Food Chemistry 115 (2009) 436–440

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Lipid and fatty acid compositions differentiate between wild and cultured Japanese eel (*Anguilla japonica*)

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ARTICLE INFO

Article history:
Received 11 July 2008
Received in revised form 8 December 2008
Accepted 9 December 2008

Keywords:
Anguilla japonica
Fatty acid composition
Japanese eel
Lipid profile

ABSTRACT

The lipid profile and fatty acid composition of muscle, liver, and plasma lipoprotein were examined in wild and cultured Japanese eel (*Anguilla japonica*). Although, the muscle lipid levels of wild eels (11.6%) were less than those of cultured eels (13.1%), both eels were classified as typical fatty fish. Compared with the liver lipid composition of cultured eels, triacylglycerol (TG) level of the liver decreased in wild eels, whilst phosphatidylcholine and phosphatidylethanolamine levels of the liver increased in wild eels, reflecting the difference of liver lipid levels in both eels. Wild eels contained more cholesteryl ester (CE) and less TG, phospholipid, and free cholesterol in their plasma than cultured eels. The ratio of TG to CE decreased, whilst that of CE to total cholesterol increased in the plasma of wild eels. Different fatty acid compositions were found between wild and cultured eels. Compared with the fatty acid compositions of cultured eels, wild eels contained high percentages of 18:2, 18:3, and 20:4 and low percentages of 22:6, 20:1, and 20:5 in their muscle, liver, and plasma lipoprotein. The lipid profile and fatty acid composition seemed to be useful criteria for distinguishing between wild and cultured eels.

1. Introduction

Fish preferentially utilise lipids rather than carbohydrates as an energy source and accumulate considerable amounts of lipids in their muscle, liver, or adipose tissue (Sheridan, 1988; Watanabe, 1982). Fish can be divided into fatty and non-fatty fish based on the lipid level of their muscle. Muscle is the main lipid reservoir in the former, whilst the latter possesses substantial quantities of lipids in the liver, but not muscle (Ando, Mori, Nakamura, & Sugawara, 1993; Sheridan, Allen, & Kerstetter, 1983; Shibata & Kayama, 1989).

Japanese eel *Anguilla japonica* is known to be a typical fatty fish (Ohshima, 1985; Ozaki, Koga, Takahashi, Adachi, & Yamauchi, 2008). Japanese eel is one of the most valuable cultured species in Japan. Some 100,000 and 300 tons of cultured and wild eels, respectively, are supplied to the food market of Japan a year (Tachiki, 1996). A commercial eel diet mainly consists of fish meal and starch and its fatty acid composition is rich in 16:0, 18:1, 22:6, and 20:5 (Ozaki et al., 2008; Suzuki, Okazaki, Hayakawa, Wada, & Tamura, 1986). Cultured Japanese eels contain more than 10% of lipids in their muscle. Most Japanese favour grilled cultured Japanese eels because they accumulate considerable amounts of lipids in their muscle.

In our previous papers, we have reported that cultured Japanese eels were in plasma hyperlipoproteinemia, reflecting their high use of lipids (Ando & Matsuzaki,1996, 1997). Plasma lipoproteins serve as major carriers of lipids and other hydrophobic compounds. Lipoproteins with high levels of lipids, such as very low density (VLDL) and low density (LDL) lipoproteins, predominated in the plasma of cultured Japanese eels, in contrast to other teleosts with high density lipoprotein (HDL) as the main component. To our knowledge, cultured Japanese eels with high level of VLDL seem to be the only teleosts reported to date, although, plasma VLDL level has been shown to increase in rainbow trout *Oncorhynchus mykiss* treated with oestradiol-17β (Wallert & Babin, 1992). On the other hand, the average plasma lipoprotein levels of wild Japanese eels were lower than those of cultured eels (36.9 vs. 54.1 mg/ml of plasma), and both VLDL and LDL levels of wild Japanese eels were reduced to one-half of cultured ones (Ando, 1999; Ando & Matsuzaki, 1996). The lipoprotein profiles found in the plasma of wild Japanese eels were different from those of cultured eels. Both HDL and VLDL were the predominant lipoproteins in the plasma of wild Japanese eels. VLDL is synthesised from triacylglycerol (TG) and apolipoproteins in the liver and secreted as TG-rich lipoprotein in the blood. TG is hydrolysed by lipoprotein lipase during the circulation of VLDL. The hydrolytic products are absorbed in the peripheral tissues, including muscle and resynthesised into TG, the final storage product. Thus the difference of plasma lipoprotein profiles between wild and cultured Japanese eels seems to influence their

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doi:10.1016/j.foodchem.2008.12.032

lipid profiles in muscle and liver. It is of interest whether wild Japanese eels are classified into fatty fish similarly to cultured eels.

In this study, we examined the lipid profiles of muscle, liver, and plasma in wild and cultured Japanese eels. We also analysed fatty acid compositions of muscle, liver, and plasma lipoproteins from both eels. The results indicate that lipid profiles of liver and plasma, and fatty acid compositions of muscle, liver, and plasma lipoproteins enable us to differentiate between wild and cultured Japanese eels.

2. Materials and methods

2.1. Fish

Wild Japanese eels (average body weight 195 g, $n = 3$) were captured from the Amori River, Kagoshima. Cultured Japanese eels (average body weight 220 g, $n = 3$) starved for two days were purchased from a local fish market.

2.2. Proximate and lipid compositions of muscle and liver

The dorsal muscle and liver from wild and cultured Japanese eels were analysed in duplicate for moisture and protein according to the usual methods (AOAC, 1984). Lipid extraction from the dorsal muscle and liver was carried out in duplicate by the method of Bligh and Dyer (1959). The total lipid was analysed quantitatively by thin-layer chromatography (TLC). The TLC plates (Kieselgel 60, a ready-made plate from Merck) were developed using *n*-hexane:diethylether:acetic acid 85:15:1 (by volume) for non-phospholipid, and chloroform:methanol:acetic acid:water 25:15:4:2 (by volume) for phospholipid. The TLC plate was sprayed with 3% copper acetate-8% phosphoric acid, heated on a hot plate, and quantified using a Pharmacia Biotech Image Master. The amount of phospholipid was calculated from lipid-phosphorus assayed using a Phospholipid-Test Wako (Wako Pure Chemical Industries, Osaka, Japan).

2.3. Plasma lipoprotein isolation and plasma lipid analysis

Blood was drawn into ethylenediaminetetraacetic acid (EDTA, 5 mg/ml of blood) dissolved in 0.15 M NaCl, by using a syringe inserted in the caudal vasculature of live Japanese eels, and maintained at a temperature of 4 °C throughout the procedure. Plasma was obtained by centrifugation (3000 g for 15 min). VLDL (density (d) < 1.006 g/ml), LDL (1.006 < d < 1.085 g/ml), HDL2 (1.085 < d < 1.100 g/ml), and HDL3 (1.100 < d < 1.210 g/ml) in the plasma were obtained by sequential ultracentrifugal flotation (Ando & Matsuzaki, 1996), using a Hitachi CS100 ultracentrifuge equipped with a RP80AT rotor (267,000 g at 15 °C). The plasma lipid composition was estimated using commercially available enzymatic kits from Kyowa Medex (Tokyo, Japan) for TG, phospholipid (PL), free (FC), and total cholesterol (TC). The amount of cholesteryl ester (CE) was calculated from the difference between TC and FC contents. Protein content was determined using Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as a standard.

2.4. Fatty acid analysis

A 1 ml aliquot of chloroform solution containing 100 mg of total lipid from muscle or liver was poured on a Sep-Pak silica cartridge (2.0 ml; Waters Chromatography Division, Milford, MA) for separating non-phospholipid and phospholipid fractions. Non-phospholipid and phospholipid fractions were eluted with 20 ml chloroform and 30 ml methanol, respectively. The total lipid was extracted from plasma lipoprotein fractions according to the method of Bligh and Dyer (1959).

Fatty acid compositions of total lipid, non-phospholipid, and phospholipid were determined after methanolysis according to Prevot and Mordret (1976), and subsequent analysis on a gas chromatograph (Hitachi type 263-30). The conditions for gas chromatography were as follows. The separation column consisted of a 40 m wide-bore capillary column G-300, equivalent to polyethylene glycol 20 M (i.d. 1.2 mm) (Chemical Inspection Association, Tokyo). The oven temperature was 190 °C, and the injection port and detector temperature were 245 °C. Helium was used as a carrier gas and peak integration was carried out with a Hitachi D-2500 Chromato-Integrator. Fatty acids were identified by comparing their retention times with those of standards and reported equivalent chain length values.

2.5. Statistics

Data were analysed using one-way analysis of variance, followed by Student's *t*-test.

3. Results and discussion

The results of this study were obtained from a limited sample size ($n = 3$ from each wild and cultured Japanese eels) and any statistical analyses of data seemed not to be valid. Nevertheless, some average values significantly differed between wild and cultured eels.

3.1. Proximate compositions of muscle and liver from wild and cultured Japanese eels

Table 1 shows the proximate compositions of muscle and liver from wild and cultured Japanese eels. The moisture contents of muscle from wild and cultured eels ranged from 67.3% to 71.1% and 66.1% to 68.2%, respectively. The protein contents of muscle from both eels were in the range of 18.3–19.4%. The lipid contents of muscle from wild and cultured eels ranged from 8.1% to 13.5% and 12.6% to 13.8%, respectively. The muscle lipid levels of wild eels seemed to be less than cultured eels, but both eels are fatty enough to be classified into typical fatty fish. In cases of American eel (*Anguilla rostrata*), the eels cultured with fish meal in earthen ponds for 18 months contained over 14.7% lipid in their muscle, whilst wild eels with similar body weight (166 g) to cultured eels averaged less than 3.4% lipid in their muscle (Otwell & Rickards, 1981/1982). The difference of muscle lipid levels between wild Japanese and American eels may be influenced by feed composition.

Compared with the proximate compositions of muscle, the moisture contents of liver increased, whilst the lipid and protein contents of liver decreased (Table 1). The moisture contents of liver from wild and cultured eels ranged from 72.9% to 74.7% and 68.8% to 72.8%, respectively. The liver protein contents of wild eels were less than those of cultured eels, ranging 13.6% to 14.8% in the

Table 1
Proximate compositions of muscle and liver from wild and cultured Japanese eels.

Component (g/100 g tissue)		Wild	Cultured
Muscle	Moisture	69.1 ± 1.9	67.4 ± 1.1
	Lipid	11.6 ± 3.1	13.1 ± 0.6
	Protein	19.0 ± 0.4	18.9 ± 0.5
Liver	Moisture	73.9 ± 1.0	70.8 ± 2.8
	Lipid	4.7 ± 1.1	7.6 ± 0.2
	Protein	14.2 ± 0.6	16.8 ± 3.3