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Fatty Acids Profile of Tropical Bagridae Catfish (*Mystus nemurus*) During Storage

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Abstract: Changes in the fatty acid composition of the fresh water catfish (*Mystus nemurus*) stored in 10°C and ice (0±2°C) for 1, 10 and 20 days were monitored. A total of 22 fatty acids were found to be present in the studied samples. The main saturated fatty acids (SFA) were palmitic (17.99%), tridecanoic (16.59%), stearic (4.40%) and myristic (2.61%). The monounsaturated fatty acids (MUFA) were dominated largely by the oleic acid (24.84%) and palmitoleic acid (4.66%). The long-chain polyunsaturated fatty acids (PUFA) were also present in significant amounts, composed of eicosapentaenoic (2.65%) and docosahexaenoic (4.44%). Results also revealed that saturated and monounsaturated fatty acid significantly increased ($p < 0.05$) during storage while polyunsaturated decreased. This should attract attention to the importance of the proper and short period storage to retain the best quality of fish meat and its lipid contents.

Key words: Fish, food quality, food safety, storage conditions, lipid decomposition

INTRODUCTION

Tropical bagrid catfish, *Mystus nemurus* is found in the rivers of Indonesia, especially in the Aceh province and in other Asian countries such as Thailand and Vietnam. It is a commercially important species for inland fisheries in Malaysia. The demand and consumption of freshwater fish are increasing because of their nutritional benefits like high content of ω -3 polyunsaturated fatty acid, low content of cholesterol and high content of good quality protein. Its perceived benefit to health has made it a more up market food. Statistics has shown that fresh water fish dominate Southeast Asian aquaculture production (29% by weight and 57% by value in 1995) (Usmani *et al.*, 2003). In Malaysia, the aquaculture production is projected to reach 600,000 metric tons by the year 2010 (Data adopted from the Malaysia Agricultural Directory Index 2003/2004). *Mystus nemurus* is a high fatty fish species (15-18.83%) with a high level of polyunsaturated fatty acid (19.29-24.51%) and 10-11% proteins. Lipid is the most important component that affect the quality attributes of fish (Menoyo *et al.*, 2004). Lipid hydrolysis and oxidation have been shown to occur during fatty fish storage and become an important factor of fish acceptance, influencing protein deterioration, texture changes, functionality loss, off-flavor development (Wang *et al.*, 1991). This is due to the deterioration of the highly unsaturated nature of the fatty acids in fish tissue (Pamela *et al.*, 1992; Gladyshev *et al.*, 2008). The lipid deterioration must also be attributed to polyunsaturated fatty acid oxidation (Maria *et al.*, 1992).

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Fish oils which are enriched with nutritional supplements like ω -3 and ω -6 polyunsaturated fatty acids (PUFAs) are known to reduce the blood pressure and triglycerides (Eristland, 2000) and have demonstrable benefits in the prevention and treatment of cardiovascular disease, stroke, lupus nephropathy, Crohn's disease, breast cancer, prostate cancer, colon cancer, hypertension, rheumatoid (Levine and Barbara, 1997; Woods, 2008), modify platelet aggregation, preventing weight loss in cancer patients and minimize inflammatory responses (Uauy-Dagach and Valenzuela, 1996).

Knowledge of the fatty acids composition of freshwater fish is limited to a few species and only few studies have been published on the storage characteristics of freshwater fish when kept in ice. The PUFA composition may vary among species of fish, little attention has been paid to the PUFA composition of different species when selecting fish for diets (Weaver *et al.*, 2008). Therefore, when fish are suggested as a mean of improving health, both fat content and PUFA distribution must be considered. Thus this study provides compositional data on the resulting fatty acids from *Mystus nemurus* during storage to highlight the nature of the decomposed lipids and the resulted fatty acids in one of the most fatty species of fish, namely *Mystus nemurus*. This in turn will determine the nature, types, classification of the resulted fatty acids and let researchers pinpoint the health impact of the resulted fatty acids on human consumers. Moreover, this study sheds light on the effect of the storage on the resulting fatty acids in fish meat and how optimal storage temperature and shortened storage period might lower efficiently the decomposed fatty acids.

MATERIALS AND METHODS

This study was conducted in the period from December 2006 to February 2008 in Selangor, Malaysia. A mixture of standard fatty acid methyl ester (FAMES) with 37 components and individual FAMES standards like lauric acid (C12:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0), linoleic acid (C18:2 ω -6), tricosanoic acid (C23:0) and lignoceric acid (C24:0) were purchased from Sigma. All other chemicals and solvents were analytical grade.

Sampling

Mystus nemurus (weighing between 1.00-1.50 kg each) was harvested commercially from Bukit Serdang Fish Farm, Selangor and transported alive to the laboratory within 20 min after harvested. Immediately upon arrival, whole fish were washed and divided into 2 groups i.e., refrigerated ($10\pm 2^{\circ}\text{C}$) and iced ($3\pm 1^{\circ}\text{C}$) storage temperatures. Samples were taken for 1, 10 and 20 days. Control was done on the first days immediately after fish death.

Proximate Composition

Moisture was determined gravimetrically by desiccation at 105°C . Ash content was determined by incinerating in muffle furnace at 600°C . The crude protein was determined by the Kjeldahl method (Cunniff, 1998)

Extraction of Sample

The fish lipids were extracted by the method of Blight and Dyer (1959). Representative samples of fish filets (320 g) were homogenized in Waring blender for 2 min with a mixture of methanol (640 mL) and chloroform (320 mL). One volume of chloroform (320 mL) was added to the mixture and blended for an additional 30 sec. The homogenate was stirred with a glass rod and filtered through Whatman No. 4 filter paper on a Buchner funnel. The residue was added with another 200 mL chloroform and blended for 30 sec before filtered. The filtrate was transferred to a separator funnel. The lower clear phase was drained into four 500 mL round bottom flask and concentrated with a rotary evaporator at 40°C . Crude lipid was flushed with nitrogen gas and butylated hydroxytoluene (BHT)

at a concentration of 0.05% (of the lipid) was added to the remaining lipid extract and the extract was stored at -25°C for further analysis.

Fatty Acid Profile

The sample for FAMES analysis was melted at 60-70°C and homogenized thoroughly before taking a test sample. Approximately 50 mg of the test sample was weighted into a 2 mL vial. 0.80 mL petroleum ester was added. The vial was closed and shaken to dissolve the oil. The cap was removed before adding 0.20 mL 10% sodium methoxide. The cap was closed quickly and the mixture was shaken vigorously for 1 min with the help of a vortex mixer. After at least 5 min, the clear upper layer of methyl esters was pipette off for further analysis. This method was identical to the AOCS Official Method Ce 2-66.

The FAMES were analyzed by Hewlett Packard 6890 Gas Chromatograph equipped with a flame ionized detector (FID) and fitted with a Supelco SP-2330 capillary column (30 meter x 0.25 mm i.d., 0.20 µm film thickness). Column temperature was programmed at 15.0°C min⁻¹ from 90°C rising to 220°C; at 2.0°C min⁻¹ for 20 min and 15.0°C min⁻¹ for 7 min. Injector and detector temperatures were 220 and 240°C, respectively. The carrier gas was helium at a flow rate of 1.4 mL min⁻¹, average velocity of 20 cm sec⁻¹ and pressure 10 psi. The split used was 1/100. Peak areas were calculated on a computing integrator (HP G1530A). Compounds were tentatively identified by comparison with the retention times of known mixture and individual standard from Supelco.

RESULTS AND DISCUSSION

The fatty acid composition of whole river catfish muscle during storage under different temperatures was studied (Fig. 1, 2). A total of 22 fatty acids were found to be present in the fish oil. The most abundant fatty acids were C18:1ω9, C16:0, C13:0, C18:2ω6, C16:1, C22:6ω3, C18:0,

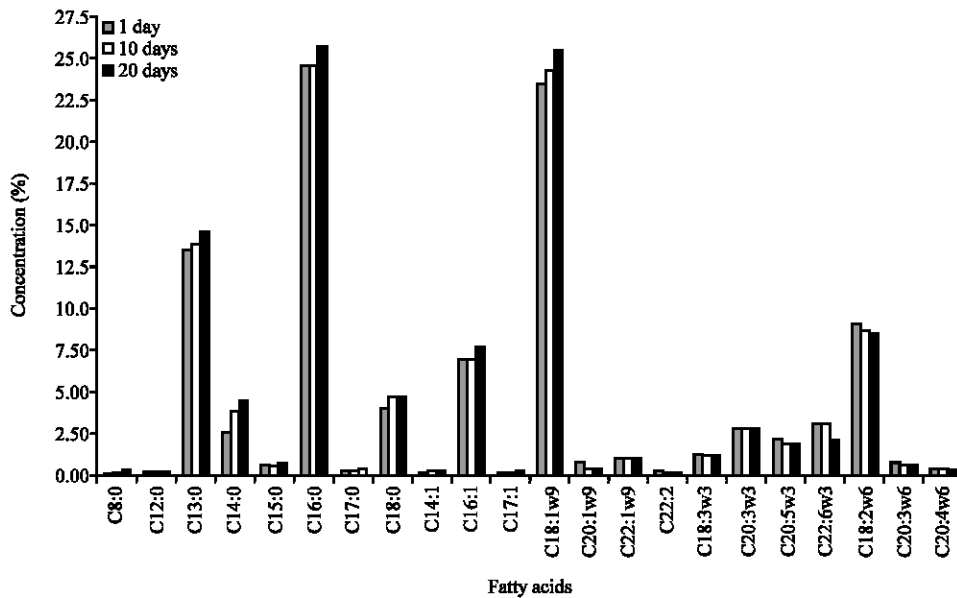


Fig. 1: Changes in saturated and unsaturated fatty acids of *Mystus nemurus* were stored for 1, 10 and 20 days at (10±2)°C storage temperature.

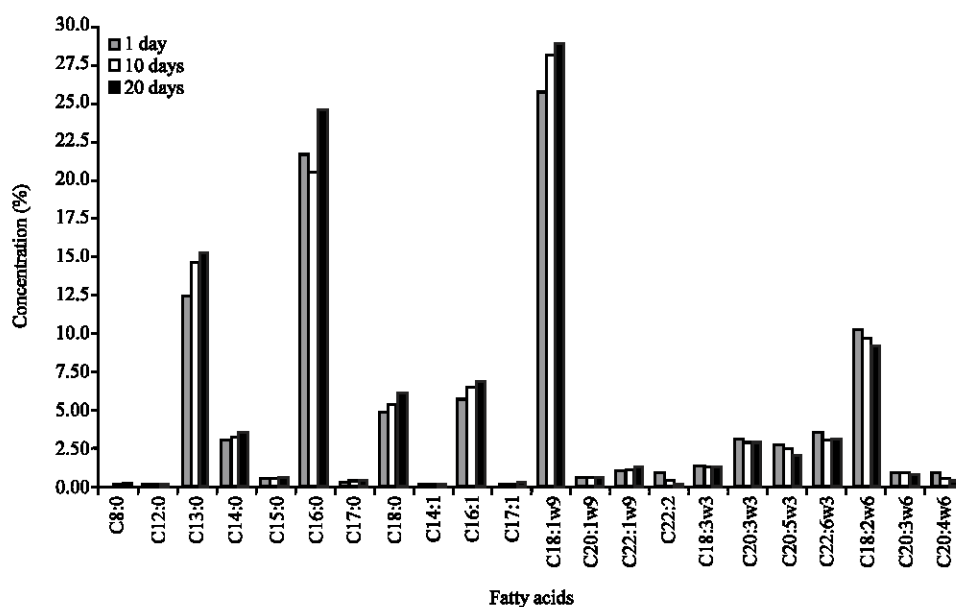


Fig. 2: Changes in saturated and unsaturated fatty acids of *Mystus nemurus* were stored for 1, 10 and 20 days at (2±1)°C storage temperature

C20:3 ω 3, C20:5 ω 3 and C20:4 ω 6. Total saturated fatty acids (SFA) content in the river catfish was 44.54%, dominated by C16:0 (17.99%) followed by C13:0 (16.59%), C18:0 (4.40%) and C14:0 (2.61%).

The monounsaturated fatty acid (MUFA) in the catfish were 30.85% and dominated largely by C18:1 ω 9 (24.84%), followed by C16:1 (4.66%). Among the polyunsaturated fatty acids (PUFA) 24.51%, C18:2 ω 6 was dominant (9.12%). The more common ω 6 and ω 3 polyunsaturated fatty acids were also present like C18:2, C18:3, C20:3, C20:4, C20:5 and C22:6. The presence of eicosapentaenoic C20:5 ω 3 and docosahexaenoic C22:6 ω 3 acids was significant and accounted for 7.09% of the total fatty acids. The ratio ω 6/ ω 3 fatty acids was 1.06, indicating the predominance of the ω 6 isomers, mainly owing to the C18:2 ω 6. The Department of Health of the UK recommends a maximum value of 4.0 for this ratio. Fish muscle lipids that contain several ω 3 and ω 6 long chain polyunsaturated fatty acid have nutritional implication because they are important for fetal and term-infant neural development. The PUFA and SFA values were different from that found by Justi *et al.* (2003), i.e., 26.3% (SFA) and 44.7% (PUFA) for Nile tilapia (*Oreochromis niloticus*). According to the data obtained, the PUFA/SFA ratio in the lipids of *Mystus nemurus* were higher (0.55) than the minimum recommended of 0.45 for healthy diet (Hu, 2001).

Results also indicated that the quantities of saturated and monounsaturated fatty acid increased during storage while content of polyunsaturated decreased along the storage periods. This is due to the highly rapid oxidation of unsaturated fatty acids. The type of the fatty acid present in the catfish tissues is a major factor in determining the oxidative stability of the lipids. As the number of double bonds increases in the fatty acids especially for the C20 and C22, the rate of auto-oxidation and the susceptibility to rancidity increase dramatically. The highly unsaturated fatty acids in the tissues of river catfish would adversely affect the storage quality of the fish.

DHA (C22:6 ω 3) and EPA (C20:5 ω 3) in fish can form four or five free radicals for every fatty acid undergoing oxidative breakdown, making the rate of peroxide formation and oxidation extremely

rapid during the propagation stage. The last step of oxidation, termination occurs when broken down products combine to form stable, non-radical products and the rate of free radical formation slow down as the number of fatty acid molecules not yet oxidized decreases (Ronald, 1998). Linolenic acid was present in low concentration in fish tissues that Cis-11,14,17-eicosadienoic acid (C20:3 ω 3) decreased significantly ($p < 0.05$) at ambient storage, whereas decreased not significantly ($p < 0.05$) at 10°C and iced storage. It was shown that linolenic acid was incorporated into the carbon chains of 20:5 and 22:6 acids of fish and was also found that the probable conversion pathway for linolenic acid was as follows: 18:3 ω 3 \rightarrow 18:4 ω 3 \rightarrow 20:4 ω 3 \rightarrow 20:5 ω 3 \rightarrow 22:5 ω 3 \rightarrow 22:6 ω 3 (Edward, 1967; Mourente and Tocher, 1994). The autoxidation of linoleic acid occurs at the storage period to forms another products (Chi and Chen, 1994).

Linoleic acid C18:2 ω 6, the major dietary source of the ω 6 PUFAs, is abundant in fish tissues and is the precursor of arachidonic acid. It was shown that a significant decrease ($p < 0.05$) was observed in C18:2 ω 6 for all storage conditions from 9.06 to 10.26%. C20:4 ω 6 showed a non-significant difference ($p < 0.05$) decrease for storage at 10°C temperature whereas significantly decreased for samples stored at icing temperature. Experiments by Kelly and Cheng (2000), gave indications that fish, like land animals, probably convert fatty dienoic acid into tetraenoic, pentaenoic and hexaenoic acids.

Taken together, it was found concluded that 22 fatty acids were found to be present in the studied samples. The SFA were palmitic (17.99%), tridecanoic (16.59%), stearic (4.40%) and myristic (2.61%). The MUFA were dominated largely by the oleic acid (24.84%) and palmitoleic acid (4.66%). The PUFA were also present in significant amounts, composed of eicosapentaenoic (2.65%) and docosahexaenoic (4.44%). Taken together, it was concluded that the saturated and monounsaturated fatty acids significantly increased during storage of *Mystus nemurus* at both ambient and refrigerator temperatures. Moreover, the longer the period of storage, the more decomposed fatty acids were found. Therefore, this species of fish, which contains a large amount of fat, needs a more proper, more consistently cold and shorter duration storage conditions than other traditionally used fish products.

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