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Lipid Quality Deteroriation of Bagridae Catfish (*Mystus nemurus*) During Storage

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Abstract: Lipid damage indices of whole freshwater catfish (*Mystus nemurus*) stored at ambient (28±2°C), chilled (10±2°C) and iced (2±1°C) temperature for 24 h, 10 and 20 days, respectively, were studied. Lipid quality deterioration was evaluated by measuring free fatty acids, peroxide values, p-anisidine value, thiobarbituric acid and polyene index. The results showed that *Mystus nemurus* is a fatty fish species with 16.8-19% of fat content. The lipid hydrolysis and lipid oxidation that occurred after 12 h at ambient, 8 days at chilled and 16 days at iced storage increased significantly ($p<0.05$) to unacceptable levels with decreased polyene. This study demonstrates evidence-based research that typical storage conditions for *Mystus nemurus* is of practical significance to producers and consumers.

Key words: *Mystus nemurus*, lipid damage, free fatty acid, peroxide value, p-anisidine value, thiobarbituric acid, polyene index

INTRODUCTION

Fish and other marine species give rise to products of great economic importance in many countries. During processing and storage, fish quality may decline as a result of several factors. One of the most important concerns, is the oxidation of the highly unsaturated lipids, (Ackman, 1989) which are directly related to the production of off-flavors and off-odors (Harris and Tall, 1994).

Quality assurance and safety of seafood is a major challenge for producers and a concern for consumers in the current century. In this sense, wild and farmed fish species are known to deteriorate after death by different mechanisms (Hsieh and Kinsella, 1989). During the chilled storage of fish, lipid hydrolysis and oxidation have been shown to occur leading to biochemical changes such as changes in the protein and lipid fractions and the formation of biogenic amines in some cases for certain species (Huidorbo *et al*., 2001). Therefore, lipid deterioration has become an important factor for fish acceptance as it influences rancidity development, protein denaturation and texture changes (Verma *et al*., 1995).

With respect to *Mystus nemurus*, efforts have been made to employ this species in the manufacturing of a large number of restructured products from fish mince in Asian countries and increasingly exported to European countries (Usmani *et al*., 2003). However, since it is also, directly consumed, research has been carried out on fish as such (Gunstone, 1996) and protein changes have been studied during frozen storage (Huidorbo *et al*., 2001) and quality changes during chilling storage have been determined (Aubourg *et al*., 1998; Smith *et al*., 1972).

There has not been a previous study that explored the lipid hydrolysis in *Mystus nemurus* sp. nor were the effects of storage conditions studied on the lipid oxidation of this species. Therefore, the current study was designed to investigate lipid damage caused by the storage of *Mystus nemurus* for 20 days at three different temperatures (28±2°C, 10±2°C and 2±1°C). And detection of primary and secondary lipid oxidation and lipid hydrolysis was carried out.

MATERIALS AND METHODS

Sampling: *Mystus nemurus* weighing 1.00±0.05 kg each was harvested commercially from Bukit Serdang Fish Farm, Selangor and transported alive to the laboratory within 20 min. Upon arrival, the fish were immediately slaughtered by immersing in iced-cold water, hypothermia,

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then divided into 3 groups and immediately packed in separate insulated styrofoam boxes. One group of fish was kept at ambient temperature (28±2°C) for 24 h. The second group was stored at chill (10±2°C) for 22 days and the remaining fish were kept in clean crush iced (2±1°C). The styrofoam boxes were provided with outlets for water drainage and stored at 4±1°C. The ratio of ice fish^{-1} (3:1) was maintained constant throughout the experiment.

The stored fish at ambient temperature were sampled 4 hourly for 24 h (0, 4, 8, 12, 16, 20 and 24 h), the fish at chilled temperature were sampled every 2 days for 10 days (0, 2, 4, 6, 8, 10 days) and the fish in iced storage were sampled every 4 days for 20 days (0, 4, 8, 12, 16, 20). Approval from the regional research ethics committee for dealing with animals was granted.

Determination of water and lipid contents: Water content was determined by weight difference of the homogenized muscle (1-2 g) before and after 24 h at 105°C. Results were calculated as g water/100 g muscle.

Fish lipids were extracted from the homogenized white muscle according to the method of Bligh and Dyer (1959) with slight modification. Representative samples of fish fillets (320 g) were homogenized in Waring blender for 2 min with a mixture of 2:1 methanol: chloroform (640: 320 mL). One volume of chloroform (320 mL) was added to the mixture and after blending for an additional 30 sec, another 320 mL distilled water was added. The homogenate was stirred with a glass rod and filtered through Whatman No. 4 filter paper on a Buchner funnel. The residue was then dissolved with another 200 mL chloroform and blended for 30 sec before filtered. The filtrate was transferred to a separatory funnel. The lower clear phase was drained into four, 500 mL round bottom flasks 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 (Kinsella *et al*., 1977).

Lipid damage measurements

Determination of free fatty acids: Free fatty acid contents were determined according to AOCS Official Method Ca 5a-40 by acidometric titration of the Bligh and Dyer (1959) extracts after adding ethanol and using phenolphthalein as an indicator. The percentage of free fatty acids in lipids was calculated as oleic acid. Each sample was analyzed in triplicates.

Determination of peroxide value: The Peroxide Value (PV) of lipids was determined by iodometric titration of the Bligh and Dyer (1959) extracts after the addition of acetic acid and incubation time of 5 min after adding potassium iodide. The procedure was otherwise similar to the AOCS Official Method Cd 8b-90. All experiments were performed in triplicates and for each experiment, at least 2 replicates were used. PV was calculated as milliequivalents per kg of lipid.

Determination of p-anisidine value: The P-Anisidine Value (AV) is defined by convention as 100 times the optical density measured at 350 nm in a 1 cm cell of a solution containing 1.00 g of the oil in 100 mL of a mixture of solvent and reagent according to the AOCS Official Method Cd 18-90. This method determines the amount of aldehydes (principally 2-alkenals and 2,4-dienals) in animal.

Determination of thiobarbituric acid: Preparation of Thiobarbituric Acid (TBA) reagent and working TEP standard solution were according to Ke procedure (Ke *et al*., 1984). The working TEP standard solution was prepared by accurately pipetting 0, 0.4, 0.8, 1.2, 1.6 and 2.0 mL into screw-cap test tubes and water was added up to 5 mL. The 5 mL of TBA reagent were added and tubes were tightly capped. The final concentrations of TEP, in 10 mL volumes, were 0, 4.0, 8.0, 12.0, 16.0 and 20.0×10^{-7} mol L^{-1}, respectively. The test tubes were heated in vigorously in a boiling water bath for 45 min and cooled in tap water. The solutions were determined for absorbance at 538 nm after 30 min of cooling by setting the blank (0.0 mL TEP) to zero. The plot of TEP concentration (μM) against absorbance at 538 nm was linear up to 2.0 μM TEP under the conditions described here. The molar extinction coefficient of the color developed (absorbance/molarity) was 1.9×10^{5} at 538 nm.

The 10 g samples of finely chopped fish were weighed and transferred to the blender jar with 35 mL of distilled water and blended for 2 mm or until the sample was finely harnessed. Afterwards, the sample homogenate was transferred to a tarred 500 mL round bottom flask that contains approximately, 100 mg of propylgallate and EDTA. Distilled water was added so that total weight of the sample and water was 105 g. The sample was flushed with nitrogen and 95 mL 4M HCI were added inside the sample. Immediately, the distillation was set out and 50 mL of distillate was collected in a volumetric flask within 35 mm or less. The distillation rate was kept at 1-2 drops sec^{-1}. The still, between samples, was rinsed with methanol and then distilled. TBA distillates were refrigerated overnight if necessary.

Samples (5 mL) of each TBA distillate and 5 mL of TBA reagent were pipetted into screw-cap tubes, covered

tightly and then treated as described in standard curve preparation. A blank of 5 mL of distilled water and 5 mL of TBA reagent were run simultaneously for absorbance at 538 nm. Sample solutions with absorbance >0.5 were diluted with distilled water or alternatively the analysis repeated using less TBA distillate.

Fatty acid composition: Lipid extracts were converted into fatty acid methyl esters and analyzed by gas chromatography. The Polyene Index (PI) was calculated as the following fatty acid ratio: (C20:5 + C22:6)/C16:0 (Lubis and Buckle, 1990).

Statistical analysis: Experimental data was analyzed using Analysis of Variance (ANOVA) and the significant difference among the means was determined by Duncan's Multiple Range Test (DMRT) using the Statistical Analysis System (SAS) software package.

RESULTS AND DISCUSSION

Water content ranged between 62 and 65% in all samples without differences in regard to temperature and storage time. Lipid contents ranged between 16.78 and 18.00%. Thus, the *Mystus nemurus* can be classified as fatty fish.

Lipid hydrolysis: Lipid hydrolysis occurred during ambient, chilled and iced storage (Table 1-3). At ambient temperature, a progressive increase of lipid hydrolysis with storage time was observed leading, at the end of the experiment, to a proportion of 6.37% free fatty acid in the lipid fraction. At 10°C storage temperature, a significant increase ($p<0.05$) in the free fatty acid contents was obtained at day 10 (12.05%). However, at iced storage, a significant increase ($p<0.05$) was occurred until the end of the storage time and like the ambient storage, a progressive increase was occurred until the end of the storage time to a 7.40%. This study showed that the free fatty acid levels reached 5% limits after 12 h of storage at ambient, 8 days at chilled and 16 days at iced storage (Table 1-3).

Lipid oxidation: The primary oxidation products such as hydroperoxides were measured and presented as PV. A fast development of primary oxidation in the first 24 h of experiment was detected at ambient temperature (Table 1), reaching the highest mean PV value at hour 20 (2.79 meq kg^{-1}). Peroxide formation in chilled (10±2°C) and iced (2±1°C) temperature, proved to be very slow (Table 2 and 3). A significant increase in PV was observed at 10±2°C storage from the initial value of 0.79 meq kg^{-1} to the 10th day storage (2.26 meq kg^{-1}). The highest mean PV was at day 8 (2.28 meq kg^{-1}). A significant increase in the rate of primary oxidation occurred till day 16 and after that only small differences were observed at 2±1°C storage temperature. The studied samples showed significant PV levels >2 meq kg^{-1} of fish, the limit of acceptability of PV, after 12 h at ambient, 8 days at chilled and 16 days at iced storage.

The secondary lipid oxidation products were measured according to the TBA method. It was shown that a progressive increase in the secondary oxidation development was obtained during ambient storage and till the end of the storage time, 17.99 µmol MDA kg^{-1} (Table 1). The same trend was observed for the chilled and iced storage (Table 2 and 3). Data showed that the higher the storage temperatures, the higher were the final TBA values. The secondary oxidation products can be

Table 1: Lipid damage detection[a] of *Mystus nemurus* during ambient storage[b]

ST (h)	FFA (%)	PV (meq kg^{-1})	AV (abs g^{-1})	TBA (µmol MDA kg^{-1})	PI
0	1.13f	0.79f	0.45e	1.36g	3.90a
4	2.25e	1.10e	0.60d	4.85f	3.79b
8	3.81d	1.47d	0.71c	6.80e	3.77b
12	4.87c	1.90c	0.76b	9.82d	3.76b
16	5.64b	2.40b	0.91a	12.47c	3.74c
20	6.33a	2.79a	0.97a	14.56b	3.69d
24	6.37a	2.69a	0.99a	17.99a	3.67d

[a]ST: Storage Time, FFA: Free Fatty Acids, PV: Peroxide Value, TBARS: Thiobarbituric Acid Reactive Substance, AV: Anisidine Value), PI: Polyene Index, [b]Means of 3 independent determinations. Values in the same column followed by different letters are significantly different ($p<0.05$)

Table 2: Lipid damage detection[a] of *Mystus nemurus* during chilled storage[b]

ST (days)	FFA (%)	PV (meq kg^{-1})	AV (abs g^{-1})	TBA (µmol MDA kg^{-1})	PI
0	1.13f	0.79e	0.45f	1.36f	3.90a
2	2.94e	0.99d	0.54e	4.14e	3.78b
4	4.17d	1.19c	0.62d	6.44d	3.77b
6	4.48c	1.29b	0.70c	8.80c	3.74c
8	4.89b	2.28a	0.78b	9.90b	3.72c
10	6.95a	2.26a	0.86a	10.22a	3.64d

[a]As indicated in Table 1, [b]Means of three independent determinations. Values in the same column followed by different letters are significantly different ($p<0.05$)

Table 3: Lipid damage detection[a] of *Mystus nemurus* during iced storage[b]

ST (days)	FFA (%)	PV (meq kg^{-1})	AV (abs g^{-1})	TBA (µmol MDA kg^{-1})	PI
0	1.13f	0.79e	0.45f	1.36f	3.90a
4	2.98e	1.30d	0.57e	2.44e	3.86a
8	3.76d	1.59c	0.67d	3.36d	3.81b
12	4.40c	1.72b	0.72c	4.11c	3.76c
16	4.95b	1.99a	0.79b	9.88b	3.61d
20	7.40a	1.98a	1.28a	11.53a	3.55d

[a]As indicated in Table 1, [b] Means of three independent determinations. Values in the same column followed by different letters are significantly different ($p<0.05$)

followed with the AV. The obtained AV at ambient temperature (Table 1), showed a similar trend as free fatty acids results for ambient temperature storage. AV increased significantly to reach the highest point 0.99 from 0.45 absorbance g^{-1} and for 10°C storage, it increased significantly from an initial value of 0.45-0.86 absorbance g^{-1} after 10 days of storage, whereas the trend for icing storage increased slowly to reach the peak (Table 2 and 3).

The *Mystus nemurus* became unacceptable when the AV exceeds to level of 0.8 absorbance g^{-1}.

The PI of *Mystus nemurus* at the three studied temperatures of storage is shown in Table 1-3. Overall, amount of the PI decreased in value throughout the storage period and temperature. At ambient temperature, PI decreased from the initial value of 3.70-3.67 after 24 h storage, to 3.64 after 10 days storage at 10°C and to 3.55 after 20 days at iced storage. The initial value of sardines (1.50) was much higher than that of sardinops sardines (0.88-0.93). The PI decreased during storage, but remained nearly stable after 6 weeks storage, especially in the case of the sardinops sardines.

Mystus nemurus can be classified as fatty fish and contains high amount of lipids. In this study, the lipid hydrolysis tested during ambient, chilled and iced storage. It was found that a progressive increase of lipid hydrolysis with storage time was observed maximally at ambient, 10°C storage temperature and least at iced storage. These findings indicated that free fatty acids increased with storage time and temperature. This hydrolysis reflects a loss of quality, giving rise to a high increase of the free fatty acid content. The production of free fatty acids can be used as degradation index of nutritional compounds (Verma *et al*., 1995). With respect to other previously studied chilled fish species, such as sardine (Aubourg *et al*., 1997), horse mackerel (Aubourg, 2001) and blue whiting (Aubourg *et al*., 1998), the lipid hydrolysis was observed at slower rate in the fish species studied here, *Mystus nemurus*.

The free fatty acid formation due to lipid (TAG) hydrolysis has provided a suitable means for assessment of fish damage during storage and can be used as a quality index for fish and other food products (De Koning and Mol, 1991; Hui and Tung, 1997). Moreover, as quality specifications for crude fish oil, Bimbo (1998) suggested maximum acceptable values of 5%. This study showed that the free fatty acid levels reached 5% limits after 12 h of storage at ambient, 8 days at chilled and 16 days at iced storage.

Regarding lipid oxidation, the primary oxidation products were measured, such as hydroperoxides and presented as PV. A fast development of primary oxidation in the first 24 h of experiment was detected at ambient temperature reaching the highest mean value at 20 h while, peroxide formation in chilled (10±2°C) and iced (2±1°C) temperature, proved to be very slow during chilled storage. A significant increase in the rate of primary oxidation occurred till day 16 and after that only small differences were observed at 2±1°C storage temperature. A decrease in the PV at the end point could be explained as a result of decomposition of hydoperoxides into smaller molecules or interaction with protein (Santiago, 1999; Melton, 1983). PV is useful during the initial storage of lipid oxidation (Gary, 1978).

The secondary lipid oxidation products were measured according to the TBA method. It was shown that a progressive increase in the secondary oxidation development was obtained during ambient storage till the end of the storage time. Data showed that the higher the storage temperatures, the higher were the final TBA values. These results were consistent with that obtained by Lubis and Buckle (1990) who studied rancidity and lipid oxidation of dried-salted sardines. The TBA value was also, correlated with the release of propenal, propanal and acetaldehyde. Pentane and hexanal production were highly correlated with TBA values and loss of linoleic acid (Leon *et al*., 1992). The limit of acceptability of TBA in *Mystus nemurus* was reached after 12 h at ambient, 8 days at chilled and 16 days at iced storage temperature.

The hydroperoxides are very unstable and decompose into secondary oxidation. At ambient temperature storage, the trend of AV increased progressively and significantly with time whereas the trend for icing storage increased slowly to reach the peak. AV measured the secondary products of lipid oxidation, which were also broken down to other chemical products. On the other hand, no much change in the AV was found for the icing storage throughout the storage process. The AV procedure was developed mainly to use the reaction of α and β-aldehydes (primarily 2-alkenals) with p-anisidine reagent because many of compounds considered responsible for the off-flavor development in oil are actually aldehydes (Pamela, 1994). Peroxides are decomposed to give secondary oxidation products that are anisidine-reactive. The *Mystus nemurus* became unacceptable when the AV exceeds the level of 0.8 absorbance g^{-1}. PI might provide a meaningful tool to measure oxidative rancidity in fishery product. PI decreased in value throughout the storage period and temperature.

PI (C20:5 + C22:6/C16:0) might provide a meaningful tool to measure oxidative rancidity in fishery product because it includes only 2 major polyenoic fatty acids that the levels of which, can be measured with reasonable accuracy as they are the larger fatty acids among the large

gas chromatography peaks. The PI decreased during storage, but remained nearly stable after 6 weeks storage. In the case of the sardinops sardines, Perez-Mateos *et al.* (2004) found that the ratio of total unsaturated to total saturated fatty acids decline during 90 day's storage at -22.3°C and then stabilized.

CONCLUSION

Taken together, lipid hydrolysis and lipid oxidation in *Mystus nemurus*, with the resulting primary and secondary products, increased significantly after 12 h at ambient, 8 days at chilled and 16 days at iced storage. However, PI decreased for all the three storage temperatures studied. Therefore, lipid hydrolysis and oxidation appeared useful to assess the quality and safety of *Mystus nemurus* storage especially, this fish species proved to be one of the fatty species and storage conditions affect largely the quality and safety of its products.

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