Biochemical, Microbiological, and Sensory Changes of Sea Bass (*Lateolabrax japonicus*) under Partial Freezing and Refrigerated Storage

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Packages of fresh sea bass (*Lateolabrax japonicus*) were stored under temperatures ranging from −3 to 10 °C. Changes in *K* value, volatile basic nitrogen, aerobic plate count, and sensory acceptability were monitored. The shelf life of stored sea bass at 5 °C was 3 days. It was extended to about 2 weeks at 0 °C. Partial freezing storage at −3 °C increased the shelf life to >4 weeks. Ice and partial freezing temperatures increased the shelf life by lengthening the lag phase of bacterial growth and retarding the nucleotide breakdown rate. A maximum *K* value of 50% was appropriate for sea bass shelf life determination. In contrast, a maximum microbial population of 3×10^6 CFU/g of fish muscle was a good shelf life indicator only at storage temperatures ≥0 °C. A rapid rise in volatile basic nitrogen could serve as an indication of substantial microbial spoilage.

Keywords: *Sea bass; storage temperature; shelf life; partial freezing*

INTRODUCTION

Fishes and shellfishes are relatively perishable protein sources for human consumption. Scientists have been constantly searching for improved methods to preserve or extend the shelf life of various aquatic food products. Their findings on seafood preservation and shelf life extension technology have been summarized in recent reviews (Sikorski and Pan, 1994; Ashie et al., 1996). Among the freshness preservation techniques, the effectiveness of partial freezing received both suspicion and applause. Sikorski and Pan (1994) stated that partial freezing might cause drip loss and possible quality degradation. In contrast, Ashie et al. (1996) and Haard (1992) said that it has more benefits than drawbacks as long as proper temperature control (at −3 ± 0.5 °C) is maintained during seafood storage. Many reports, e.g. Kato et al. (1974), Lee and Toledo (1984), and Uchiyama and Kato (1974), indicated that partial freezing did improve the shelf life of stored seafood with little side-effect. A reexamination of the influence of partial freezing on stored seafood quality appears to be worthwhile.

The quality of seafood degrades because microbial spoilage and biochemical reactions occur during storage. Several spoilage indicators have commonly been used to assess the quality of stored seafood. Change in microbial population is a traditional quality index of fresh fish (Martin et al., 1978). Numerous reports concentrated on the microbiological quality of fish (Gram and Huss, 1996). Total volatile basic nitrogen (TVB-N or VBN) was also regarded as a good quality criterion (Botta et al., 1984; Malle and Poumeyrol, 1989). Postmortem nucleotide catabolism of fish results in different breakdown products of adenosine triphosphate. Different ratios of these compounds, such as the *K* value (Saito et al., 1959) and K_i indicator (Karube et al., 1984), have been proposed. Ehira and Uchiyama (1987) reported that the *K* value was a good indicator for fish freshness. Surette et al. (1988) proved that both autolytic and bacterial enzymes contributed to the changes in nucleotide compounds. It is known that biochemical changes and microbiological spoilage are closely related. However, the method of choice for fish quality assessment seems to remain a personal preference.

Sea bass has white flesh, mild taste, and low fat content (Boyd et al., 1992). These attributes have made several bass species popular around the world. Striped bass (*Morone saxatilis*) and its hybrid with *Morone chrysops* seem to be the favorite genus in the United States. In Europe, sea bass (*Dicentrachus labrax*) is popular. In China, Japan, Korea, and Taiwan, people raise the species *Lateolabrax japonicus* in ponds to meet the needs of domestic consumption. Boyd et al. (1992) found that bass fillets had a shelf life of 8 days in refrigerated storage. The shelf life was extended to 21 days for fillets stored with chillpack at −2 °C. They suggested that hypoxanthine formation and aerobic plate counts were good indicators of quality deterioration. In addition, Eifert et al. (1992) and Handumrongkul and Silva (1994) examined the effect of postharvest cooling, CO_2 treatment, and CO_2 packaging on the quality change of striped bass. Karahadian et al. (1995) reported that the freshness of aquacultured striped bass fillets was maintained for up to 10 days, much longer than that of wild-captured counterparts (up to 5 days) after refrigerated storage. In contrast, little information is available on the quality changes of Japanese sea bass (*L. japonicus*). Only Uchiyama and Kato (1974) and Kato et al. (1974) investigated the changes of some quality indicators during storage in ice and at −3 °C.

Doyle (1991) reviewed the literature regarding cold

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storage temperature and found that refrigerated foods were held by some consumers at ≥10 °C and by some retailers at 12.8 °C. Sergelidis et al. (1997) also revealed that ~55% of domestic and 32% of retail store refrigerators had temperatures ≥9 °C in Greece. Therefore, it is most likely that fresh muscle foods are exposed to some periods of temperature abuse before human consumption. Until now, no shelf life data were available for sea bass above the storage temperature of 0 °C.

The objectives of this study were to examine the influence of partial freezing and refrigerated temperature on the quality of cultured sea bass (*L. japonicus*) during storage and to compare the changes in several commonly used freshness indicators.

MATERIALS AND METHODS

Raw Material and Preparation. Sea bass (*L. japonicus*) were raised in aquaculture ponds at Chia-Yi, Taiwan. After being captured with cast net, they were stored in ice and transported to our laboratory within 16 h. Each individual fish sample was scaled, gutted, eviscerated, and rinsed with tap water. Afterward, it was placed on a polypropylene plastic tray and overwrapped with polyethylene film. Dozens of packed sea bass were stored under a partial freezing temperature of −3 °C and under refrigerated temperatures of 0, 5, and 10 °C. At selected time intervals, three packages were removed from each storage temperature and tested for property changes.

Aerobic Plate Count. Twenty-five grams of fish muscle was homogenized with 225 mL of sterilized deionized water. A series of 1:10 dilutions were made. Into each of the triplicate plate count agars was poured 0.1 mL of sample solution with different dilution ratios. The plates were then incubated at 35 °C for 48 h.

Chemical Analysis. The trichloroacetic acid (TCA) microdiffusion method was employed to analyze the VBN. Ten grams of sea bass muscle was homogenized with 7% TCA and filtered with a Toyo no. 2 filter paper. The volume was then adjusted to 100 mL. One milliliter of TCA extract was analyzed as described in Cobb et al. (1973). The microdiffusion sample was kept at 37 °C for 90 min. Hydrochloric acid (0.02 N) was then used for titration. The blank was 1 mL of 7% TCA.

To analyze the nucleotide breakdown products, 5 g of sea bass muscle was homogenized with 6% perchloric acid at 0 °C for 2 min (20 000 rpm). The homogenate was centrifuged at 4 °C, 3000*g*, for 20 min. The supernatant was filtered through a Toyo no. 2 filter paper. The filtrate was immediately neutralized with 1 M potassium hydroxide to pH 6.5. To ensure complete recovery of nucleotide compounds, the sediment was extracted according to the above procedure two more times. After standing at 0 °C for 30 min, all of the filtered supernatants were combined and diluted to 100 mL. The sample was stored at −40 °C before HPLC analysis.

Nucleotide compounds were analyzed by HPLC (Waters Associate model 510 liquid chromatograph), fitted with a 490E programmable multiwavelength detector, and an Inersil ODS column (25 cm × 4.6 mm, filled with 5 μm ODS from GL Science, Japan). Twenty-five microliters of PCA extract, filtered through a 0.20-μm nylon membrane, was injected. Separation was achieved by using 0.05 M phosphate buffer (pH 6.50) at an elution rate of 0.8 mL/min. The rest of the procedure was the same as the gradient elution procedure described by Suwetja et al. (1989). The eluent was monitored by UV absorption at 254 nm. The amounts of nucleotide breakdown compounds were determined by comparing with standards (Sigma). The results were expressed as *K* values (Saito et al., 1959; López-Gálvez et al., 1995), calculated by using the following formula: *K* values (%) = 100 (inosine + hypoxanthine)/(ATP + ADP + AMP + IMP + inosine + hypoxanthine).

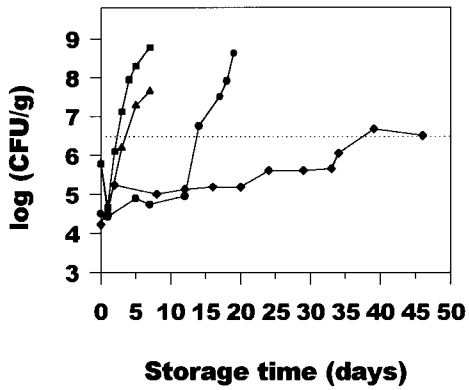

Figure 1. Changes in aerobic plate count of packaged sea bass with storage time at different temperatures: (◆) −3 °C; (●) 0 °C; (▲) 5 °C; (■) 10 °C.

Sensory Evaluation. The attribute rating method (IFT, 1981) was employed to monitor the sensory change of sea bass during storage. A panel of 10 members was requested to rate the appearance, texture, odor, and overall grade of the raw fish muscle samples taken from stored sea bass. A rating scale of 1−9 points was used, with 9 equivalent to fresh sea bass of top quality and 5 being indicative of acceptable borderline freshness. Panelists were randomly selected from a group of 20 graduate students. In a preliminary test, all panelists had experienced observing the change in sensory attributes of stored fresh sea bass. The average scores of the overall grades in acceptability are presented.

Shelf Life Criteria. For determining the aerobic plate count shelf life, the sanitation standard (3×10^6 CFU/g) for frozen seafood was used (DOH, 1996). The maximum VBN level (15 mg/100 g) recommended for frozen foods (DOH, 1996) was used for establishing VBN shelf life. After a survey of 104 fish samples from commercial retail outlets in Japan, Ehira and Uchiyama (1987) reported that raw fish in medium-quality sushi shops had an average *K* value of 52.2 ± 8.9%. With reference to their finding, *K* values of 50% and 55% were adopted in this study to determine the shelf life of sea bass.

Shelf Life Plot. As pointed out by Labuza (1985), the shelf life of a particular quality change could be related to the storage temperature by the equation $\theta_s = \theta_0\, e^{-bT}$. θ_0 is the shelf life at a reference temperature (e.g. 0 °C, 273.15 Kelvin), and θ_s is the shelf life at storage temperature *T*. By making a semilogarithmic plot of shelf life versus temperature, we obtained the slope of the line: $b = \ln Q_{10}/10 = 0.503E_A/[T(T + 10)]$. The slope was obtained by performing the least-squares linear regression for the quality change data using SigmaPlot software (formerly a product of Jandel Scientific, San Rafael, CA; currently a product of SPSS Inc., Chicago) installed on a personal computer. The activation energy (E_A) and Q_{10} (the ratio of shelf life values between any two temperatures 10 °C apart) of a quality change were then calculated.

RESULTS AND DISCUSSION

Microbiological Quality Change. Figure 1 shows the changes in aerobic plate count (APC) of sea bass during storage. Using 3×10^6 CFU/g as the microbial safety criterion, we found the shelf lives at 10, 5, 0, and −3 °C to be 2, 3, 14, and 37 days, respectively. Ice temperature storage increased the shelf life to ~2 weeks. Partial freezing at −3 °C extended the shelf life to >5 weeks. The microbes in packed sea bass grew rapidly during storage at 5 and 10 °C. On the contrary, the lag phase became apparent for samples stored at 0 and −3 °C. These data revealed that ice temperature and partial freezing storage effectively inhibited the microbial growth by prolonging the lag phase.

VBN. The VBN of stored sea bass (Figure 2) remained relatively low during the initial period of stor-

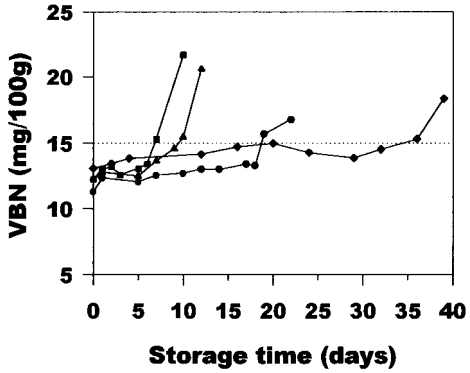

Figure 2. Changes in VBN of packaged sea bass with storage time at different temperatures: (◆) −3 °C; (●) 0 °C; (▲) 5 °C; (■) 10 °C.

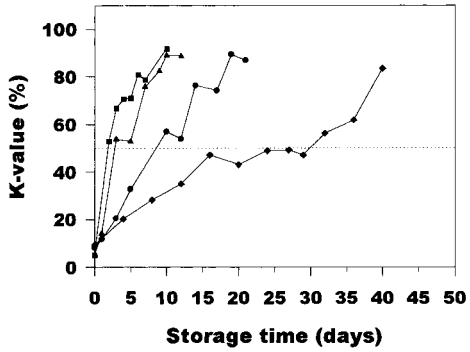

Figure 3. Changes in *K* value of packaged sea bass with storage time at different temperatures: (◆) −3 °C; (●) 0 °C; (▲) 5 °C; (■) 10 °C.

age. Using 15 mg/100 g as the acceptable level of VBN, the VBN shelf life would be 6, 9, 18, and 34 days at storage temperatures of 10, 5, 0, and −3 °C, respectively. The VBN shelf life values were much longer than the microbial shelf lives. From Figures 1 and 2, one can find that VBN did not change noticeably until the number of microbes increased to $\approx 10^6$ (−3 °C) or 10^7 (0 °C and above). The more rapid rise of VBN at high microbial numbers signaled the stage of substantial spoilage. Even still at a relatively low VBN level (15 mg/100 g of fish muscle), the shelf life values were longer than those determined by other methods. Therefore, the measurement of VBN was not a good measure for ensuring the safety of overwrapped sea bass packages. The results of Botta et al. (1984) indicated that VBN measured with the TCA microdiffusion method tends to be lower than VBN values determined by using other extraction procedures. However, the trend determined by TCA microdiffusion is representative of the actual VBN change in samples. As a consequence, the VBN levels for shelf life expiration would depend on the extraction methods. Methods other than the TCA microdiffusion procedure may lead to higher VBN values. The use of a critical VBN value for shelf life determination is therefore not practical unless a standard method of analysis is widely accepted.

Nucleotide Degradation. The *K* values of sea bass increased with storage time (Figure 3). In contrast to that observed for microbial growth and VBN change at 0 and −3 °C, the *K* values increased without any lag phase at all temperatures. The *K* value is an indicator of enzymatic conversion of nucleotides into inosine and hypoxanthine. Although Surette et al. (1988) have demonstrated that the degradation enzymes may be

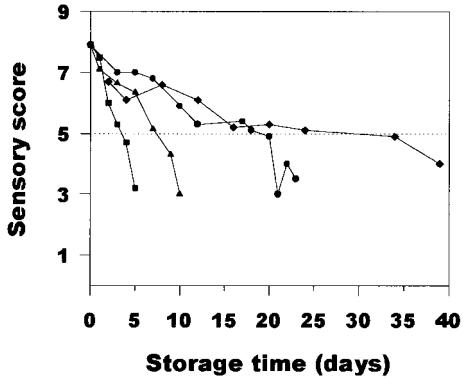

Figure 4. Changes in average sensory score of packaged sea bass with storage time at different temperatures: (◆) −3 °C; (●) 0 °C; (▲) 5 °C; (■) 10 °C.

endogenous or microbial, a substantial amount of microbial enzymes seemed not to be a prerequisite for nucleotide catabolism. This resulted in the immediate rise in *K* value upon storage. If we used a *K* value of 50% as the limit for shelf life expiration, we would have shelf lives of 2, 3, 8, and 30 days, respectively, for 10, 5, 0, and −3 °C storage. In almost all conditions, these shelf life values were lower than the shelf life values determined by all other quality measures. The only exception happened at −3 °C, where the *K* value shelf life (30 days) was longer than sensory shelf life by 1 day. The shelf life of sea bass, when the *K* value reached 55%, was 2, 6, 13, and 31 days, respectively, at each storage condition. These values were closer to the sensory shelf life values, but they sometimes were longer than the microbial shelf life values. The above results corresponded with the average *K* value of 52.2 ± 8.9% (Ehira and Uchiyama, 1987) for fish samples in Japanese medium-quality sushi shops.

Sensory Quality Change. The average overall sensory scores of sea bass decreased continuously with time (Figure 4). It was generally believed that partial freezing might cause protein denaturation and result in drip loss and less desirable textural properties. However, the panelists did not notice those objectionable quality degradation phenomena for the samples stored at −3 °C. The reason might be that, in this study, the sea bass samples contained skin, bone, and abdominal membrane, which helped to prevent the loss of water during partial freezing storage. Some fluctuation in sensory evaluation results occurred for samples stored at 0 and −3 °C. The changes in sensory attributes for samples stored at these lower temperatures were more subtle to detect. Since panel members for each test were not exactly the same, we suspect personal rating difference could also contribute to the variation in average overall scores between tests. In addition, the fish samples that panelists had to evaluate after various storage periods were different; these samples might have individual differences by nature. Nevertheless, we notice that the sensory quality did show a general trend of decreasing with storage time. The degradation rate increased with increasing temperature. The sensory shelf lives, when the average score became ≤5, were 3, 7, 19, and 29 days, respectively. The sensory shelf life was longer than the shelf life values determined by objective indicators at 0 °C and higher storage temperatures. On the contrary, it was comparable to the *K* value shelf life when sea bass was stored at −3 °C. The above results suggested that a maximum *K* value of 50%

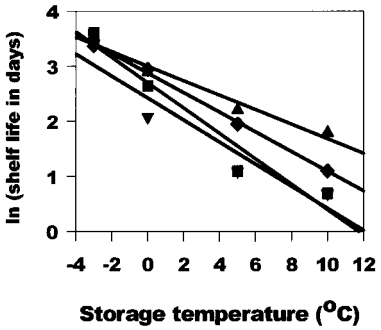

Figure 5. Shelf life plots for different quality indicators of sea bass: (■) aerobic plate count; (▲) VBN; (▼) *K* value; (◆) sensory score.

Table 1. Activation Energy (E_A) and Ratio of Shelf Life Values between Any Two Temperatures 10 °C Apart (Q_{10}) of Different Quality Indicators

quality indicator	E_A (kcal/mol)	Q_{10}	correlation coefficient (R^2)
APC (−3 to 10 °C)	35.4	10	0.9380
−3 to 5 °C	46.5	23	0.9998
5−10 °C	12.6		*a*
VBN (−3 to 10 °C)	20.2	3.7	0.9648
−3 to 5 °C	24.2	5.1	0.9860
5−10 °C	12.6		*a*
K value (−3 to 10 °C)	30.7	7.4	0.8992
−3 to 5 °C	41.3	16	0.9486
5−10 °C	12.6		*a*
sensory scores	27.4	5.9	0.9974

[a] Interpolation through shelf life values at 5 and 10 °C was used to calculate these values.

was appropriate for determining the shelf life of sea bass within the temperature range investigated in this study. A maximum microbial population of 3×10^6 CFU/g of fish muscle seemed to be a good shelf life indicator only at storage temperatures ≥0 °C. Objective quality measurements are much more helpful to the protection of consumer safety at higher storage temperatures because sensory detection of spoilage lagged behind microbiological or chemical shelf life under those conditions.

The studies of Doyle (1991) and Sergelidis et al. (1997) revealed the widespread incidence of high refrigeration temperature (≥9 °C) in the life cycle of refrigerated foods. The APC and *K* value shelf lives of sea bass at the refrigeration temperatures of 5 and 10 °C were 3 and 2 days, respectively. These data suggest that if one could not be sure whether refrigeration temperature has been improperly raised to ≥5 °C, then a packed fresh sea bass had better be consumed within 1 or 2 days. On the contrary, if strict temperature control around 0 and −3 °C could be assured during the distribution cycle of fresh sea bass, then its shelf life could be extended to approximately 2 and 4 weeks, respectively.

Shelf Life Plots. The shelf life values of sea bass determined from different criteria are plotted in Figure 5. The calculated Q_{10} and activation energy (E_A) values are shown in Table 1. Over the whole temperature range investigated in this study, the E_A and Q_{10} for *K* value were the closest to those for the change in sensory scores. This helped to explain why so many researchers have found good correlation between *K* value and sensory evaluation results in seafood storage tests. However, between −3 and 5 °C, the E_A and Q_{10} values for VBN and sensory scores were closer. This resulted in a good agreement between VBN and sensory shelf life values. The E_A and Q_{10} for aerobic plate count were the highest among all quality indicators. It corresponded with the temperature-sensitive nature of microbial growth and led to a high effectiveness of partial freezing at −3 °C on reducing the microbial growth rate. A single slope, i.e., one activation energy value, fitted the shelf life plot for each property change reasonably well. However, it was found that aerobic plate count, VBN, and *K* value data could be better represented by two E_A values (Table 1). This suggested that at ∼5 °C, the temperature sensitivity of bacterial growth, microbial VBN product generation, and nucleotide degradation changed. Changes in these objective freshness indicators were more sensitive to storage temperature when sea bass samples were stored at a temperature below 5 °C. The reason might be that the predominant spoilage microbes >5 °C were different from the ones that predominate below that temperature.

Objective quality indicators are useful when they can foretell the shelf life before a fresh fish becomes spoiled. From that viewpoint, a maximum microbial population of 3×10^6 CFU/g of fish muscle or a maximum *K* value of 50% seemed to be appropriate criteria for sea bass shelf life determination at storage temperatures ≥0 °C. The maximal *K* value of 50% could also be used for determining shelf life at −3 °C. A rapid rise in VBN indicated substantial microbial spoilage. Partial freezing storage was quite effective for extending the shelf life of fresh sea bass. It seemed to have more benefits than disadvantages. Compared with the abundance of literature on the modified-atmosphere packaging of fresh fishes, fewer studies have been done on the pros and cons of partial freezing on the quality change and safety of fresh seafood. Partial freezing appears to be an effective freshness preservation method for fishes. It deserves more investigations.

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