was optimum for histamine formation in albacore and that whole fish became spoiled by day 5, and APC was almost 10⁷ CFU/g. Iced albacore stored at 0°C had the longest shelf-life among all the temperature tested, 0–37°C (Fig. 3). APC was below 10⁶ CFU/g until 7 days, and increased slowly thereafter. APC of whole fish increased faster than those of dressed fish as had been observed with fish stored at 25°C. Whole fish developed thick slime on the skin by day 15, but it was not observed on dressed fish.

Pérez-Villarreal and Pozo (1990) reported that albacore had a longer shelf-life than any other scombroid fish. They reported 12 days as the maximum storage time that fish could be kept in ice with good quality. The increase in trimethylamine concentration was negligible over 25 days storage in ice. Price et al. (1991) reported APC of albacore stored in ice increased gradually for 9 days and reached 10⁶ CFU/g between 12 and 16 days. Marrakchi et al. (1990) reported that iced sardines had a shelf-life of 9 days. The initial APC of iced sardine, 3.16 × 10⁵ CFU/g, reached the limit counts of 10⁶–10⁷ CFU/g at day 9 in iced storage, while the counts exceeded these limits within 24h at ambient temperature. According to Ryder et al. (1984), jack mackerel had a shelf-life of 7 days in ice based on sensory results. During 23 days of storage in ice, APC did not exceed 10⁶ CFU/g until day 11, and K value reached 20% after 7 days.

The gill and intestines of fish are the main reservoirs of normal microflora in seawater (Taylor and Speckhard, 1983). Bleeding and evisceration is considered essential for some fish to maximize quality (Jacoby, 1987). In our study, APC increased showing distinctive differences between whole and dressed fish held at 0°C. Whole fish reached the maximum level, 10⁶ CFU/g, in 15 days, while dressed fish reached the same level in 18 days. However, no significant difference in APC was observed between whole and dressed fish, when incubated at 30 or 37°C. The trends in APC increase of whole and dressed albacore demonstrated that it was necessary to store fish below 4°C even after evisceration and degilling.
were more susceptible to histamine formation than dressed fish. No histamine was found in whole or dressed fish stored in ice up to 18 days (Fig. 3).

Haaland et al. (1990) showed the relationship between the formation of free amino acids and amines in mackerel stored at 2°C and 20°C. The contents of several amino acids decreased when fish were stored at 20°C with the resultant formation of phenylethylamine, tyramine, putrescine, histamine and cadaverine. However, no obvious changes were reported in fish stored at 0°C. Wendakoon et al. (1990) reported a similar result with no amines formed in mackerel during iced storage, but several amines including histamine were found at high concentrations at 20°C. The rates of amine formation and its accumulation were higher in dark than in white muscle. Ababouch et al. (1991) reported that APC reached 5 × 10^8 CFU/g and the histamine level increased to 235 mg/100g after 24h incubation of sardine at ambient temperature. Frank et al. (1981) reported that the optimum temperature for histamine formation in sardine was 37.7°C based on microbial enzyme activity, and that APC and histamine content were 2.8 × 10^8 CFU/g and 343 mg/100g, respectively, on day 1 at that temperature. Price et al. (1991) reported histamine formation in round, bled and dressed albacore stored at 0°C. Histamine was not detected for 27 days. Two bled albacore samples were found on day 33 to contain histamine at 49.2 and 82.5 mg/100g. They suggested that histamine formation was inhibited at 0°C or below, but histamine production could continue due to preexisting histidine decarboxylase. According to López-Sabater et al. (1994), histamine levels higher than 2,000 ppm were found in canned sardine, mackerel and tuna. In our results, histamine content in albacore was lower than those of other scombroid fish reported, and high levels of histamine developed after fish became decomposed and unsuitable for consumption. López-Sabater et al. (1996) reported similarly that when tuna (Thunnus thynnus) were properly stored at low nonfreezing temperatures, histamine formation did not present a serious health risk to consumers.

**Changes in bacterial counts of frozen albacore**

Initial APC of previously frozen albacore was ≈2.0 × 10^9 CFU/g. During storage at 30°C (Fig. 6), APC remained at 10^2 CFU/g during day 1, and then started to increase rapidly. APC reached 7.2 × 10^3 CFU/g within 36h, and fish developed apparent signs of spoilage, such as, pungent odor, slime formation and soft texture. Although the initial APC of frozen fish were higher than those of iced fish, APC increased slowly resulting in prolonged lag periods before exponential bacterial growth occurred. Also the pattern of APC increase in frozen albacore was quite different from that of iced albacore.

Raw frozen foods would have the same microbial flora as the raw food prior to freezing, although freezing favors survival of Gram-positive bacteria (ICMSF, 1980). Dominant microflora in fish caught in cold waters are usually gram-negative psychophilic and psychrotrophic bacteria (Bennour et al., 1991). Baranowski et al. (1990) reported that psychrophiles were primarily spoilage bacteria at 0°C, while mesophiles predominated at 21 and 32°C. However, 30°C is not the optimum temperature for growth of psychrotrophic bacteria but the upper limit for growth. Therefore, we postulated that the rapid increase in APC of albacore after the lag phase was due to recovery of injured cells and contamination by mesophilic bacteria. APC of frozen albacore reached 7.4 × 10^3 CFU/g by day 2 at 25°C, and the rate of APC increase was 1 log cycle lower than that of AP in fish kept at 30°C (Fig. 6). No apparent differences were observed at 25°C between iced and frozen albacore samples. Psychrotrophic bacteria in frozen fish exhibits the most rapid growth rate between 20–24°C, and mesophilic bacteria in iced fish can also grow well in that range (Jacoby, 1987).

**Histamine in frozen albacore**

Histamine was not detected at 30°C during 5 day storage, although APC reached the maximum level, 1.0 × 10^9 CFU/g, and fish developed apparent signs of spoilage (Fig. 6). At 25°C (Fig. 6), histamine was not detected until day 4. Histamine content started to increase slowly on day 5, when the fish were spoiled. The highest histamine content was 7.14 mg/100g on day 7, when storage was discontinued.

Baranowski et al. (1990) reported freezing mahimahi, prior to storage at −20°C, inhibited subsequent histamine formation. When mahimahi was incubated for 24h at 32°C after frozen storage for 40 wk, a negligible level of histamine was found. In comparison, control fish, which had not been previously frozen, showed a histamine level of 266 mg/100g. They proposed that the drastic reduction in surviving bacteria was due to microbial destruction during prolonged frozen storage. Ryser et al. (1984) isolated several psychrotrophic bacteria, _Pseudomonas fluorescens_, _Pseudomonas putida_ and nonfluorescent _Pseudomonas_ spp. from raw sashimi tuna, and tested their abilities to form histamine at 21°C. The maximum histamine level found in their 48-h cultures was low, 3.4 mg/100 mL. Taylor and Speckhard (1983) reported that no histamine-producing bacteria were isolated from any of the muscle samples obtained from frozen skipjack tuna.

The production of histamine is not related to the total number of bacteria but rather to the number capable of synthesizing histidine decarboxylase (Bennour et al., 1991). The formation of histamine is

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Fig. 3—Changes in APC and histamine content of iced albacore during storage at 0°C. Fish were incubated whole and dressed. Samples were taken at 1 week and every 24h thereafter. APC was determined using plate count agar supplemented with 0.5% NaCl and the plates were incubated at 15°C for 5 days. Histamine content was analyzed by the AOAC fluorometric method.

Fig. 4—Changes in histamine content of iced albacore during storage at 30 and 37°C. Fish were incubated whole and dressed. Histamine content was analyzed by the AOAC fluorometric method.
mainly due to Enterobacteriaceae (López-Sabater et al., 1994). Although many different bacteria can produce histamine, including Proteus vulgaris, Proteus mirabilis, Clostridium perfringens, Enterobacter aerogenes, and Vibrio alginolyticus, only Morganella morgani, Klebsiella pneumoniae, and Hafnia alvei have been reported in foods incriminated in scombroid poisoning (Omura et al., 1978; Eitenmiller et al., 1982; Yoshinaga and Frank, 1982; Taylor and speckhard, 1983; Middlebrooks et al., 1988). Among them, only M. morganii and K. pneumoniae have been reported as prolific histamine formers. Several investigators showed that the optimum temperature for decarboxylating activity was 20 to 30°C (Middlebrooks et al., 1988; Fletcher et al., 1995). Therefore, we concluded that histamine-producing bacteria in previously frozen albacore do not recover their activity to decarboxylate histidine during incubation at 30°C but may recover it slowly at 25°C.

REFERENCES


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