Preventive method of color deterioration of yellowtail dark muscle during frozen storage and post thawing

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ABSTRACT: The aim of this study was to develop a practical preventive method for color deterioration of sliced or filleted yellowtail muscle, especially of dark muscle, during frozen storage and post thawing. When the sliced meats were packaged in a vacuum with low oxygen permeable flexible films and then stored frozen below \(-40^\circ C\), no significant discoloration or browning of dark muscle was observed for 9 months or more. For higher temperature storage at \(-20^\circ C\) or \(-30^\circ C\), nitrogen gas substituted packaging was a useful practical method for storing sliced meats for 6 weeks. In order to prevent color deterioration of sliced meats after thawing and subsequent storage at \(0^\circ C\), the efficacy of materials of packaging was investigated. The most desirable result was obtained by using a film with oxygen permeability of 50–90 cm\(^3/m^2\) per 24 h.

KEY WORDS: color deterioration, dark muscle, discoloration, fish meat, meat color, packaging, yellowtail.

INTRODUCTION

In the southern parts of Japan, the culture of yellowtail (153 000 tons in 2001) is commercially prosperous and forms the foundation of the indigenous marine products industry. Although the cultured yellowtail is conventionally shipped in rounds to fish markets or customers, shipment methods have changed rapidly from conventional rounds to fillets. Presently, a relatively large proportion of the fresh raw yellow tail trade is in iced and chilled fillets for short-term storage for a day or so during transport to customers. In contrast, freezing is superior in terms of quality maintenance for long-distance or international transportation to other regions. However, undesirable discoloration, especially rapid darkening or browning of dark muscle containing a considerable amount of myoglobin, generally occurs when fish meat fillets or slices are frozen and thawed.

Brine-frozen bonito can be stored for 3 months at \(-30^\circ C\) or for more than 6 months in \(-40^\circ C\) storage. When tuna is stored at \(-35^\circ C\) or below, the dark muscle color is retained for more than 3 months.1–4 Hashimoto and Watabe reported that discoloration of tuna meat, taking the metmyoglobin (metMb) to myoglobin (Mb) ratio as a parameter, proceeds very slowly below \(-40^\circ C\).5 Although metMb formation was accelerated at \(-20^\circ C\), the water-holding capacity and pH of tuna meat remains relatively constant for 1 year. Chow et al. clearly demonstrated that the browning of tuna meat that was frozen then thawed proceeded remarkably faster than that of unfrozen meat.6 There has been much research on quality control for various seafoods with gas substituted or vacuum packaging during storage in low temperatures.7–16 Ueoka et al. reported that a nitrogen-gas substituted atmosphere effectively prevented browning of dark muscle of cultured yellowtail in ice storage.7,8 However, there are only few reports available regarding frozen storage and chilled storage after thawing of yellowtail dark muscle.

The aim of the present study was to develop a practical preventive method for dark muscle browning of cultured yellowtail in filleted or sliced forms after thawing as well as during frozen storage, because dark muscle browning develops remarkably after thawing.

MATERIALS AND METHODS

Preparation of dark muscle

After cultured yellowtail was killed (average length 67 cm, average weight 5.4 kg, \(n = 10\)), dark muscle,
which was taken from the fillet under the lateral line, was immediately cut into four pieces, approximately 20 g in size (8.0 cm length, 4.0 cm width, 0.6 cm thickness) for use as measurement samples.

Packaging materials

The packaging materials with different degrees of oxygen permeation (cm$^3$/m² per 24 h, 25°C, 100% relative humidity) used were as follows: no. 1 (NY/K-EVA/LDPE), 1.5; no. 2 (CPP/PVDC/PP), 24; no. 3 (OPP/EVA/LDPE), 54; no. 4 (LDPE/NY), 89; no. 5 (PET/CPP), 100; and no. 6 (HDPE), 1100; where NY is nylon, K-EVA is K-ethylene vinyl acetate copolymer, LDPE is low density polyethylene, CPP is casting polypropylene, PVDC is poly (vinylidene chloride), PP is polypropylene, OPP is oriented polypropylene, EVA is ethylene vinyl acetate, PET is poly (ethylene terephthalate) and HDPE is high density polyethylene.

Storage conditions

Dark muscle samples (20 g each) were packed in vacuum- and in gas-modified atmospheres using gas substitution equipment (TVG 447-S; Nisihara, Hiroshima, Japan) with different degrees of oxygen permeation films. After slow freezing by placing them in freezers (MDF-192; Sanyo, Tokyo, Japan) at atmosphere temperatures of −20 ± 1°C, −30 ± 1°C, −40 ± 1°C and −70 ± 1°C, they were stored at −20°C, −30°C, −40°C and −70°C, respectively. In the case of quick freezing, dark muscle in the package was frozen in a contact freezer at −40°C, then stored at −20°C, −30°C, −40°C and −70°C. The temperature changes during the freezing process were monitored with a digital temperature recorder (DR015; Chino Works, Tokyo, Japan). In the case of unfrozen storage, samples were stored at 0°C, 10°C and 20°C. Frozen samples were thawed by dipping in cold water at 5°C with the package and then the color was measured.

Hunter color measurements

Color measurements were performed using a color difference meter (Color Ace TCA-A; Tokyo Denshoku, Tokyo, Japan) with the Hunter L, a, b scale standardized using a white and black standard plate. As reported by Ochiai et al., the redness value ‘a’ of fresh raw dark muscle is high and ‘b’ (yellowness) is low because of the high level of Mb. When Mb is autoxidized into metMb, the a-value significantly decreases, whereas L- and b-values increase to some extent as browning progresses. Ochiai et al. demonstrated that the a/b ratio is a useful parameter for evaluating the browning of tuna meat because of a good inverse correlation with the metMb percentage in the meat. Therefore, the a/b ratio decreases with the progress of meat discoloration. For convenience, the b/a ratio was used as an indicator of browning progress. When the value of b/a exceeds approximately 0.5, browning is only slight; when the ratio exceeds 0.8, browning becomes unmerchantable. The reported values are an average of five measurements that were taken per sample for two samples per treatment.

RESULTS

Browning of dark muscle during frozen storage

The temperature changes at the center of meats are shown in Figure 1 during slow freezing (in air) and quick freezing on a contact freezer. Even in air at −70°C, the meat sample took 30 min to freeze, whereas it took only 1 min on a contact freezer at −40°C. In order to examine the effect of temperature on browning of dark muscle of cultured yellowtail during frozen storage, samples were stored at various temperatures (−20°C, −30°C, −40°C and −70°C) after vacuum packing.

Fig. 1 Temperature changes at the center of the meat. The meat samples were frozen in refrigerators at (a) −20°C, (b) −30°C, (c) −40°C and (d) −70°C. (e) For quick freezing, the meat was frozen on a contact freezer at −40°C.
with packaging material no. 1. Figure 2 shows the changes in degree of browning (b/a) during frozen storage. For dark muscle stored at both \(-20^\circ\text{C}\) and \(-30^\circ\text{C}\) after slow freezing, the browning progressed rapidly in the first stage and the browning degree exceeded 0.5 within 2 weeks. Frozen storage at \(-30^\circ\text{C}\) after quick-freezing resulted in similar results to slow freezing. Therefore, the freezing rate was not critical. The browning degree became as large as approximately 0.8 after 1 month of storage. However, in samples stored at \(-40^\circ\text{C}\) and \(-70^\circ\text{C}\) after slow freezing, the browning degree was 0.24 and 0.16, respectively, even after 9 months; their browning progress was slight. Therefore, when dark muscle was stored at low temperatures, below \(-40^\circ\text{C}\), metMb formation as a cause of browning was well retarded.

A method enabling the storage of dark muscle at relatively high temperatures (\(-20^\circ\text{C}\) or \(-30^\circ\text{C}\)) was also examined because of the expensive running costs of low temperature storage, below \(-40^\circ\text{C}\), and the cost of a cold store. Yellowtail dark muscle was stored frozen at \(-20^\circ\text{C}\) or \(-30^\circ\text{C}\) by gas substitution packaging with nitrogen gas and carbon dioxide gas using a bag (no. 1) as well as in a vacuum. The change in degree of browning (b/a) was measured over time during storage (Figs 3,4). The dark muscle color was preserved for 2 weeks with vacuum packaging. It was extended for 6 weeks with nitrogen gas substituted packaging. Nitrogen gas substitution was able to avoid browning for a long time. Carbon dioxide also depressed the browning, but was less effective.
**Browning of dark muscle after thawing**

Because frozen yellowtail meat is not suitable for merchandise because dark muscle browning is remarkably accelerated after thawing, a method for preventing the browning of thawed dark muscle was examined.

For comparison, first, the effect of storage temperatures above 0°C on browning of fresh raw dark muscle was examined (Fig. 5). To prevent evaporation of moisture, raw dark muscle was wrapped with packaging material no. 1 and stored at 0°C, 10°C and 20°C. The browning degrees of the meat reached 0.45, 0.52 and 0.72, respectively, after 6 h. Thus, the raw meat color was preserved as bright red during storage at 0°C and 10°C for at least 6 h.

The influence of frozen storage temperature on the browning of thawed dark muscle was investigated (Fig. 6). Dark muscle packaged in nitrogen gas was stored at various temperatures (−20°C, −30°C and −70°C) for a month. The frozen samples were thawed, immediately wrapped with packaging material no. 1 and then stored at 0°C. The browning degree rapidly rose in all samples regardless of frozen storage temperatures and freezing rate. Even in frozen storage at −70°C, the browning of thawed meat was so serious that its acceptability was rapidly lost within a few hours. Furthermore, when the storage temperature was set at 10°C or 20°C after thawing, browning progressed faster than that in storage at 0°C (data not shown). In comparison with the browning of raw dark muscle, these results verify that browning progresses remarkably quickly in frozen and thawed meat.

**Influence of oxygen concentration on browning of thawed dark muscle**

In order to investigate the effect of re-packaging of thawed meat on the browning of dark muscle during ice storage, frozen dark muscle that had been stored in vacuum packaging at −70°C for 2 months was thawed. To minimize air exposure, it was immediately re-packaged in high to low oxygen modified atmospheres and stored at 0°C (Fig. 7). It was found that, until 10 h after thawing and storage, browning was most significant in the modified atmosphere containing 90% nitrogen and 10% oxygen (i.e. lower oxygen concentration resulted in faster browning progression). However, when storage exceeded 15 h, packaging with higher oxygen concentration caused gradual browning. Packaging with 100% oxygen induced more significant browning after 23 h. However, in 100% nitrogen gas and vacuum packages, the degrees of browning were 0.07 and 0.11, respectively, 1 day later and browning did not proceed significantly. As a result, it was concluded that browning of dark muscle could be prevented if the meat was packaged immediately after thawing by nitrogen substitution or vacuum packaging without exposure to air.

![Fig. 5](image-url)  
Change in color of fresh raw red muscle during storage. (■) 0°C; (●) 10°C; (▲) 20°C.
Influence of packaging materials on browning of thawed dark muscle

After thawing, dark muscle is usually exposed to air for 1–2 h because of the processing or cutting of sliced meats. After keeping thawed dark muscle at 0°C exposed to air for 1 h, samples were vacuum packed in bags that consisted of various materials with different oxygen permeation levels and were re-stored at 0°C (Fig. 8). The air exposure increased the b/a value from below 0.1 just after thawing to 0.2–0.4 (Fig. 8 at time zero). Packaging materials with low oxygen permeation (materials no. 1 and no. 2) temporarily caused high a b/a value immediately after storage and up to 2 h later. In contrast, packaging materials with oxygen permeation from 54 to 89 cm³/m² per 24 h (no. 3 and no. 4) caused no temporal rise in the b/a value. Furthermore, the elevated b/a value with air exposure decreased over time, and samples returned to their original dark muscle color 5 or 6 h later. This change was also observed visually. It was found that prevention from browning during storage of more than 4 days would be possible if packaging materials with oxygen permeation from 54 to 89 cm³/m² per 24 h were used (data not shown).

DISCUSSION

The amount and state of Mb in meat are the most important determinants of meat color. It has long been known that the browning of tuna and bonito meat is caused by generation of metMb.1–4 It is inferred that the browning of the dark muscle of cultured yellowtail also originates from Mb oxidation. Hashimoto and Watabe reported that tuna meat becomes brown colored because Mb in meat is oxidized to metMb.5 They also reported that the same change occurs in bonito and that 3 months at -30°C is the limit of storage.3 However, when the dark muscle of cultured yellowtail was stored at -30°C, the storage limit was approximately 1 month even with vacuum packaging using a low oxygen permeation bag (no. 1); its browning was faster than that of bonito. Storing yellowtail meat in nitrogen gas substituted packaging extended the storage period to up to 6 weeks at -20°C or -30°C and was more effective than vacuum packaging and carbon dioxide gas substituted packaging. It is presumed that oxygen gas might permeate more into a vacuum bag than into a bag that is pressurized with nitrogen gas. The dilution of permeated oxygen gas with nitrogen gas could be another reason.

The browning of cultured yellowtail dark muscle after thawing occurred faster than the discoloration of unfrozen meat during ice storage. Thawed fish lost its commercial value in a short time. A similar phenomenon is known in tuna meat. Chow et al. have reported that the rate of metMb formation in thawed tuna meat is higher than that of metMb in raw tuna meat kept in ice.6 The acceler-
ated browning in thawed muscle is caused by easy diffusion of oxygen gas into disintegrated muscle tissues and cells, resulting from freezing and thawing.

Frozen cultured yellowtail meat is usually sliced after thawing. This process causes an increase in the b/a value of dark muscle as a result of air exposure for 1–2 h (see time zero in Fig. 8). Nevertheless, this increase does not lead to the browning of dark muscle, because the meat color is still bright red. It is well known that there is equilibrium between the reduced form of Mb (purple in color) and the oxygenated form (oxyMb), which is bright red. In both forms, the heme iron is reduced to a ferrous state. On exposure of slices to air, the conversion of Mb to oxyMb is a result of the covalent binding of molecular oxygen to the free binding site of heme. This conversion increases the b/a value from 0.28 for Mb to 0.55 for oxyMb. If the oxidation of oxyMb occurs successively, a significant increase in the b/a value would be expected because of the formation of metMb (brown in color). However, the value in the present study remained below 0.5, even if a high oxygen gas permeable bag (no. 6) was used. When using a packaging material with oxygen permeation of 54–89 cm³/m² per 24 h, the possible length of storage exceeded 4 days at 0°C if processed in this manner. Therefore, the formation of metMb must be slow in vacuum packaging at 0°C. The results mean that oxyMb and the small amount of metMb formed can be reduced in reverse to Mb under specific conditions, depending on de-oxygenation of oxyMb, metMb-reducing activity and oxygen availability. Yamanaka et al. reported an inverse correlation between the concentration of metMb (browning) and metMb-reducing activity in bigeye tuna meat. However, metMb-reducing mechanisms in fish meat remain unclear. As the influence of thawing methods on dark muscle color was not addressed in the present study, future study is needed.

The preventive methods are summarized as follows: (i) fresh raw yellowtail fillets are sliced before freezing and packaged in a vacuum; they are kept frozen and stored at 0°C in the packaging after thawing; the package should be opened just before eating (within 4 days of thawing); (ii) fresh raw fillets are packaged in a vacuum and frozen; after thawing, the fillets are sliced in air and re-packaged in a vacuum in a bag with oxygen permeation of 54–89 cm³/m² per 24 h; they should be consumed within 4 days of storage at 0°C. Although nitrogen gas substituted packaging is more effective than vacuum packaging in preventing browning for storage between −20°C and −30°C, it is bulky and costly.

ACKNOWLEDGMENTS

This study was partly supported by a grant from the Fisheries Agency of Japan.

REFERENCES


