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Lipid composition and deposition of cultured yellowtail *Seriola quinqueradiata* muscle at different anatomical locations in relation to meat texture

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ABSTRACT: The present study was undertaken to assess the lipid composition and deposition in muscle at three anatomical locations in cultured yellowtail and to investigate the effect of lipid composition and deposition on meat texture. Lipid deposition in muscle was studied by histochemical staining of lipid with Sudan dye. Lipid class composition analysis showed that neutral lipids were the main constituents of lipid in cultured yellowtail and accounted primarily for the variation in muscle lipid content with the anatomical location of meat, as well as with season, whereas the polar lipid content remained almost constant. Furthermore, muscle neutral lipid content was correlated negatively with meat breaking strength; however, no correlation was observed between muscle polar lipid content and meat breaking strength. The histochemical study revealed that, in yellowtail muscle, lipid is preferentially deposited in the myosepta and, with increases in muscle lipid content, additional fat is deposited along sparsely distributed thin connective tissue. It was also observed that the greater the lipid deposition in collageneous connective tissue, the lower the meat breaking strength; presumably, higher lipid deposition in the connective tissue resulted in weakening of the muscle structure.

KEY WORDS: **anatomical location, cultured fish, lipid composition, lipid deposition, meat texture, yellowtail.**

INTRODUCTION

Yellowtail *Seriola quinqueradiata* farming is a well-developed practice in Japan, with aquaculture contributing approximately three-quarters of total yellowtail production, according to the annual report (1999) of the Fisheries Agency, Japan (http://www.jfa.maff.go.jp/). The use of a high-energy diet in aquaculture has led to improved growth performance but, at the same time, there has been concern regarding product quality, especially flavor, color and texture. Kora *et al.*[1] reported that cultured red seabream is less preferable compared with the wild fish because of considerable fat accumulation with obesity. Yellowtail meat is traditionally and popularly eaten raw as Sashimi. Meat texture is a dominant sensory characteristic influencing consumer acceptance and remains a challenging property to devise to suit consumer preference. Our previous study showed a negative correlation between muscle lipid content and meat breaking strength of cultured yellowtail;[2] however, the mechanism of meat texture softening with increases in muscle lipid content remains to be elucidated.

Lipid composition and distribution between and within tissues in fish vary from species to species and are influenced by seasonal and dietary variations.[3,4] In fact, total lipid and its composition in fish vary more than any other nutrient component. Christie[5] reported that neutral lipids show more changes related to dietary variation and the physiological state of fish than do polar lipids. Furthermore, Mohr[6] emphasized that lipid composition and deposition have a considerable effect on fish meat texture. Thus, to gain an insight into the meat texture softening mechanism in yellowtail with increasing lipid deposition in muscle, it is essential to assess lipid distribution in muscle. Lipid distribution in the tissues of several fish species, such as capelin, herring, mackerel and Atlantic salmon, has been studied by histochemical staining of muscle lipid;[6,7] however, to the best of our knowledge, no

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such study has been reported in the literature from the rheological viewpoint to explain the effect of lipid deposition on muscle firmness of commercially farmed fish. The aims of the present study were to assess lipid composition and mode of lipid deposition at three anatomical locations in cultured yellowtail muscle and to investigate the effect of lipid composition and deposition on meat texture.

MATERIALS AND METHODS

Experimental material

Live cultured yellowtail were purchased from a local market. Sampling was conducted from March 1999 to March 2000 and, at each sampling, three specimens were used, except for March 1999, when two fish were sampled; the weight of individual fish during the study ranged between 3.10 and 7.65°kg, as reported previously.[2] Fish were killed immediately after being taken live from the tank by spiking in the brain, they were bled by cutting the spinal chord at the tail end, packed in crushed ice and transported to the laboratory, where they were weighed, measured and processed. After measuring the biological data, fish were gutted, eviscerated and filleted on one side. The excised fillet was used for the lipid histochemical study. The other side of the fish was divided along the length into three parts namely the predorsal part, the anterior dorsal region; the dorsal part, middle dorsal region; and the tail parts, the posterior dorsal region. In all parts, sampling was performed on the location above the lateral line of the fish. Three slices of meat (10 mm thick) were excised from each body part of the fish and the meat breaking strength was measured at three points on each slice; details of this method have been described previously.[2] In brief, a cylindrical plunger, 3 mm diameter, was allowed to pierce the meat slice parallel to the orientation of the muscle fiber and the maximum force of insertion was recorded by a rheometer (model CR-200D; Sun Scientific, Tokyo, Japan) as the breaking strength; the meat breaking strength value for each body part of the fish is expressed as a mean of nine values measured. The remaining white dorsal meat from all three parts was excised, homogenized well with a knife, packed separately in polyethylene packs and stored at –85°C until chemical composition analysis. Meat samples were collected at 3 h after fish death, prerigor state, for all analyses. Sample preparation and analytical details are the same as reported previously.[2]

Lipid class composition

Muscle lipid was extracted with chloroform : methanol (2 : 1 v/v) according to the method of Bligh and Dyer.[8] Extracted lipid was kept at – 85°C until lipid class composition analysis. Total lipid was separated into 'neutral' and 'polar' fractions using a Sep-Pak silica cartridge (Waters, Milford, MA, USA).[9] A 1 mL aliquot of chloroform extract containing 100 mg lipid was transferred to the top of the Sep-Pak silica cartridge. The 'neutral' and 'polar' lipids were eluted with 20 mL chloroform and 30 mL methanol, respectively.

Lipid deposition

Previous studies on lipid distribution in fish muscle have used different histochemical techniques[7,10–12] and this prompted us to undertake a preliminary trial to establish a suitable lipid-staining protocol for cultured yellowtail muscle. The major technical limitation in the lipid histochemical study was to fix the muscle lipid, because diffusion of lipid from an improperly fixed sample during staining is a commonly encountered problem. Adams[13] reported that lipid in tissue blocks stored in fixative (formalin + Ca^{2+} ion) for 1–3 months leads to very little loss of lipid for histochemical study. In preliminary trials, we tested different time periods of fixation, as well as different ratios of fixative volume to the material. It was found that a fillet (skin on) fixed in 10% formalin containing 2% calcium acetate at a volume ratio of 1 : 20 (material : fixative) resulted in proper fixation of lipid in muscle. In the present study, we used Sudan II for lipid staining. Sudan dye is the most commonly used dye for lipid staining.[13] Aursand *et al.*[7] also studied lipid distribution in Atlantic salmon (*Salmo salar*) muscle by staining the muscle lipid with Sudan solution. In our preliminary trials, we also standardized the steps such as washing, staining and differentiation in the process to determine the best staining conditions for the experiment. An outline of the staining procedure is is given in Fig. 1.

Statistics

To evaluate the level of significance, a *t*-test was used and significance was defined at $P < 0.05$. Linear regression analysis was performed using Excel version 5.0 (Microsoft, Redmond, WA, USA).

RESULTS AND DISCUSSION

Lipid class composition

The lipid class composition of cultured yellowtail muscle at different anatomical locations is given in Table 1. The neutral lipid content of the meat from predorsal and dorsal parts was significantly higher than that of meat from the tail part. In contrast, no significant difference was observed in the polar lipid content among the different anatomical locations of meat. Lipid class composition analysis showed that neutral lipids were invariably the main constituents of lipid in cultured yellowtail and that the content of neutral lipids decreased from the head to the tail. Polar lipids were only minor constituents, with content ranging between 0.5 and 0.9 g/100 g meat, which remained almost constant irrespective of the anatomical location of the meat, as well as the season. Polar lipid content observed in the present study is in agreement with the report of Ackman,[3] who emphasized that membrane lipids are generally less than 1% of fish muscle weight. In the present study, neutral lipid content varied considerably with season; however, polar lipid content remained almost constant over the study period. The results show that the large variation in yellowtail muscle lipid[2] is due to variations in the neutral lipid content. This finding corroborates the earlier report of Christie,[5] who also found large variations in the neutral lipid fraction, whereas the polar lipid fraction remained constant. Takeuchi and Watanabe[14] reported that, in a starvation trial on carp and rainbow trout, decreases in muscle lipid were due to decreases in triglyceride and the amount of polar lipids was almost constant during starvation. The level of neutral lipids found in the present study along different anatomical locations was similar to levels reported previously for farmed yellowtail.[15]

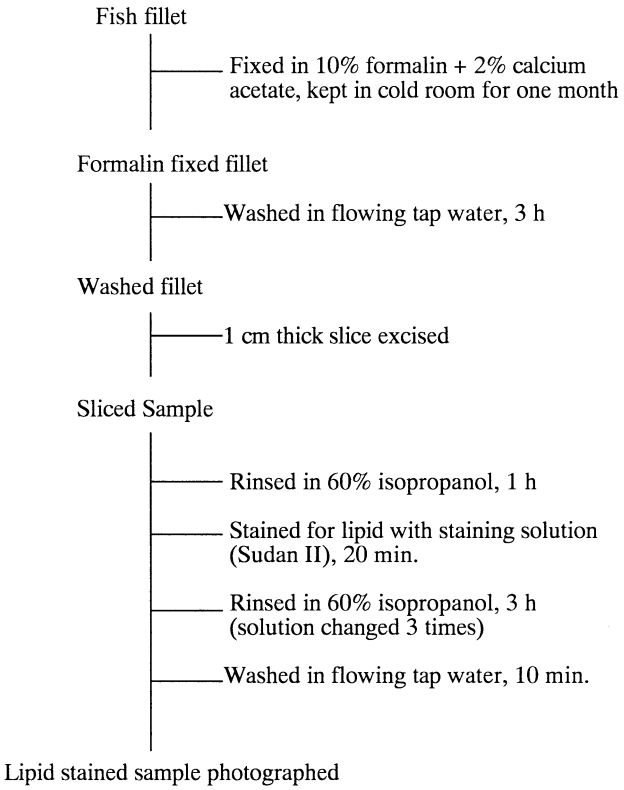

Fig. 1 Flow chart illustrating the outline of the lipid staining procedure.

Lipid composition affecting meat texture

To elucidate the effect of different lipid fractions on yellowtail meat texture, a correlation was drawn

Table 1 Lipid class composition (g/100 g meat) of cultured yellowtail meat at different anatomical locations

	Overall Sampling date						
Anatomical location	16 March 1999	14 May 1999	27 August 1999	8 October 1999	16 November 1999	4 March 2000	mean*
Pre-dorsal meat							
Neutral lipids	14.8	5.3 ± 0.7	4.2 ± 0.6	6.7 ± 1.4	13.7 ± 0.6	11.2 ± 1.7	9.0 ± 4.1[a]
Polar lipids	0.5	0.8 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	0.7 ± 0.3	1.2 ± 0.3	0.8 ± 0.3
Dorsal meat							
Neutral lipids	12.8	5.2 ± 0.7	3.8 ± 0.9	6.2 ± 0.9	13.4 ± 0.6	10.4 ± 2.0	8.4 ± 3.9[a]
Polar lipids	0.6	0.7 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.9 ± 0.2	0.7 ± 0.2
Tail meat							
Neutral lipids	7.0	3.5 ± 0.2	1.8 ± 0.3	3.1 ± 0.6	7.4 ± 0.7	6.7 ± 1.0	4.8 ± 2.3[b]
Polar lipids	0.7	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.2

Data are the mean ± SD ($n = 3$), except for March 1999 ($n = 2$).
*Different superscripts indicate significant differences among body parts ($n = 17$; $P < 0.05$).

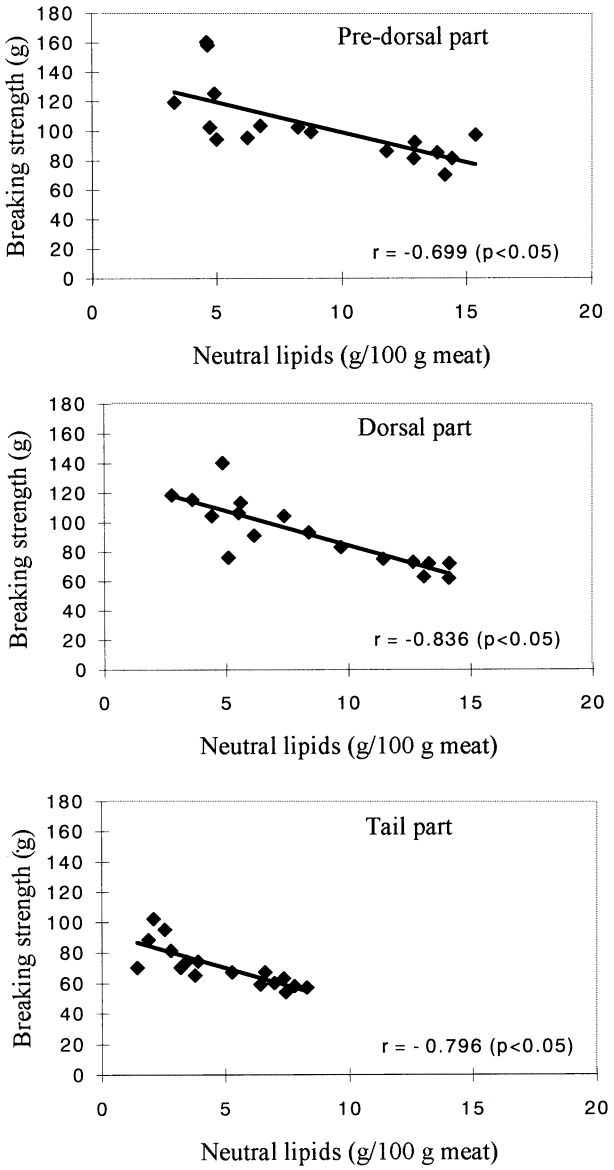

Fig. 2 Correlation between muscle neutral lipid content and meat breaking strength at different anatomical locations in cultured yellowtail. The meat breaking strength data are the same as reported previously.[2]

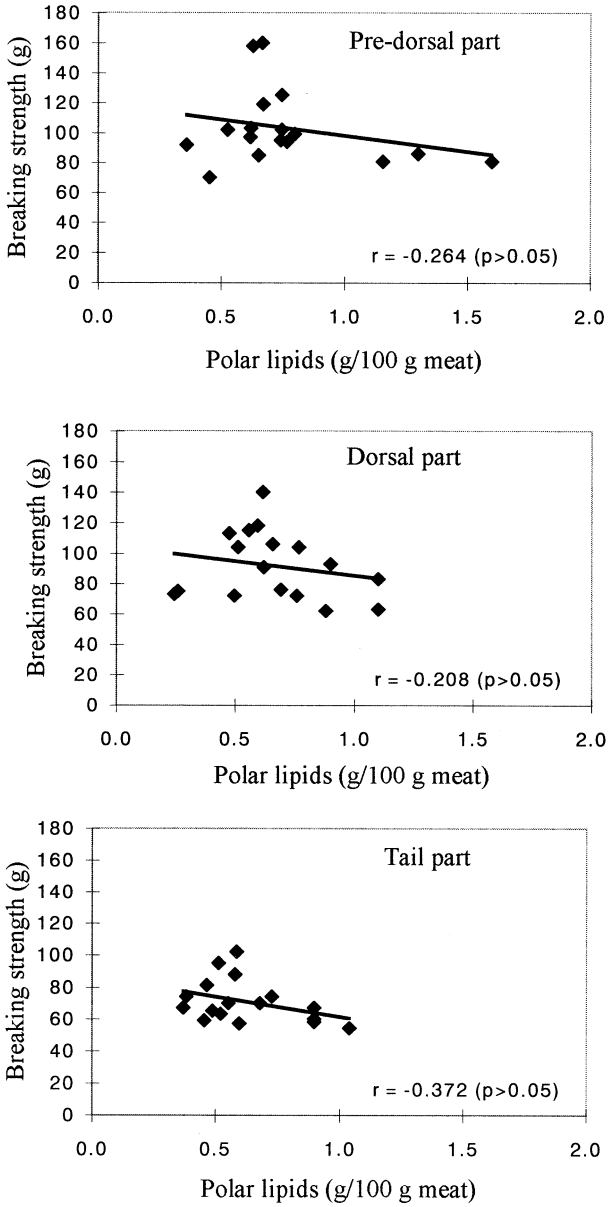

Fig. 3 Correlation between muscle polar lipid content and meat breaking strength at different anatomical locations in cultured yellowtail. The meat breaking strength data are the same as reported previously.[2]

between meat breaking strength and lipid fraction content for different anatomical locations in the body (Figs 2,3). A significant negative correlation was observed between neutral lipid content and meat breaking strength for all anatomical locations from which samples were taken. In contrast, no correlation was observed between polar lipid content and meat breaking strength. The results show that the neutral lipid fraction is responsible for the softening of meat texture in cultured yellowtail with increasing muscle lipid content and that cell-bound polar lipids had no significant bearing on meat texture. This is in agreement with the contention of Dunajski,[16] heralding the role of neutral lipids in the softening of meat texture of fish with increasing muscle lipid content. Dunajski[16] explained further that neutral lipids are immobilized by the physical structure of the musculature and tend to dilute the structural elements, thus decreasing the overall mechanical strength of meat in fatty fish.

Connective tissues are known to provide strength and integrity to the muscle. The mechanical stability of the intramuscular connective tissue

depends not only on intermolecular cross-liking, but also on the size and orientation of collagen fibrils.[17] Sheridan[18] observed that lipid is normally found as adipocytes in fish white muscle when recovered lipid exceeds approximately 1% and that adipocytes are found in isolation or in clumps throughout the loose connective tissue.[19] Furthermore, Zhou *et al.*[20] reported that, in Atlantic salmon, the myosepta serves as the site of lipid storage and neutral lipids account for most of the lipids stored in myosepta. From the above discussion, it is apparent that excessive neutral lipid deposition in the myosepta may affect the mechanical stability of the connective tissue network and, ultimately, muscle firmness. Consequently, there is a clear need to assess the mode of lipid deposition in yellowtail muscle to gain further insight into the meat texture softening related to increased muscle lipid content.

Mode of lipid deposition in white muscle

Photographs of lipid-stained white muscle of fatty fish and lean fish for different anatomical locations are presented in Fig. 4. The neutral lipid content of meat of the predorsal, dorsal and tail parts of fatty fish was 14.8, 12.8 and 7.0 g/100 g meat, respectively, whereas that of lean fish was 4.2, 3.8 and 1.8 g/100 g meat, respectively (Table 1). Dark orange lines in the figure represent the lipid-stained tissue. Lipid staining results showed that lipid was deposited along thick orange lines, myosepta, as well as along thin branched orange lines between the myosepta. It was also noted that with an increase in muscle lipid content, the lipid-stained lines became thicker throughout the meat sample. In addition, branched stained lines were densely distributed between the myosepta in samples with a high lipid content, whereas only a few lipid-stained lines were observed between myosepta in samples with a low lipid. The qualitative estimation of lipid deposition in yellowtail muscle by histochemical staining was in agreement with the quantitative estimation of lipids by chemical analysis. Furthermore, the results showed that the lipid distribution pattern in yellowtail white muscle was the same at different anatomical locations, except that more thickly stained lines were observed in meat from predorsal and dorsal parts compared with meat from the tail part. Obviously, this is due to the higher lipid content in meat from predorsal and dorsal parts compared with meat from the tail part.[2]

The present study showed that, in yellowtail white muscle, lipid was preferentially deposited in the myosepta and, with increases in muscle lipid content, additional fat was deposited along sparsely distributed thin connective tissue. The lipid deposition pattern observed in the present study is in agreement with previous reports on fish lipid histochemistry.[7,11,12] Furthermore, it was observed that densely distributed lipid-stained lines were present in the area near the skin and there were fewer lipid-stained lines towards the center of the meat. This indicates that, in cultured yellowtail muscle, higher amounts of fat are deposited in tissues near the skin and less is deposited near the inner part of the muscle. This is in agreement with the results of Undeland *et al.*,[21] who observed that muscle lipid content of herring decreased from the under-skin layer towards the inner part of the fillet. Furthermore, they observed that the proportion of triglyceride to phospholipid decreased through the fish from the under-skin layer towards the inner part of the fillet.

Lipid deposition affecting meat texture

Previous studies on the morphological properties of fish muscle to explain textural variations of meat had focused solely on the distribution and structural change of connective tissues in the muscle.[22–24] Although the lipid content in muscle is known to have an effect on meat texture, none of the lipid histochemical studies on fish had been conducted from a rheological viewpoint. Figure 5 shows photographs of lipid-stained meat from the dorsal part of cultured yellowtail. In Fig. 5, photographs of representative samples having high, medium and low muscle lipid content are shown for comparison. The neutral lipid level in samples having high, medium and low muscle lipid content was 12.8, 5.2 and 3.8 g/100 g meat, respectively (Table 1), and the meat breaking strength of these samples was found to be 69, 90 and 124 g, respectively (data as reported previously[2]).

The results show that increases in lipid deposition along the connective tissue correspond to the decreases in meat breaking strength, suggesting that higher lipid deposition along the connective tissue in cultured yellowtail had resulted in a softer meat texture. Seemingly, excess lipid deposition along the collagenous connective tissue may have caused structural weakening of the muscle, leading to a softer meat texture. This contention is in accordance with the observation of Dunajski,[16] who reported that deposition of liquid neutral lipids in muscle dilutes the structural elements of muscle and decreases the overall mechanical strength of meat in fatty fish. Although no lipid histochemical study has been reported previously on fish to explain the meat textural properties, the findings

of the present study corroborate the earlier report of Nishimura *et al.*[25] on bovine muscle, who found that a higher level of lipid deposition in the perimysium led to muscle softening. Furthermore, they noted that the development of adipose tissue in muscle disorganized the connective tissue and contributed to the tenderization of beef during the late fattening period. In a study on ayu muscle, Ito and Toyohara[26] reported that the soft meat texture during the spawning season was caused by structural changes in pericellular connective tissue, as the pericellular connective tissue got thinner and became more disintegrated during spawning. Lipid deposition and mobilization seems to be the

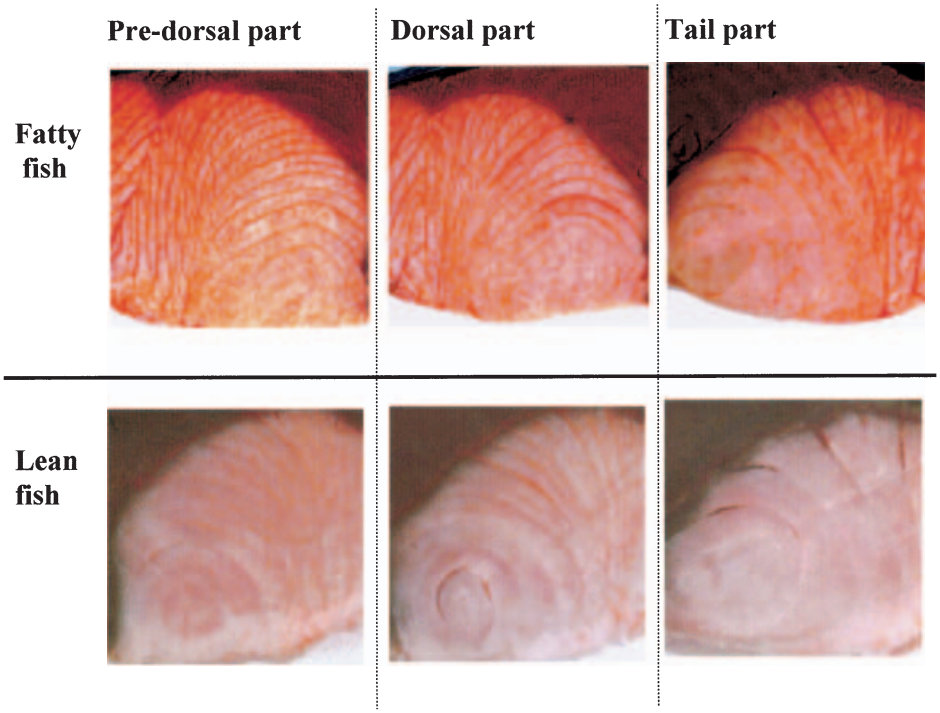

Fig. 4 Photographs showing lipid-stained muscle of cultured yellowtail from different anatomical locations.

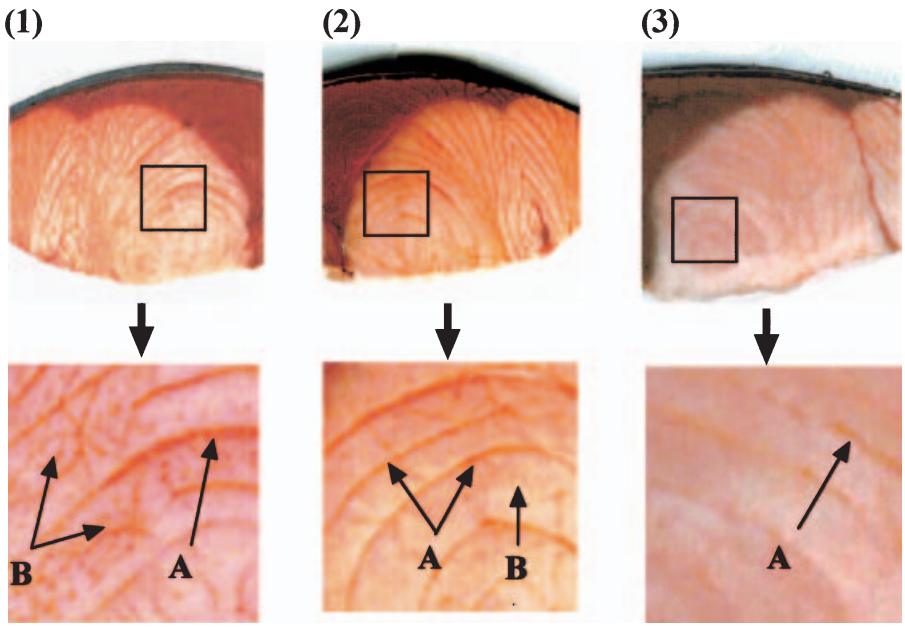

Fig. 5 Photographs showing the dorsal part muscle of yellowtail stained for lipid (representative samples with high (1), medium (2) and low (3) lipid content are shown). The bottom figures show an enlarged view of the indicated sections on the original lipid-stained sample. A, lipid deposited in the myosepta; B, lipid deposited between myosepta.

most likely phenomena to affect connective tissue structure in yellowtail muscle, because muscle lipid content varies more than any other muscle constituent according to the physiological and nutritional state of the fish.

It is clear from the above discussion that excess lipid deposition in muscle is responsible for the softening of meat texture and the effect may be caused by structural changes brought about in the connective tissue network. Light *et al.*[27] reported that the arrangement of the perimysial network, as well as the size of the collagen fiber, has a direct effect on the toughness of beef. Liu *et al.*[28] had found highly positive correlations between the shear-force value and the thickness of the perimysium in chicken skeletal muscle and suggested that the structure of the perimysium is a major factor determining meat toughness. Recently, Ando *et al.*[29] reported that lipid cells were present in the intercellular space of chub mackerel muscle. They also demonstrated that during postmortem softening of muscle, the decrease in meat breaking strength corresponded with an enlargement of the intercellular space. It is likely that excessive lipid deposition in the intercellular space may decrease the intercellular binding force in yellowtail muscle, thus resulting in soft meat texture. However, to reach any definitive conclusions, it is essential to study the microstructure of yellowtail muscle, especially the effect of excess lipid deposition on the connective tissue network. A further consideration is that collagen fiber size and its orientation may be affected by excess lipid deposition along the collagenous connective tissue during the fattening of fish and this remains a subject for further study. Moreover, the findings of the present study, showing lipid deposition in collagenous connective tissue together with a negative correlation between muscle lipid content and muscle collagen content,[2] raises the question as to what is the possibility of any physiological relationship between lipid metabolism and collagen metabolism in fish?

Conclusions

The present study suggests that neutral lipids are the dominant lipid class that account for the large variation in muscle lipid content in cultured yellowtail. It was also observed that the neutral lipid content of muscle had a direct effect on meat texture: a higher neutral lipid content led to softer meat texture. Polar lipids were only minor constituents of muscle lipid and their levels remained almost constant throughout the year; polar lipids had no direct influence on the meat texture of cultured yellowtail. Lipid histochemical studies demonstrated that, in cultured yellowtail white muscle, lipid is preferentially deposited in the collagenous connective tissue and excessive lipid deposition in the connective tissue results in a softer meat texture. Furthermore, the lipid histochemical study revealed that the lipid distribution pattern in the white muscle of cultured yellowtail is similar, irrespective of the anatomical location of the meat. The present study provides valuable information on lipid deposition in cultured yellowtail muscle and serves as basic knowledge for further study. A microscopic study on lipid and connective tissue distribution in yellowtail muscle, to explain the textural properties of meat, is in progress in our laboratory.

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