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Compositional characteristics of muscle and visceral oil from steelhead trout and their oxidative stability

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Abstract

The quality of lipid from steelhead trout viscera, a byproduct of steelhead trout industry, was evaluated and compared with that of the muscle. Steelhead trout viscera had a higher lipid content than muscle and the visceral oil differed from muscle oil in its lipid class composition. Neutral lipids, mainly triacylglycerols (TAG), comprised the major lipid class in both muscle and visceral lipid. However, as expected, muscle contained a remarkably higher level of phospholipids (PL) than viscera, and the ratio of total neutral lipids to polar lipids was lower in muscle than in viscera. Visceral and muscle lipid had similar fatty acid compositions, with the concentration of muscle polyunsaturated fatty acids (PUFA) slightly higher than that of visceral PUFA. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were the major *n*-3 PUFA present in both muscle and visceral lipid; docosapentaenoic acid (DPA) was present at about 1.61–1.76%. With respect to minor components, muscle lipid had higher α-tocopherol and total carotenoids contents than visceral lipid. Oxidative stability of visceral and muscle lipid, as determined by conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS), showed that visceral lipid was more stable against oxidation than muscle lipid, probably due to their different lipid profiles. Besides, carotenoids, which were present more abundantly in muscle, might have acted as prooxidants and therefore, decreased the oxidative stability of muscle lipid. These results suggest that steelhead trout viscera may serve as a good source of lipid and could be utilized for production of omega-3 oils.

Keywords: Steelhead trout; Muscle; Viscera; Composition; Oxidative stability

1. Introduction

Steelhead trout (*Oncorhynchus mykiss*) is mainly a seawater fish species exclusively native to the Pacific slope of North America, subsequently introduced to many parts of the world. They are widely reared in North America and European countries in a commercial scale. Rainbow trout, which belongs to the same species, is the freshwater counterpart of Steelhead trout. Although Rainbow trout can spend their entire life in freshwater, they are also capable of living in seawater. Steelhead trout grows up to an average length of 50–100 cm and weight around 2.5–10 kg with a maximum lifespan of 4–5years (Passi et al., 2004). Both steelhead trout and rainbow trout are popular in Western dishes and are often farmed for food.

Fish oil, which is a rich source of long-chain *n*-3 polyunsaturated fatty acids (PUFA), mainly EPA (eicosapentaenoic acid) and docosahexaenoic acid (DHA), has received much attention in the scientific and industrial communities because of its positive role in human health (Glomset, 1985; Schmidt & Dyerberg, 1994). The potential health benefits of fish oil include reduced risk of cardiovascular diseases, hypertension and atherosclerosis as well as inflammatory and autoimmune disorders. DHA (22:6*n*-3) is now

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recognized as a physiologically essential nutrient in the brain and retina for neural functioning and visual activity, respectively (Holub, 2001). During intrauterine life, DHA is incorporated into phospholipid membranes of retina and brain and continues to accumulate during the first two years of life after birth (Newton, 2001). Hence, fish oils are increasingly being proposed for incorporation into foodstuffs in different forms. The challenge of incorporation of fish oils into foodstuff has been overcome through novel refining techniques, which are capable of eliminating unacceptable odors and impurities (Newton, 2001). Microencapsulation has also helped in protecting the oil from oxidation and for easy incorporation into foods (Barrow, 2006).

Presently, most of fish oils available in market are produced by pressing/heating of headed and gutted pelagic fish to release oil mainly concentrated in the flesh of fish (Pigott, 1996). However, there is a potential of producing oil from by-products (Aidos et al., 2003). The by-products of fish processing, including heads, skins, frames, and viscera, often end up in landfills or rendering plants. Major by-products of salmon and Pollock industries in the USA were 90,000 and 700,000 metric tons, respectively in 2000 (Crapo & Bechtel, 2003). There are reports on producing good quality fish oils from herring (Aidos et al., 2003) and catfish (Sathivel, Prinyawiwatkul, King, Grimm, & Lloyd, 2003) by-products. There is also a growing world market for high quality fish oils for inclusion into food and as dietary supplement.

In rainbow trout, lipids are mainly stored in the flesh and to lesser extent in the gut and ovaries (Nassour & Leger, 1989). However, viscera of rainbow trout, which contains a substantial amount of fat ranging from 6% to 31% (Nassour & Leger, 1989) can be used to recover oil that can possibly be converted to good quality edible oils. Recovery of oil may add value to viscera, which is currently a processing waste.

The degree of unsaturation of PUFA makes fish oil extremely vulnerable to oxidation and development of off flavors and unpleasant odors leading to quality deterioration (Finley & Shahidi, 2001). Therefore, protection of fish oil during processing and storage is important. Rainbow trout contains carotenoids and tocopherols, which are natural antioxidants that prevent lipid oxidation. However, the prooxidant character of carotenoids has also been reported and associated with their instability against heat and light (Subagio & Morita, 2001).

The lipid classes and fatty acid profiles of major fish species and their muscle tissues have been determined (Ackman, 1989), however, there has been very few studies on lipid classes and fatty acid profiles of viscera and other by-products. Oliveira and Bechtel (2005) reported the lipid composition of Alaska pink salmon (*Oncorhynchus gorbuscha*) and Alaska walleye pollock (*Theragra chalcogramma*) by-products. The aim of this study was to determine the lipid classes and fatty acid profiles as well as minor components of crude oil extracted from viscera of steelhead trout and to assess the oxidative stability of the oil in comparison with those of the oil from the muscle.

2. Materials and methods

2.1. Materials

Cultured steelhead trout (*O. mykiss*) were provided by Veterinary Laboratory, St. Alban's, NL. All chemicals used were purchased from Fisher Scientific Ltd., Ottawa, ON, Canada, or Sigma–Aldrich Canada Ltd., Oakville, ON, Canada. The solvents were of ACS grade, pesticide grade or HPLC grade.

2.2. Lipid extraction

Lipids were extracted from steelhead trout muscle and visceral samples using chloroform/methanol according to Bligh and Dyer (1959) and quantified gravimetrically. Briefly, 200 ml of methanol and 100 ml of chloroform were added to 100 g of each sample and the mixture was homogenized for 2 min using a Polytron PT 3000 (Brinkmann Instruments, Rexdale, ON) homogenizer. Another 100 ml of chloroform were added to the mixture, followed by further blending for 30 s. The homogenate was filtered and the residue re-extracted with 100 ml of chloroform for 30 s and filtered. The filtrates were combined, and 100 ml of distilled water were added and mixed well. The mixture was allowed to stand in a cold room for separation and the chloroform layer containing lipid was collected. The solvent was evaporated in a rotary evaporator (Büchi Rotavapor, Flawil, Switzerland) at 40 °C. Total lipid content was calculated gravimetrically.

2.3. Lipid classification

Muscle and visceral lipid classes were determined by thin layer chromatography-flame ionization detection (TLC-FID) with an Iatroscan MK-5 TLC-FID Analyzer System (Iatroscan Laboratories Inc., Tokyo, Japan) equipped with TSCAN data handling software (Scientific Products and Equipment, Concord, ON). Separation of lipid classes was carried out following a three-stage development procedure described by Christopher (1999). The rods spotted with sample and standards were developed twice in hexane/diethyl ether/formic acid (99:1:0.05, v/v/v) and partially scanned at pps (partial pyrolysis selection) 25 after drying. The second development was conducted in hexane/diethyl ether/formic acid (79:20:1, v/v/v) and rods were partially scanned (pps 11). The rods were then developed twice in 100% acetone followed by development in chloroform/methanol/distilled water (5:4:1, v/v/v) and fully scanned afterwards. The three chromatograms were considered together, and each peak identified by comparing the retention times with those of known standards. Quantification of each lipid class was achieved by calculating the area percentage of a specific peak to the total peak area.

2.4. Fatty acid analysis

Fatty acid composition of muscle and visceral lipid was analyzed by gas chromatography (GC). Fatty acids were converted to fatty acid methyl esters (FAMEs) using 6% sulfuric acid in methanol, as described by Hamam and Shahidi (2005), and analyzed using a Hewlett-Packard 5890 Series II gas chromatograph (Agilent, Palo Alto, CA) equipped with a fused capillary column (Supelcowax-10, 30 m length, 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON). The temperature of the injector and detector (FID) was both set at 250 °C. The oven temperature was programmed to increase from 220 to 240 °C at a rate of 30 °C/min. Ultra high purity (UHP) helium was used as the carrier gas at a flow rate of 15 ml/min. Data were analyzed with a Hewlett-Packard 3365 Series II Chem Station Software (Agilent, Palo Alto, CA). The FAMEs were identified by comparing their retention times with those of authentic standards. Results were expressed as area percentage of each fatty acid in total fatty acids.

2.5. HPLC-MS analysis of α-tocopherol

Minor components in lipid extracts were determined in terms of α-tocopherol and carotenoids. Lipid extracts were saponified according to Ryynänen, Lampi, Salo-Väänänen, Ollilainen, and Piironen (2004) with some modifications and the unsaponifiable matter was analyzed for α-tocopherol content. Briefly, 0.5 g of lipid extract was mixed with 5 ml of ethanol, 2 ml of deionized water, 0.5 ml of KOH solution (50%) and 0.1 g of ascorbic acid and sealed under nitrogen. The mixture was then heated for 25 min at 100 °C. Ethanol (2.5 ml) and deionized water (2.5 ml) were added to the above mixture and unsaponifiable matter was extracted three times with hexane. The solvent from combined hexane extracts was removed under a stream of nitrogen and the residue was redissolved in 1 ml of hexane. Samples and standard tocopherol solutions were filtered using a 0.45 μm syringe-filter and then used for HPLC analysis.

Tocopherol contents in steelhead trout muscle and visceral lipid were determined by normal phase high performance liquid chromatography (HPLC)–mass spectrometry (MS). An Agilent 1100 HPLC system (Agilent, Palo Alto, CA) with a UV-diode array detector (UV-DAD) was employed and separation was achieved on a Supelcosil LC-Si column (250 mm × 4.6 mm i.d., 5 μm, Sigma–Aldrich Canada Ltd., Oakville, ON) coupled with a Supelcosil LC-Si guard column. α-Tocopherol was eluted with an isocratic solvent system containing hexane/2-propanol (99:1, v/v) at a flow rate of 1.2 ml/min. Fifty microliters of tocopherol standard and sample were injected. α-Tocopherol was detected at 290 nm by the UV detector. LC flow was analyzed on-line by the mass spectrometric detector system (LC-MSD-Trap-SL, Agilent, Palo Alto, CA) with a positive ion APCI (atmospheric pressure chemical ionization). The operating conditions used were 121 V for the fragmentor voltage, 350 °C for drying temperature, 400 °C for APCI temperature, 60 psi for the nebulizer pressure, and 7 l/min for the drying gas flow. Tocopherol concentrations in samples were obtained from a standard curve, and expressed as mg tocopherol per kg of oil.

2.6. Determination of total carotenoids

The total carotenoids content of muscle and visceral lipid extracts was measured spectrometrically. Lipid extract was dissolved in hexane at a concentration of 30 mg/ml followed by filtration. The absorbance of resultant solution was recorded at 450 nm using a Hewlett-Packard model 8452A diode array spectrophotometer (Agilent, Palo Alto, CA). A standard curve was obtained using varying concentrations of β-carotene. Total carotenoid content in lipid samples was expressed as mg of β-carotene equivalents per kilogram of oil.

2.7. Determination of oxidative stability

Oxidative stability of muscle and visceral lipid extracts was evaluated by measuring the conjugated dienes and thiobarbituric acid reactive substances (TBARS) at different time intervals. Lipid extracts in loosely capped vials were allowed to oxidize at 60 °C in a forced air oven (Schaal oven conditions) for a period of 10 days. Samples were removed on 0, 1, 3, 5, 7 and 10 day from the oven, cooled to room temperature, flushed with nitrogen and stored at −20 °C until analyzed.

Conjugated dienes (CD) in the oils were measured spectrometrically according to IUPAC (1987). A specified amount of oil (0.02–0.03 g) was dissolved in 25 ml of isooctane and the absorbance of the solution was read at 234 nm. Pure isooctane was used as the blank. Conjugated diene values were calculated using the equation $CD = A/(C \times L)$, where A is the absorbance at 234 nm, C is the concentration of the oil in g/100 ml, and L is the length of the cuvette in cm.

TBARS values of the oils were measured according to the AOCS (1990) method. Malondialdehyde (MDA) precursor, 1,1,3,3-tetramethoxypropane (TMP) was used as a standard. Sample solutions were prepared by dissolving approximately 0.2 g of oil samples in 25 ml of 1-butanol. Five milliliters of sample and standard solutions were added to 5 ml of TBA reagent (2 mg/ml of TBA in 1-butanol) in dry test tubes. The contents were heated in a water bath at 95–100 °C for 120 min and cooled down on ice. The absorbance of the resultant solutions was measured at 532 nm. A standard curve (absorbance versus TMP concentration) was constructed. Concentrations of MDA equivalents in oil samples were read from the standard curve and TBARS values expressed as the total μmol of MDA equivalents per gram of oil.

3. Results and discussion

The chloroform/methanol extraction yielded 12.2% lipid from steelhead trout muscle tissue, which is close to that in the literature (12.86%) for rainbow trout fillet (Nielsen et al., 2005). As expected, viscera had a higher lipid content (40.2%) than muscle, as muscle was composed mainly of moisture (data not shown). However, much lower levels were reported for visceral lipid of other fish species, such as 13.2% for herring headless by-products (Aidos, Masbernat-Martinez, Luten, Boom, & Van der Padt, 2002) and 14.32% for Pollack viscera (Oliveira & Bechtel, 2005). The lipid class composition of steelhead trout muscle and viscera is given in Table 1. As observed in most oils, triacylglycerols (TAG) comprised the major lipid class of muscle and viscera. TAG accounted for 74.1% of total lipid in steelhead trout viscera. Similar result has been reported for Pollock viscera, where TAG were present at a level of 72.14% of total visceral lipid (Oliveira & Bechtel, 2005). Muscle had a lower proportion of TAG than viscera. Passi et al. (2004) reported that a 3-year-old rainbow trout contained 61.4% TAG in its muscle lipid, a much higher value than that of 51.8% in our study, possibly due to the existing differences in age, sex, location and diet of the fish under investigation. Phospholipids (PL) were the second most abundant lipid class next to TAG in both muscle and viscera. In animals, TAG are known to serve as the most concentrated form of energy storage, whereas PL are one of the major components of cell membranes with limited use as an energy source. Lipids from muscle tissue are characterized by a high proportion of PL, as also found in steelhead trout. The content of PL in steelhead trout muscle was 32.9% of the total muscle lipid, which was quite high in comparison with that in viscera (13.7% of the total visceral lipid). Lipid classes, including free fatty acids (FFA), sterols (ST), diacylglycerols (DAG), and acetone-mobile polar lipids (AMPL), mainly glycolipids, were detected as minor components, with the exception of visceral DAG, which was present at a level of 12.4% of the total visceral lipid. The ratio of total neutral lipids to total polar lipids was considerably higher in viscera than in muscle. This is in accordance with the fact that fat from adipose tissues acts mainly as energy provider and energy reserves while muscle lipid is used for structural and functional purposes.

Fatty acid composition of muscle and visceral lipid is shown in Table 2 and Fig. 1. Steelhead trout muscle and viscera had similar fatty acid patterns. Monounsaturated fatty acids, particularly oleic acid (C18:1), were predominant in both muscle and viscera, followed by polyunsaturated fatty acids (PUFA) and saturated fatty acids. Steelhead trout lipid was rich in PUFA, especially *n*-3 PUFA. The PUFA content was slightly higher in muscle than in viscera. DHA and EPA were the major *n*-3 PUFA,

Table 1
Lipid classes of muscle and viscera of steelhead trout

% of total lipid	Muscle	Viscera
TAG	51.8 ± 6.24	74.1 ± 3.36
FFA	0.89 ± 0.47	tr
ST	0.66 ± 0.59	0.33 ± 0.09
DAG	2.32 ± 1.44	12.4 ± 1.20
AMPL	4.59 ± 2.00	1.57 ± 0.30
PL	32.9 ± 5.31	13.7 ± 1.84
∑Neutral	55.7 ± 5.67	86.8 ± 3.02
∑Polar	37.5 ± 4.34	15.3 ± 1.79

Results are mean ± SD of three replicates. Symbols are: TAG, triacylglycerols; FFA, free fatty acids; ST, sterols; DAG, diacylglycerols; AMPL, acetone-mobile polor lipids; PL, phospholipids; and tr, trace.

Table 2
Fatty acid composition (area %) of steelhead trout muscle and visceral lipid

Fatty acids	Muscle	Viscera
12:0	0.06 ± 0.01	0.06 ± 0.00
14:0	2.84 ± 0.06	3.43 ± 0.09
14:1	0.06 ± 0.00	0.08 ± 0.01
15:0	0.23 ± 0.01	0.23 ± 0.23
16:0	15.9 ± 0.11	15.7 ± 0.03
16:1	6.80 ± 0.13	7.04 ± 0.07
17:0	0.22 ± 0.01	0.22 ± 0.01
17:1	0.58 ± 0.01	0.53 ± 0.04
18:0	4.39 ± 0.09	4.50 ± 0.01
18:1	28.5 ± 0.36	28.6 ± 0.13
18:2	8.72 ± 0.09	9.00 ± 0.11
18:3 *n*-6	0.10 ± 0.00	0.15 ± 0.01
18:3 *n*-3	0.96 ± 0.03	0.92 ± 0.02
20:0	0.10 ± 0.01	0.09 ± 0.00
20:1	3.34 ± 0.11	3.08 ± 0.00
20:2	0.67 ± 0.01	0.63 ± 0.01
20:3 *n*-6	0.32 ± 0.01	0.40 ± 0.02
20:3 *n*-3	0.80 ± 0.03	0.88 ± 0.04
20:4 *n*-6	0.11 ± 0.00	0.10 ± 0.00
20:5 *n*-3	3.28 ± 0.15	3.35 ± 0.14
22:1	0.28 ± 0.02	0.28 ± 0.01
22:4 *n*-6	0.15 ± 0.01	0.16 ± 0.01
22:5 *n*-3	1.76 ± 0.03	1.61 ± 0.03
22:6 *n*-3	9.70 ± 0.48	7.98 ± 0.21

Results are mean ± SD of three replicates.

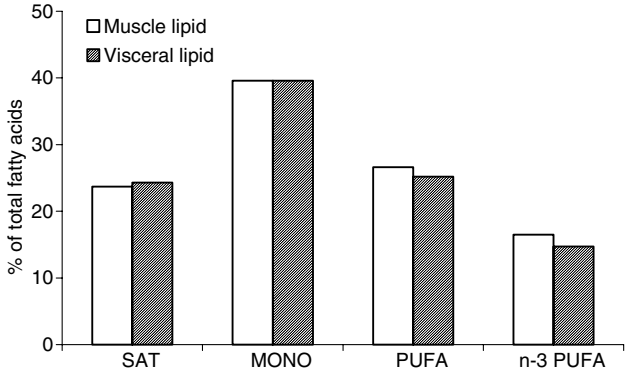

Fig. 1. Fatty acid profile of steelhead trout muscle and visceral lipid (SAT, saturated fatty acids; MONO, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids).

accounting for 9.70% and 3.28% of total fatty acids, respectively, in muscle and 7.98% and 3.35%, respectively, in viscera. Muscle contained a higher level of DHA than viscera. The corresponding concentrations of these two fatty acids in the literature were, respectively, 10.6% and 4.3% in the fillet of rainbow trout (Nielsen et al., 2005), slightly higher than results of this work; no published values were found for viscera. Docosapentaenoic acid (DPA, C22:5 *n*-3) was present in lesser amounts than DHA and EPA and had a concentration of 1.76% and 1.61% of total fatty acids in muscle and viscera, respectively. It is indicated that lipid from steelhead trout viscera possesses comparable value to muscle lipid as a source of important PUFA such as EPA, DPA and DHA, which are believed to be essential and health beneficial to humans. Moreover, from quantitative point of view, viscera had a much higher lipid content than muscle and therefore higher yields of fatty acids. Although being a by-product of steelhead trout industry, viscera could be utilized for production of essential fatty acid concentrates for a variety of applications.

α-Tocopherol and carotenoids are minor components in fats and oils. However, they make great contribution to the quality of the oil with respect to its oxidative stability. α-Tocopherol is one of the most important antioxidants in biological systems and the only form of tocopherol homologue detected in steelhead trout (Fig. 2). Lopéz, Satué, González, and Agramont (1995) reported that farmed rainbow trout contained 190 mg α-tocopherol/kg oil in their muscle, a somewhat higher level than that of 130 mg/kg oil in muscle in this study (Table 3). The difference may be caused by the diet that fish receive, as farmed fish might have their feed supplemented with vitamin E. It has been demonstrated that tissue α-tocopherol concentration is proportional to dietary α-tocopherol concentrations, as observed in many fish species (Baker & Davis, 1997; Chaiyapechara, Casten, Hardy, & Dong, 2003). Steelhead trout viscera had a lower content of α-tocopherol (84 mg/kg oil) than muscle, although this value was very high compared to that of other fish species, such as α-tocopherol in headless by-products of herrings (4 mg/kg oil) (Aidos et al., 2002).

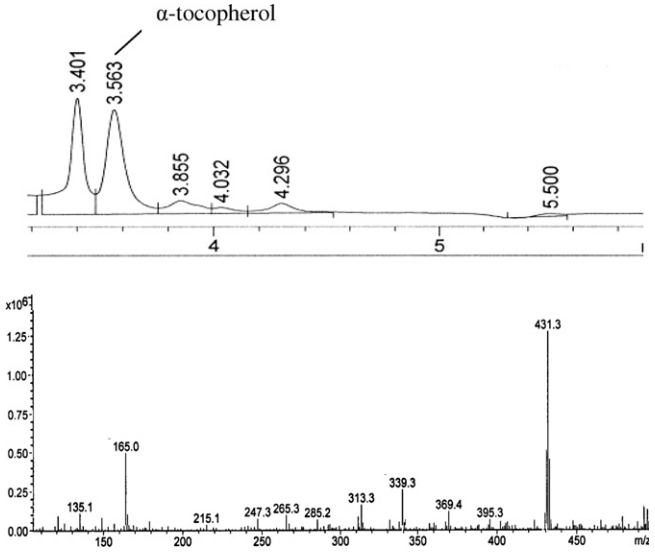

Fig. 2. LC chromatogram and mass spectrum of α-tocopherol in steelhead trout visceral lipid. The MS peak at *m*/*z* 431.3 represents the $[M+H]^+$ ion of α-tocopherol.

Table 3
Minor components (α-tocopherol and carotenoids) of steelhead trout muscle and visceral lipid

Minor components	Muscle	Viscera
α-Tocopherol (mg/kg oil)	130 ± 7.21	84.0 ± 3.04
Total carotenoids (mg β-carotene eq./kg oil)	99.6 ± 0.30	19.2 ± 8.40

Results are mean ± SD of three replicates.

Carotenoids are basically recognized as antioxidants that effectively inhibit lipid oxidation. However, carotenoids at high concentrations or at high partial pressure of oxygen, may lose their effectiveness as antioxidants *in vivo* or exert a prooxidant effect *in vitro* (Kiokias & Gordon, 2004; Young & Lowe, 2001). High doses of β-carotene and lycopene were found to promote lipid oxidation and thus decrease the oxidative stability of TAG or vegetable oils (Subagio & Morita, 2001; Vajdak, Schmidt, Lizicarova, Zahradnikova, & Sekretar, 2004). Lutein acted as a prooxidant even at a low concentration (Subagio & Morita, 2001). Steelhead trout flesh is pink in color and is known to be a rich source of carotenoids. The total carotenoids in muscle lipid was 99.6 mg β-carotene equivalents/kg oil (Table 3), while a dramatically lower concentration of total carotenoids was observed in visceral lipid (19.2 mg β-carotene equivalents/kg oil).

Stability of lipids of steelhead trout muscle and viscera against accelerated oxidation was determined by measuring CD and TBARS as primary and secondary oxidation products over 10 days of storage, respectively (Figs. 3 and 4, respectively). The CD and TBARS values of muscle and visceral lipid followed the same increasing trend during

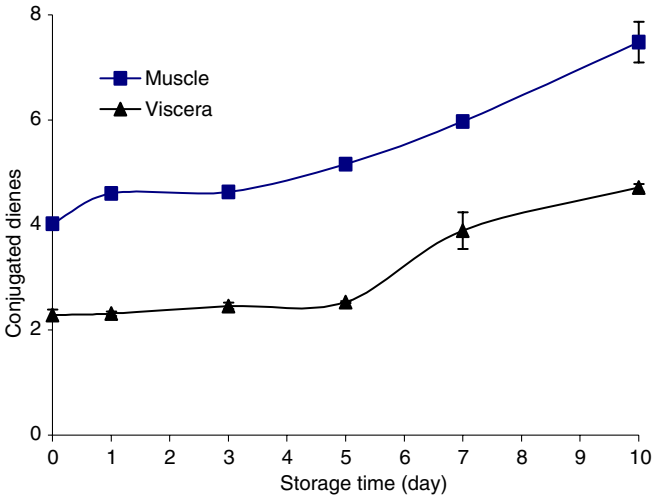

Fig. 3. Conjugated dienes of steelhead trout muscle and visceral lipid during a 10-day storage at 60 °C.

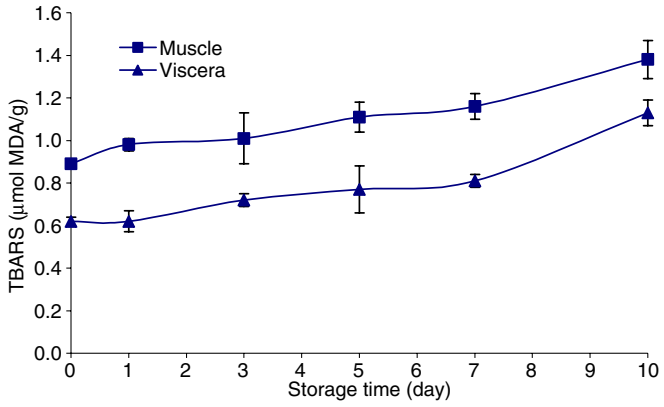

Fig. 4. TBARS of steelhead trout muscle and visceral lipid during a 10-day storage at 60 °C.

the 10-day storage period. Muscle lipid had higher concentrations of CD than visceral lipid at any time interval, which strongly correlated with the results for TBARS, suggesting that visceral lipid exhibited a better oxidative stability than muscle lipid.

Muscle lipid was more susceptible to oxidation than visceral lipid, probably due to a slightly higher level of PUFA, particularly DHA, than viscera, which may contribute to its higher susceptibility to oxidation. Furthermore, muscle tissue differed from viscera in lipid class composition with its PL content being remarkably higher than visceral PL. PL have been proven to play an important role in oxidative rancidity of meat. Jittrepotch, Ushio, and Ohshima (2006) found that the PL fraction rather than TAG was responsible for oxidative deterioration in cooked turkey meat. Assuming this is also true in oils, muscle lipid of steelhead trout containing more PL would therefore, exhibit lower oxidative stability than visceral lipid, which is in agreement with the finding in this study.

In addition to fatty acid and lipid class composition, minor components such as α-tocopherol and carotenoids may also affect the oxidative stability of oils. Muscle lipid had a higher concentration of α-tocopherol and total carotenoids than visceral lipid, and presumably should be less oxidized if both α-tocopherol and carotenoids have acted as antioxidants. In contrast, visceral lipid was found more stable than muscle lipid against oxidation. However, it should be noted that carotenoids not only show antioxidants effectiveness, they may also exhibit prooxidant activity under certain circumstances, as discussed earlier. It is possible that the carotenoids in steelhead trout may exert a prooxidant effect on the oil and hence increased lipid oxidation. Although the prooxidant potency of carotenoids *in vivo* may be inhibited by many unknown mechanisms, they may shift their antioxidant activity into prooxidant activity *in vitro*, such as in post-mortem fish.

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