*Asian Fisheries Science* **29** (2016):232-244

ISSN 0116-6514
E-ISSN 2073-3720
https://doi.org/10.33997/j.afs.2016.29.4.006

Asian Fisheries Science
The Journal of the Asian Fisheries Society

# Effect of Freezing and Thawing on the Quality of Northern Bluefin Tuna *Thunnus Thynnus* (Linnaeus 1758)

**HIROKO SEKI, KAORI NAKAZATO, KAZUNORI KOBAYASHI, TAE SOO LEE, MIO SAKURADA and NAOKO HAMADA-SATO***

Tokyo University of Marine Science and Technology, 4-5-7, Konan, Minato-ku, Tokyo, 108-8477

## Abstract

Global tuna populations are in decline. Maintaining post-catch fish quality is important to prevent wastage of precious food resources. Tuna is often preserved by freezing; however, changes in tuna quality post-thawing have been poorly investigated. Therefore, the aim of this study was to evaluate the quality of frozen/thawed and raw flesh of northern bluefin tuna *Thunnus thynnus* **(**Linnaeus 1758) over 7 or 8 days period using characteristic indicators of flesh quality such as colour, bacterial count, K-value, pH and odour. The results showed that colour was significantly influenced by freezing and thawing. The colour of thawed tuna flesh deteriorated after 3 days, while that of raw flesh was maintained for 6 days. In contrast, bacterial count, K-value, pH and odour were not significantly influenced by freezing and thawing. The K-value was approximately 20% after 3 days at 4 °C in both raw and frozen/thawed flesh. Tuna should not be eaten raw if it was stored for longer than 3 days at 4 °C, regardless of whether the tuna was frozen and then thawed or kept raw. Freezing preserves the quality of fish flesh for a long period and is thus a good strategy to prevent tuna spoilage.

## Introduction

In recent years, global tuna populations have been steadily declining (Fisheries Research Agency 2015). To prevent further decline, it is necessary to effectively regulate marine resources and fisheries in order for tuna to be caught sustainably. In addition, it is imperative that the highest possible yield is sustained by ensuring that the quality of tuna is being maintained after catch. As it takes several years for tuna to reach sexual maturity, the overall catch needs to be limited to help populations recover. Although northern bluefin tuna *Thunnus thynnus* (Linnaeus 1758) reaches maturity faster than any other species, 95-99% of current catches were sexually immature (Japan Fisheries Research and Education Agency 2014; Hamasaki et al. 2014). As this species is listed as endangered (IUCN 2014), regulations on tuna catch have been tightened to help populations recover.

*Corresponding author. E-mail address: hsnaoko@kaiyodai.ac.jp

Given these considerations, it is crucial to control the quality of tuna after catch to prevent these precious food resources from being wasted. Normally, northern bluefin tuna are processed by removing blood immediately after catch and are subsequently frozen to maintain the quality. However, the protein in tuna flesh is denatured and flesh quality deteriorates as a result of the freezing/thawing process (Yoneda et al. 2006). Therefore, raw tuna is being increasingly preserved in water with ice at approximately 0 °C without freezing. Raw tuna is highly valuable and sold at a high price; long-term storage, however, is difficult. Generally, the quality of the fish does not deteriorate during freezing, and changes in quality are thus dependent upon the thawing process. Despite the importance of the thawing process in maintaining quality, the effects of freezing/thawing on changes in the quality of tuna flesh have been poorly investigated.

The quality of marine products deteriorates with time and other parameters are known to influence quality. Generally, although bacterial count is the most important factor in the evaluation of food quality, the evaluation of freshness is obtained by calculating the K value from the quantity of degraded products of ATP (ATP = adenosine triphosphate → ADP = adenosine diphosphate → AMP = adenosine monophosphate → IMP = inosinic acid → HxR = inosine → Hx = hypoxanthine) and is also an extremely important parameter in the evaluation of the quality of marine food products. In addition, the freshness of marine products is evaluated by observations of colour, odour and pH values. In addition to these parameters being evaluated individually, it is also widely accepted that they influence each other. Bacteria influence the degradation of ATP-related compounds (Matsumoto and Yamanaka 1991; Yokoyama et al. 1996; Hayashi and Nakata 2003; Seki and Hamada-Sato 2014), while the K value is related to pH (Koseki et al. 2006). Therefore, various parameters should be evaluated comprehensively to assess the quality of marine food products. In this study, the influence of freezing/thawing on the tuna flesh quality using indicators such as bacterial counts, K value, colour, pH and odour was evaluated and compared with that of raw flesh.

## Materials and Methods

### *Raw and frozen tuna samples*

Two northern bluefin tuna (each ~50 cm in length) were caught from the wild in Nagasaki, Japan. Raw tuna was filleted and transported under cold conditions (at 4 °C) to the laboratory at Tokyo University of Marine Science and Technology. Tuna to be frozen was filleted and frozen at −20 °C for 24 h and transported under cold conditions (at 4 °C) to the laboratory. The frozen fillets were thawed at room temperature for 4 h; both fillets were preserved at 4 °C (±0.5 °C). The day that raw fish arrived in the laboratory it was determined as day 0, while for frozen tuna the day 0 was determined the day that the frozen tuna fillets were thawed.

***Bacterial count***

The flesh bacterial count was obtained according to previously published methods described by Seki et al. (2016). Dorsal tuna flesh (10 g) was blended with 90 mL of 0.9% NaCl in sterile water in a sterilized bag (PYXON-30; ELMEX Ltd.) using a stomacher (EXNIZER-400; ORGANO Co., Ltd.) (30 s, 260 rpm). After blending, to obtain a general count of viable bacteria, the supernatant was diluted 10 times using 0.9% NaCl in sterile water. The diluted supernatant (100 µL) was then smeared on a standard agar plate and stored at 35 °C for 48 h to obtain the flesh bacterial count via the plate count method. To obtain a count of marine, salt tolerance bacteria from tuna flesh, the supernatant was smeared onto a standard agar plate with 1% NaCl and stored at 20 °C for 168 h. Previous reports (Fujii 1985; Fukuda et al. 2012) indicated that bacteria isolated from seawater on fresh fish grew better on PCA containing 1% NaCl and incubated at 20 °C than on PCA without NaCl at 35 °C. The bacterial count was determined on both plates every other day for 8 days, starting from day 0.

***Measurement of ATP-related compounds and K-values***

Methods for these analyses followed those described in previous publication (Seki et al. 2016). ATP-related compounds were extracted from 2 g of dorsal flesh in 5 mL of 10% perchloric acid by shaking. Briefly, flesh was removed by centrifugation (11,509×*g*, 10 min, 5 °C), the supernatant was transferred to a 50 mL centrifuge tube, and 10% perchloric acid was added to achieve a volume of 25 mL. The mixture was neutralized using KOH, and the precipitate was removed by centrifugation (13,697×*g*, 5 min, 5 °C). The supernatant was then transferred to a 15 mL centrifuge tube and diluted using sterilized water to achieve a volume of 10 mL. The dilution was then filtered (Millex-LG 0.20 µm) and ATP-related compounds were measured via HPLC (high-performance liquid chromatography) (pump: Hitachi L2130, column: Shodex GS-320 HQ, moving phase: 200 mM $NaH_2PO_4$, flow rate: 0.6 $mL \cdot min^{-1}$, temperature: 30 °C, detector: Hitachi L7420, wavelength: 260 nm). The quantity of ATP-related compounds and K-values were measured daily for 7 days. The K-value was calculated as follows:

$$\text{K-value (\%)} = (\text{HxR} + \text{Hx})/(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}) \times 100.$$

***Measurement of colour, pH and odour***

L*a*b* values, parameters of lightness and hue, of the dorsal flesh were measured using a colorimeter (CR-13; KONICA MINOLTA, Inc.). pH values were measured using a pH meter (D-74; HORIBA, Ltd.) from the dorsal flesh of tuna. The odour of the dorsal flesh was judged by sensory evaluation. The panel for sensory evaluation comprised five untrained individuals. The flesh was smelled, and acceptability was evaluated on the basis of odour. Colour, pH and odour were monitored daily for 7 days from day 0.

***Statistical analyses***

Data were subjected to a one-way ANOVA using the least significant difference method and the Student's *t*-test. Differences were considered statistically significance at a p value of < 0.05.

## Results

***Time-dependent changes in bacterial counts***

The counts of flesh bacteria and marine, salt tolerance bacteria from tuna flesh are shown in Fig. 1A and B, respectively. The flesh bacterial count of frozen/thawed tuna was < $10^2$ CFU·g$^{-1}$ during the 8-day period. Few flesh bacterial counts were confirmed for these periods. The flesh bacterial count in raw tuna was approximately $10^2$ CFU·g$^{-1}$ for 8 days, where little change was observed. The marine bacterial count in frozen/thawed tuna was $10^1$-$10^2$ CFU·g$^{-1}$ at day 0, but it was not detected after 2 days. The marine bacterial count in raw tuna was $10^2$-$10^3$ CFU·g$^{-1}$ at day 0, and although it decreased to $10^1$-$10^2$ CFU·g$^{-1}$ at day 4, the count increased again to $10^2$-$10^3$ CFU·g$^{-1}$ by day 8. The bacterial count and marine bacterial count in raw tuna were higher than those observed in frozen/thawed tuna over the course of 8 days.

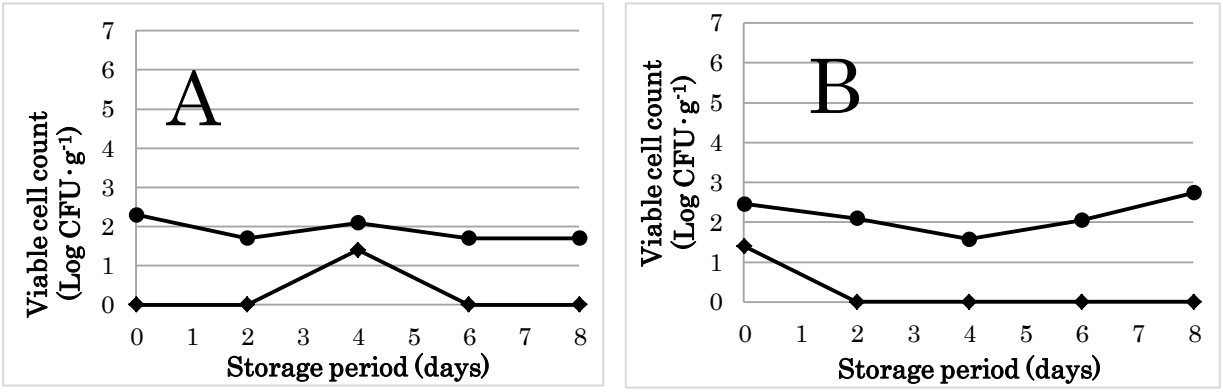


**Fig. 1.** Bacterial count in northern bluefin tuna flesh. Changes were observed in (A) flesh bacterial count, cultured on standard agar plates at 35°C, 2 days, and (B) marine bacterial count, cultured on standard agar plates with 1% NaCl at 20°C, 7 days. ●: raw tuna flesh, ◆: frozen/thawed tuna flesh.

***Time-dependent changes in ATP-related compounds and K-value***

Time-dependent changes in ATP-related compounds in northern bluefin tuna flesh under raw and freezing/thawing conditions are shown in Fig. 2A and 2B, respectively. ATP, ADP, and AMP were hardly detected in both raw and frozen/thawed tuna at day 0 as ATP, ADP, and AMP had already degraded. IMP levels in raw tuna were maintained between 11 µmol·g$^{-1}$ and 14 µmol·g$^{-1}$, for 7 days. In contrast, IMP levels in frozen/thawed tuna were 16 µmol·g$^{-1}$ at day 0, increasing to 17 µmol·g$^{-1}$ by day 2, after which they decreased with time to 11 µmol·g$^{-1}$ by day 7 ($p < 0.05$). IMP levels in raw and frozen/thawed tuna were 12 µmol·g$^{-1}$ and 16µmol·g$^{-1}$, respectively, on day 0.

IMP levels in frozen/thawed tuna were higher than those in raw tuna during the 7-day period. HxR (inosine) levels in raw tuna were 0.93 $\mu mol \cdot g^{-1}$ on day 0 and 5.2 $\mu mol \cdot g^{-1}$ on day 7, showing an increase with time ($p < 0.05$). HxR levels in frozen/thawed tuna were 1.2 $\mu mol \cdot g^{-1}$ on day 0 and 1.8 $\mu mol \cdot g^{-1}$ on day 7; the levels also increased significantly with time ($p < 0.05$). HxR levels accumulated faster in raw tuna than in frozen/thawed tuna, such that HxR levels were three times higher in raw tuna on day 7 than in frozen/thawed tuna. Hx (hypoxanthine) levels in raw tuna were 0.071 $\mu mol \cdot g^{-1}$ on day 0, and slowly increased to 0.69 $\mu mol \cdot g^{-1}$ on day 7 ($p < 0.05$). In contrast, Hx levels in frozen/thawed tuna were 0.15 $\mu mol \cdot g^{-1}$ on day 0, and increased to 1.8 $\mu mol \cdot g^{-1}$ on day 7 ($p < 0.05$). Hx levels in frozen/thawed tuna accumulated faster than in raw tuna. Time-dependent changes in the K-value of northern bluefin tuna in response to raw and freezing/thawing conditions are shown in Fig. 3. The K-value of raw tuna was 7.6% on day 0, which increased over time to 37% on day 7 ($p < 0.05$). The K-value of frozen/thawed tuna was 7.9% on day 0, which increased over time to 30% on day 7 ($p < 0.05$). Although the K-values of both raw and frozen/thawed tuna did not differ significantly on day 0 ($p > 0.05$), the K-value of raw tuna was significantly higher than that of frozen/thawed tuna after day 1 ($p < 0.05$), with the exception of day 6 ($p > 0.05$).

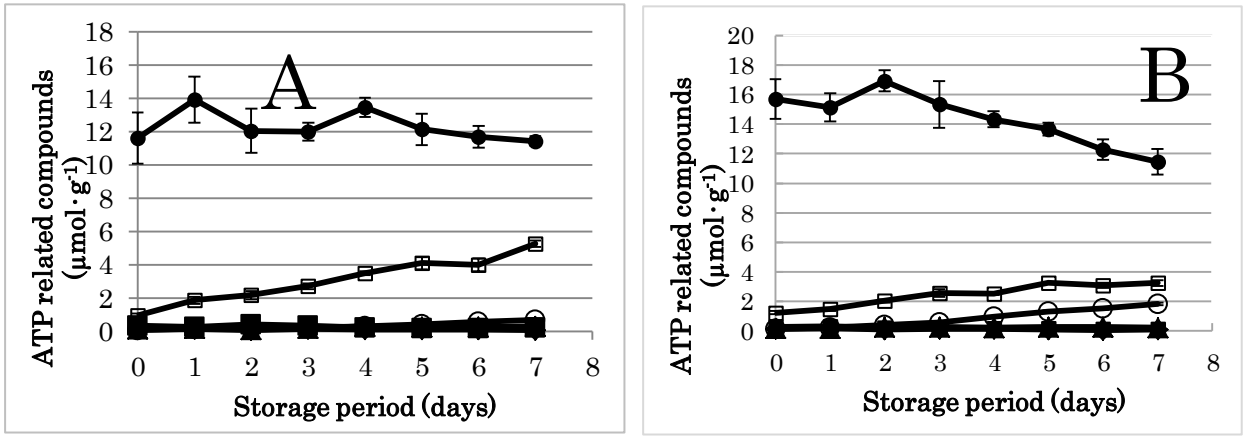


**Fig. 2.** Time-dependent changes in the quantity of ATP-related compounds in northern bluefin tuna flesh. Changes were observed with time in (A) raw tuna flesh and (B) frozen/ thawed tuna flesh. Error bars denote standard deviations of the mean (n = 9). ◆ ATP, ■ ADP, ▲ AMP, ● IMP, □ HxR, ○ Hx.

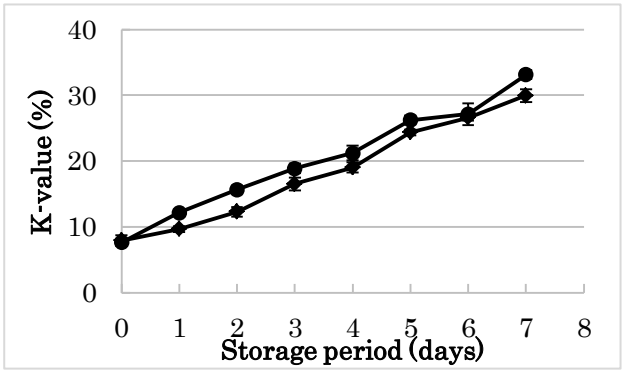


**Fig. 3.** Time-dependent changes in K-values in northern bluefin tuna flesh. Error bars denote standard deviations of the mean (n = 9). ●: raw tuna flesh, ◆: frozen/thawed tuna flesh.

***Colour, pH and odour***

Time-dependent changes in colour were measured using the L*a*b* value, where the L* value represents the degree of whiteness, the a* value represents redness, and the b* value represents yellowness. When whiteness, redness, and yellowness increase, so do their corresponding values. Time-dependent changes in the L*a*b* value of northern bluefin tuna in response to raw and freezing/thawing conditions are shown in Fig. 4A and 4B, respectively. An increase in L* value denotes a deterioration in quality (Pong et al. 2000; Tsukamasa 2005). In raw tuna, the L* value was between 28 and 31 and did not significantly ($p > 0.05$) increase over the course of the 7-day period. In frozen/thawed tuna, the L* value was observed to be 30 on day 0, decreased to 27 on day 2, increased after day 4, and ultimately reached 32 on day 7 ($p < 0.05$). A significant difference in the L* value was only observed between raw and frozen/thawed tuna on day 7 ($p<0.05$), with no significant ($p > 0.05$) differences noted from days 0 to 6. In raw tuna, the a* value ranged between 6.5 and 7.8, where no significant ($p > 0.05$) differences were observed over the course of the 7-day period. In contrast, in frozen/thawed tuna, the a* value significantly ($p < 0.05$) decreased with time, where the value was observed to be 12 on day 0 and 2.8 on day 7. Differences in the time course of the a* values in both raw and frozen/thawed tuna were observed. In raw tuna, the b* value ranged between 5.8 and 7.0, where no significant ($p > 0.05$) differences were observed from days 0 to 6, followed by an increase in b* value 8.6 on day 7. In frozen/thawed tuna, the b* value was 9.2 on day 0, significantly ($p < 0.05$) decreased to 6.6 on day 1, and then increased to 12 on day 7.

Time-dependent changes in the pH of northern bluefin tuna in response to raw and freezing/thawing conditions are shown in Fig. 5. The pH values ranged between 5.8 and 5.9 for raw tuna and between 5.9 and 6.0 for frozen/thawed tuna during the 7-day period. No significant ($p > 0.05$) differences in pH values were observed in raw or frozen/thawed tuna over the course of the observational period. However, the pH of frozen/thawed tuna was significantly ($p<0.05$) higher than that of raw tuna during the entire 7-day period. In addition, the odour of both raw and frozen/thawed tuna was acceptable over the 7-day period.

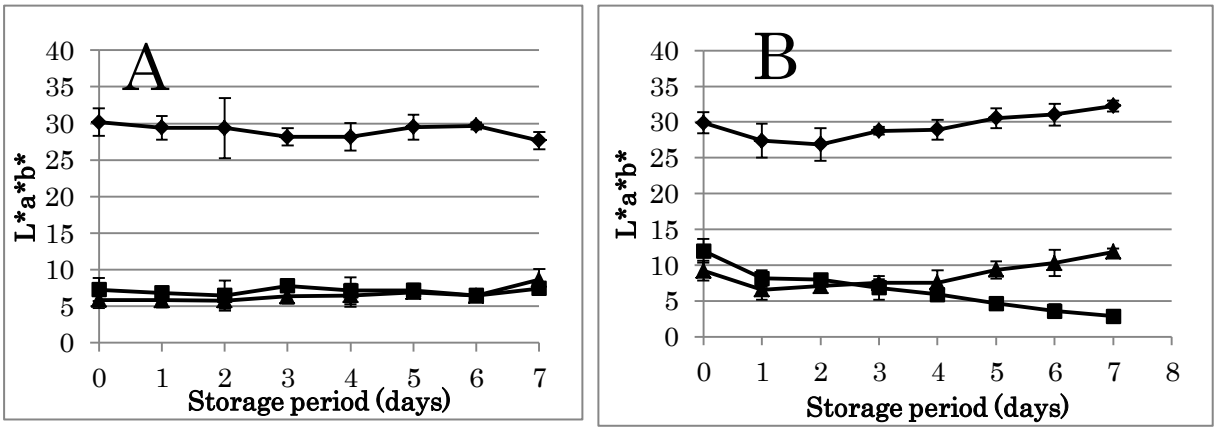


**Fig. 4.** Time-dependent change in the colour of northern bluefin tuna flesh. Changes were observed in (A) raw tuna flesh and (B) frozen/thawed tuna flesh. Error bars denote standard deviations of the mean (n = 3). ◆: L* value, ■: a* value, ▲: b* value.

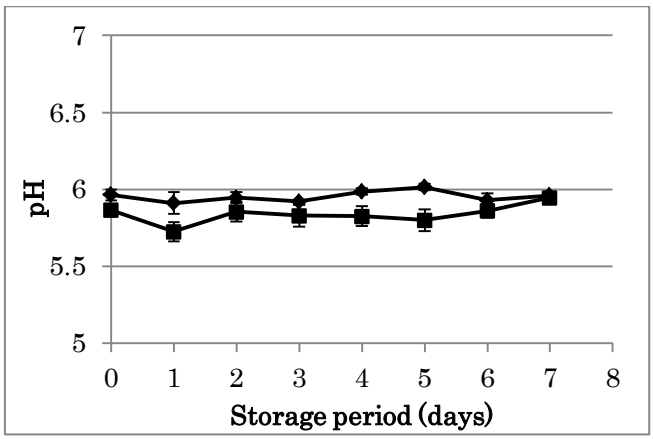


**Fig. 5**. Time-dependent changes in the pH values of northern bluefin tuna flesh. Error bars denote standard deviations of the mean (n = 2). ■: raw tuna flesh, ◆: frozen/thawed tuna flesh.

## Discussion

### *Time-dependent changes in bacterial count*

The flesh bacterial count and marine bacterial count in frozen/thawed tuna were not detected over the course of 8 days (Fig. 1B). However, the bacterial count in yellowfin tuna *Thunnus albacares* (Bonnaterre 1788) was reported to be higher than the findings in the present study, measuring between $10^1$ and $10^3$ CFU·g$^{-1}$ for 4 days and varied widely between $10^0$ and $10^3$ CFU·g$^{-1}$ after day 4 (Gill et al. 1987). One possible explanation for the difference in the bacterial count results obtained by Gill et al (1987) and the present study could be due to the differences in the storage temperatures used in the two studies. Bacteria generally do not exist in live fish muscle, and thus any bacteria detected in the dead fish muscle tissues are from the external surface of the body or from organs. Therefore, it is easy to detect bacteria in dead fish muscle if the fish had died for a longer period. As the tuna in this study were filleted and frozen immediately after catch, it is not surprising that bacteria was hardly detected. In addition, the flesh bacterial count and marine bacterial count were $10^1$–$10^2$ CFU·g$^{-1}$ on day 4 on day 0, respectively (Fig. 1B). It is a distinct possibility that flesh bacterial count samples were contaminated during processing, as flesh bacterial count was not detected on days 3 and 5. A previous study that measured the marine bacterial count in marine food products revealed that the incidence of psychrotrophic bacteria was markedly elevated just after thawing (Fukuda et al. 2012). Likewise, the presence of marine, salt tolerance bacteria from tuna flesh was detected on day 0 in frozen/thawed tuna in the present study.

The flesh bacterial count of raw tuna was $10^2$ CFU·g$^{-1}$ over the course of the 8-day period (Fig. 1A). The bacterial count in raw flesh from the Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel 1844) reared under culture conditions showed $10^3$ CFU·g$^{-1}$ during a 6-day period (Tsukamasa 2005). Similar to the present study, the bacterial count in raw tuna flesh did not increase over time. Furthermore, Tsukamasa (2005) obtained a higher flesh bacterial count than that observed in the present study. One possibility is that the previous study involved a longer fillet-processing time, thus giving bacteria ample time to invade the muscle tissues.

In the present study, the marine bacterial count in raw tuna decreased until day 4, after which it increased until day 8 (Fig. 1A). It was previously reported that the psychrotrophic bacterial count in both Pacific bluefin tuna (Tsukamasa 2005) and Atlantic cod *Gadus morhua* Linnaeus 1758 (Magnusson and Martinsdottir 1995) increased after day 4, as was observed in this study. The flesh bacterial count and marine bacterial count were higher in raw tuna than in frozen/thawed tuna during the 8-day period of observation (Fig. 1A and B). A previous study showed that, in general, although a portion of bacteria are killed by freezing, counts increase again after thawing (Nagase and Wakisaka 2008). However, as this increase was not observed in this study, it is possible that any existing bacteria in the tuna flesh perished in the freezing process, and that flesh was not contaminated by bacteria owing to rapid filleting/processing.

***Time-dependent changes in ATP degradation and K-value ATP***

In the present study, ATP, ADP and AMP were not detected in raw or frozen/thawed tuna over the course of the 7-day period, although a high quantity of IMP was detected on day 0 (Fig. 2). Kimura et al. (2014) reported that ATP in Pacific bluefin tuna is already degraded to IMP on day 0. The quantity of IMP in both raw and frozen/thawed tuna comprised 70% of all ATP-related compounds on day 7 in the present study. The quantity of IMP in Pacific bluefin tuna and spotted mackerel *Scomber australasicus* Cuvier 1832 was at its highest on day 0 and comprised 70% of all ATP-related compounds on day 7 (Shiraita et al. 2012; Kimura et al. 2013; Kimura et al. 2014), as seen in the present study. IMP is degraded by IMP-degrading enzyme (IMPase) that is present in fish muscle. In the present study, IMP levels were higher in frozen/thawed tuna than in raw tuna. IMPase activity in frozen/thawed New Zealand golden Snapper *Pagrus auratus* (Forster 1801) decreased quickly from day 0 to day 1, although IMPase activity in raw fish decreases slowly from day 0 to day 3 (Nedachi and Hirota 1991).

Therefore, in the present study, it can be surmised that freezing influenced IMPase activity in northern bluefin tuna. Inosine (HxR) was accumulated at higher levels in raw tuna than in frozen/thawed tuna, while hypoxanthine (Hx) levels were higher in frozen/thawed tuna than in raw tuna during the 7-day period. In line with these observations, Hx accumulation in frozen fish tissues has been previously reported in bigeye tuna *Thunnus obesus* (Lowe, 1839) (Sakaguchi 1996) and yellowfin tuna (Gill et al. 1987). Thus, freezing might promote the breakdown of HxR to Hx. Although Fletcher and Statham (1988) reported that bacteria promote this degradation, a bacterial count in frozen/thawed tuna was not detected in the present study (Fig. 1), eliminating this as a possibility. As ATP-related compounds are degraded by enzymes present in fish muscle, freezing undoubtedly influences the activities of the enzymes responsible for the breakdown of IMP and HxR.

The K value of raw tuna was higher than that of frozen/thawed tuna over 7 days (Fig. 3). However, the K values of Pacific bluefin tuna changed from year to year (Shiraita et al. 2012; Kimura et al. 2013; Kimura et al. 2014) and can vary by 2–3% between the dorsal and ventral flesh of the same fish (Nakamura et al. 2006). As the K value is highly correlated with IMPase activity (Tomioka and Endo 1984), the effect of freezing/thawing on IMPase should be investigated in the future.

***Colour, pH and odour***

Although no differences in L*a*b* values were observed during the 7-day period in raw tuna, in frozen/thawed tuna, the L* value increased, the a* value decreased, and the b* value decreased at first and then increased again. As the L* value represents whiteness, a decrease of transparency and turbidity will increase the L* value (Okamoto 2010). The increase in the L* value observed in frozen/thawed tuna in the present study indicates an increase in turbidity with time. The L* value is dependent upon the freezing temperature, being suppressed below −30 °C and increasing above −20 °C (Chow et al. 1988). In the present study, as tuna flesh was frozen at −20 °C, the L* value of frozen/thawed tuna increased. However, while an increase in the L* value was not observed in raw tuna, increases in the L* value of tuna flesh stored in 100% relative humidity at 4 °C have been previously reported by Pong et al. (2000). The effects of relative humidity and of individual differences will need to be further investigated.

The a* and b* values represent the redness and yellowness parameters, respectively, and a*/b* = 1 gives a colour degradation reference value. The colour is acceptable when a*/b* is > 1 in *T. thynnus* and *T. albacares* (Ochiai et al. 1988). The change in the a*/b* value of raw and frozen/thawed tuna over time is shown in Table 1. In frozen/thawed tuna, the values of a*/b* were > 1 during the first 2 days, but decreased to a*/b* < 1 after day 3, which is an indication that the colour deteriorated. In contrast, in raw tuna the value of a*/b* > 1 during the first 5 days, and changed to a*/b* = 1 on day 6, and then decreased to a*/b* < 1 on day 7. Thus, the colour of raw tuna deteriorated 3 days later than it did in frozen/thawed tuna. Similar reports exist indicating that frozen/thawed tuna discolours more quickly than unfrozen tuna (Ochiai et al. 1988) and that this discolouration occurs at temperatures below freezing (Hashimoto and Watabe 1983; Matthews 1983; Watabe and Hashimoto 1987; Imamura et al. 2012).

In addition, when tuna flesh is stored between −20 °C and −80 °C, the discolouration of the flesh stored at −20 °C is the fastest (Chow et al. 1988), the same temperature at which frozen/thawed tuna flesh was stored in the present study. It is important to limit the amount of metmyoglobin (metMb) in tuna flesh to maintain the flesh colour. Based on the report by Yamanaka et al. (1973), metMb reductase activity decreased markedly at −20 °C, thus the tuna flesh in the current study was preserved at −20 °C. Since the discolouration of tuna flesh is reported to occur at temperatures between −4°C and −7°C (Sugimoto 1987), it is important to decrease the storage time at these temperatures to prevent flesh discolouration. In the present study, storage times in this temperature range were not shortened.

The ΔE values, which indicate colour differences, are shown in Table 2. ΔE values ranged from 3.4 to 5.7 on days 0 and 2 and increased from 2.8 on day 3 to 7.5 on day 7, indicating that there were colour differences between frozen/thawed and raw tuna. ΔE was quantified according to The National Bureau of Standards (NBS) rates, where ΔE values of 3.0-6.0 are considered to represent a ‘marked change’ and ΔE values of 6.0−12.0 are considered to represent an ‘extremely marked change’ (Ergun and Nagas 2007). The difference in colour becomes noticeable as the value of ΔE increases, thereby affecting the value of tuna. In the present study, the pH of frozen/thawed was higher than that of raw tuna (Fig. 5).

The pH of fish flesh is known to decrease immediately after death (Fukuda 1995; Koseki et al 2006), and is not influenced by freezing/thawing, but rather depends on the state of freshness at the time the flesh was frozen (Fukuda 1995). As the tuna flesh in this study was already fillet-processed, the pH of the flesh had already decreased. It has been reported that the pH of frozen/thawed bluefin tuna ranged from 5.4-5.6 (Kodani et al. 2009) and 6.0-6.2 (Kojima and Muraji 1977), while the pH values of raw bluefin tuna range between 5.9 and 6.0 (Kimura et al. 2014) and 5.7 and 6.0 (Nakamura et al. 2006), indicating possible individual differences in pH.

In this study, no flesh bacteria or marine bacteria were detected over the 7-day period, and thus there was no odour of both raw and frozen/thawed tuna making it acceptable regardless of storage regime.

**Table 1**. Changes in a*/b* values over time in frozen/thawed and raw northern bluefin tuna flesh.

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| Frozen/ thawed tuna | 1.3 (0.078) | 1.3 (0.17) | 1.1 (0.21) | 0.91 (0.21) | 0.82 (0.24) | 0.50 (0.10) | 0.36 (0.12) | 0.24 (0.070) |
| Raw tuna | 1.2 (0.097) | 1.2 (0.18) | 1.1 (0.14) | 1.2 (0.14) | 1.1 (0.29) | 1.1 (0.16) | 1.0 (0.076) | 0.88 (0.098) |

Values represent the mean of six independent determinations (n = 6). Standard deviations are given in parentheses.

**Table 2.** Changes in ΔE values over time in frozen/thawed and raw northern bluefin tuna flesh.

| | Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---|---|---|---|---|---|---|---|---|
| ΔE | 6.7 (3.7) | 3.4 (2.0) | 5.7 (4.3) | 2.8 (0.49) | 4.0 (1.9) | 3.8 (1.2) | 5.4 (1.2) | 7.5 (1.2) |

Values represent the mean of six independent determinations (n = 6). Standard deviations are given in parentheses. Values were recalculated using the formula $[(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$

## Conclusion

This study reports the outcome of a comparison of changes in the quality of frozen/thawed bluefin tuna flesh and raw bluefin tuna flesh for a period of 7 or 8 days, as well as the effect of freezing/thawing. Significant differences in colour were observed between frozen/thawed tuna and raw tuna. The colour of frozen/thawed tuna deteriorated 3 days sooner than it did in raw tuna, which deteriorated after day 6. However, no differences in flesh bacterial counts and marine bacterial counts, K values, pH values, or odour were detected between frozen/thawed tuna and raw tuna. In addition, the K-value was approximately 20% at day 3 at 4 °C in both raw and frozen/thawed flesh, regardless of storage regime.

As seafood can typically be eaten raw when the K value is below 20%, this value indicates that the storage limit for raw consumption is 3 days at 4 °C. Since freezing preserves the long-term quality of fish flesh, this storage strategy is vital to prevent the unnecessary wasting of this precious resource.

## References

Chow, C.J., Y. Ochiai, S. Watabe and K. Hashimoto. 1988. Effect of freezing and thawing on the discoloration of tuna meat. Nippon Suisan Gakkaishi 54:639-648.

Ergun, G. and I. C. Nagas. 2007. Color stability of silicone or acrylic denture liners: an in vitro investigation. European Journal of Dentistry 1:144-151.

Fisheries Research Agency. 2015. The current status of international fishery resources: Pacific Bluefin Tuna, *Thunnus orientalis*. http://kokushi.fra.go.jp/H27/H27_04S.pdf. Accessed 10 November 2016.

Fletcher, G.C. and J.A. Statham. 1988. Shelf-life of sterile yellow-eyed mullet (*Aldrichetta forsteri*) at 4 °C. Journal of Food Science 53:1030-1035.

Fujii, T. 1985. Comparative studies on methods for determination of bacterial counts in seafoods. I. Comparisons of media, incubation temperatures and plating methods. Bulletin of Tokai Regional Fisheries Research Laboratory 118:71-79. (in Japanese with English abstract).

Fukuda, Y. 1995. Effect of the meat quality by pH decrease caused in and after the death of fish meat. Fisheries Research 14:36-41. (in Japanese).

Fukuda, T., M. Furushita and T. Shiba. 2012. Viable counts of bacteria determined on food fishes by the standard-agar-plate method incubated at 35 °C and nutrient-agar-plate method incubated at 20 °C. Journal of National Fisheries University 60:183-188. (in Japanese with English abstract).

Gill, T.A., J.W. Thompson, S. Gould and D. Sherwood. 1987. Characterization of quality deterioration in yellowfin tuna. Journal of Food Science 52:580-583.

Hamasaki, H., N. Kato and T. Ikuta. 2014. Fishery sustainability through stock management case study of Pacific bluefin tuna in Iki, Nagasaki. FRI Research Report, No.418. http://www.fujitsu.com/jp/group/fri/en/economic/publications/report/2015/report-418.html. Accessed 31 August 2016.

Hashimoto, K. and S. Watabe. 1983. Changes in color and water holding capacity of tuna meat during frozen storage. Nippon Suisan Gakkaishi 49:203-206. (in Japanese with English abstract).

Hayashi, M. and K. Nakata. 2003. Effect of contaminating bacteria on the inosinic acid content of chicken meat. Journal of Home Economics of Japan. 54:441-448. (in Japanese with English abstract).

Imamura, S., M. Suzuki, Y. Murata, M. Kimura, T. Kimiya, E. Okazaki and Y. Hiraoka. 2012. Prevention of thaw-rigor during frozen storage of bigeye tuna *Thunnus obesus* and meat quality evaluation. Fisheries Science 78:177-185.

IUCN. 2014. Global appetite for resources pushing new species to the brink – IUCN Red List. https://www.iucn.org/content/global-appetite-resources-pushing-new-species-brink-%E2%80%93-iucn-red-list. Accessed 8 September 2016.

Japan Fisheries Research and Education Agency. 2014. Resources and biology of tuna. Seizando-shoten, Tokyo. pp 51–52, 104–105.

Kimura, G., R. Suzuki and H. Matsubara. 2013. Methods to maintain the freshness of Pacific Bluefin tuna (*Thunnus orientalis*). Report of Aomori Prefectural Industrial Technology Research Center Food Research Institute 4:18-23. (in Japanese).

Kimura, G., M. Takeuchi and H. Matsubara. 2014. Effect of "Kaimin" processing on freshness of Pacific Bluefin tuna (*Thunnus orientalis*) under storage in ice. Report of Aomori Prefectural Industrial Technology Research Center Food Research Institute 5/6:1-6. (in Japanese).

Kodani, Y., M. Honda, A. Kato, M. Matsumoto and H. Nakano. 2009. The studies on the techniques of freezing and storage of *Thunnus thynnus* (1st report). Industrial Technology Institute, Tottori Prefecture Reports of the Industrial Research Institute of Tottori Prefecture 11:18-24. (in Japanese with English abstract).

Kojima, T. and T. Muraji. 1977. Thawing time and quality of some frozen foodstuff after vacuum-, water- and air-thawing. The Refrigeration 52:577-585. (in Japanese with English abstract).

Koseki, S., S. Kitakami, N. Kato and K. Arai. 2006. Rigor mortis of fish and shellfish and evaluation of freshness of their muscles as K value. Journal of the School of Marine Science and Technology, Tokai University 4:31-46. (in Japanese with English abstract).

Magnusson, H. and E. Martinsdottir. 1995. Storage quality of fresh and frozen-thawed fish in ice. Journal of Food Science 60:273-278.

Matsumoto, M. and H. Yamanaka. 1991. Influences of antibiotics chloramphenicol on post-morten biochemical changes in the muscle of kuruma prawn during storage. Nippon Suisan Gakkaishi 57:2291-2297. (in Japanese with English abstract).

Matthews, A.D. 1983. Muscle colour deterioration in iced and frozen stored bonito, yellowfin and skipjack tuna caught in Seychelles waters. International Journal of Food Science & Technology 18:387-392.

Nagase, N. and Y. Wakisaka. 2008. Survival and recovery of bacteria in the commercial chicken after refrigeration. Kenmei Girl School Junior College, Research Bulletin 43:15-24. (in Japanese).

Nakamura, Y., M. Ando, M. Seoka, K. Kawasaki and Y. Tsukamasa. 2006. Changes in physical/chemical composition and histological properties of dorsal and ventral ordinary muscles of full-cycle cultured Pacific bluefin tuna, *Thunnus orientalis*, during chilled storage. Journal of Food Science 71:E45-E51.

Nedachi, K. and N. Hirota. 1991. Changes in ATP related compounds and IMP-degrading enzyme activity of New Zealand golden snapper. Nippon Suisan Gakkaishi 57:329-335. (in Japanese with English abstract).

Ochiai, Y., C.J. Chow, S. Watabe and K. Hashimoto. 1988. Evaluation of tuna meat discoloration by Hunter color difference scale. Nippon Suisan Gakkaishi 54:649-653.

Okamoto, A. 2010. A trial of the high quality maintenance technology development of squid. Development of Fisheries. 2010 105. http://www.marinelabo.nagasaki.nagasaki.jp/news/suisankaihatsu/no105.pdf. Accessed 31 August 2016. (in Japanese).

Pong, C.Y., T.K. Chiou, M.L. Ho and S. T. Jiang. 2000. Effect of polyethylene package on the metmyoglobin reductase activity and color of tuna muscle during low temperature storage. Fisheries Science 66:384-389.

Sakaguchi, M. 1996. Quality and flavor of frozen and unfrozen tuna meat. Studies on Flavor and Texture of Live Fish Meat 06453178:46-67. (in Japanese).

Seki, H. and N. Hamada-Sato. 2014. Identification of bacteria that contribute to IMP degradation in horse mackerel. Journal of Food Processing & Technology 5:363. DOI: 10.4172/2157-7110.1000363

Seki, H., K. Nakazato, K. Kobayashi, M. Sakurada, T. S. Lee and N. Hamada-Sato. 2016. Effect of different packaging materials on the quality of tuna and use of corrugated cardboard as suitable packaging material for fisheries logistics. International proceedings of chemical, biological and environmental engineering 92:8-17.

Shiraita, T., Y. Kado and H. Matsubara. 2012. Maintenance of freshness of spotted mackerel (*Scomber australasicus*). Report of Aomori Prefectural Industrial Technology Research Center Food Research Institute 3:9–11. (in Japanese).

Sugimoto, M. 1987. Improvement of the preserving quality of foods by refrigerating. Food composition. Natural coloring matters. The Refrigeration 62:1295-1301. (in Japanese).

Tomioka, K. and K. Endo. 1984. K value-increasing rates and IMP-degrading activities in various fish muscles. Nippon Suisan Gakkaishi 50:889–892. (in Japanese with English abstract).

Tsukamasa, Y. 2005. The quality change and the color tone maintenance of cultured bluefin tuna meat under refrigeration. The 21st-century COE program of Kinki University. The supportive-type research base of the fish culture industry of bluefin tuna and others reports. pp. 163–166. (in Japanese).

Watabe, S. and K. Hashimoto. 1987. Improvement of the preserving quality of foods by refrigerating. Marine products. Quality deterioration of tuna meat during frozen storage. The Refrigeration 62:1302-1308. (in Japanese).

Yamanaka, H., M. Takamizawa and K. Amano. 1973. Relation between the colour of tuna meat and the activity of metmyoglobin reductase– Ⅱ Change in the activity of metmyoglobin reductase during frozen-storage and subsequent thawing of tuna meat. Nippon Suisan Gakkaishi 39:673-681. (in Japanese with English abstract).

Yokoyama, Y., M. Sakaguchi, Y. Azuma, F. Kawai and M. Kanamori. 1996. Postmortem changes of ATP and its related compounds in oyster tissues in the presence of antibiotic chloramphenicol. Fisheries Science 62:312-316.

Yoneda, C., M. Kasai, K. Hatae, Y. Hirota and J. Nakamura. 2006. Quality of frozen tuna meat thawed by three different methods. Journal of Cookery Science of Japan 39:16-21. (in Japanese with English abstract).

*Received: 03/10/2016; Accepted: 13/12/2016 (MS16-65)*