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Lipid quality in benni (*Barbus sharpeyi*) fillets during ice storage

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Abstract

This research was conducted to evaluate qualitative changes of Benni Fish (*Barbus Sharpeyi*) during its maintenance in ice storage for 20 days. To do so, chemical spoilage indicators including peroxide (PV), thiobarbituric acid (TBA), free fatty acids(FFA), total lipid (TL), moisture (M), heme iron (HI), and also organoleptic parameters (tissue, gill appearance, gill smell, general appearance, and eyes) were measured. Fat quality of sample fish (in terms of oxidative and hydrolytic rancidity) showed a significant reduction during the maintenance period ($p<0.05$). Peroxide index changes from 3.73 to 7.52 (meq/kg) and TBA from 0.5 to 6.6 (mg MDA/kg) was recorded as markers of oxidative spoilage and FFA changes from 2.05 to 6.58 (expressed as % of oleic acid) were recorded as indicator of hydrolytic rancidity. Each one of sensory tests were rated as excellent to good until the fourth day and their quality was acceptable until the tenth day and then organoleptic results dropped significantly. In general, the best time of fish Shelf life in ice storage was determined to be 7 to 10 days.

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Introduction

Fish and aquatic products are considered as important economic products in many countries (Aubourg *et al.*, 2002) Benni fish with scientific name of *Barbus sharpeyi* belongs to Barbusse genus and cyprinidae family (coad ,1996). Benni fish is one of the commercial fishes of Khuzestan wetlands which is more popular and more favorite than other farmed fishes especially because of the relatively appropriate growth, tolerance to unfavorable environmental conditions, and high economic value (twice or three times as expensive as Chinese carp fish). Due to its high percentage of polyunsaturated fatty acid and protein it is a very perishable food and if it is kept in poor conditions, enzymatic and microbial activities will cause spoilage and degradation of fish meat (Ozogal *et al.,* 2006). Spoilage begins since the time that the fish dies and complicated changes occur due to enzymatic, chemical, and microbial activities(Gray and huss, 1996) and if kept improperly, quality and nutritional value of the fish meat and fat decreases rapidly (Mazorra-Manzano, 2000). Spoilage process decreases with decreasing temperature. When the temperature is lowered enzymatic activities and further growth of microorganisms will reduce. One of the most convenient and the cheapest ways to cool off the fish is to use ice (Balachandran, 2001). Even though during the fish maintenance in ice storage the growth of microorganisms and the rate of enzymatic and chemical activities reduce, oxidation and fat spoilage processes won't stop and spoilage trends will progress slowly(Fisher and Deng,1977). If fat spoilage develops in the fish, while creating terrible smell (Barr *et al.*, 1996), undesirable changes will occur in taste (Balachandran, 2001), tissue (Ben-Gigirey *et al.*, 1999), color, Characteristics and nutritional value of the fish (Clark *et al.*, 1997). Therefore, fat spoilage is considered as the most important factor that lowers the fish quality (Connell, 2002). Since the goal is to eat more fish and to have more convenient and easier access to health and fresh aquatic products and as this kind of fish is mainly distributed to the market while keeping in ice storage, therefore changes that occur in the fish fat and appearance during the maintenance time and also identifying the best time to maintain the fish in ice storage were investigated. The aim of this study was to evaluate Lipid quality in benni (*Barbus sharpeyi*) fillets during ice storage

Materials and methods

Sample preparation and Storage conditions of fish

Benni fish were taken alive form the fish farming pond in Azadegan fish farming site.To carry out the experiments 30 fish were randomly selected from the similar size health fish which were caught with the average weight of 300-400 gr. In order to separate external waste materials from their body surface, the fish were washed in fresh water. Then the fish were placed in alternate layers of crushed ice relatively as thick as 5 cm inside unlit boxes. During the experiment almost every day some ice was added to the box in order to compensate for the melted ice and to fix the internal temperature of the boxes (1-3 ^{o}C). The internal temperature of the boxes containing under-experiment fish was constantly measured by a mercury thermometer. The average measured temperature ranged from 1 to 3 ^{o}C.

Sampling for analysis

In order to measure chemical parameters of lipid spoilage, in every time of experiment (1st, 4th, 7th, 10th, 13th, and 20th day of maintenance in ice storage) three Benni fish were used. To do so, the fish were washed after abdominal drainage, cutting off the fins and separating the waffles and then the fish meat was ground and homogenized and then tested. At each stage of the experiment sensory changes tests were carried out on three fish and sensory evaluation was done by five ones. Chemical indicators related to fat changes were examined in three samples of fish.

Chemical analyses

Lipid content of fish by the (Bligh and Dyer, 1959) method, moisture content of fish by AOAC method (1990) was estimated. Peroxide value (PV) and free fatty acid (FFA) content were determined in the lipid extract by the Egan (1997) method. FFA analysis, expressed as % of oleic acid, PV expressed in

milliequivalents of peroxide oxygen per of kilogram.the thiobarbituric acid (TBA) (Mg malondialdehyde kg-1 flesh muscle) was determined in a 5% trichloracetic acid extract according to the method of Kirk and Sawyer (1991).Heme iron by (Clark *et al*., 1997) method was determined.

Sensory assessment

Organoleptic parameters (tissue, gill appearance, gill smell, general appearance, and eyes) by five trained panelists, according to the method of (Lin and Morrissey, 1994) were measured (Table 1).

Statistical Analysis

The SPSS software was used for statistical analysis. Kolmogorov-Smirnov normality test was performed to evaluate the data. One-way ANOVA to determine significant differences (95% confidence level) values of each parameter measured at different time intervals were used. For determining the presence or absence of statistically significant difference between the mean indices measured at different times, Duncan test was used. Express relationships between indicators and their significance measured by the correlation test were performed**.** Check for significant differences in mean sensory testing on different days, the sensory data obtained from tests using Kruskal-Wallis and Mann-Whitney tests were analyzed.

Results

The measured values of chemical indicators of fat spoilage and sensory evaluation indicators during the fish maintenance in ice storage and also the correlation coefficients of binary combinations of fat spoilage indicators of Benni fish are shown in tables 2, 3, and 4 respectively.

Table 1. Descriptive sensory evaluation definitions and descriptors.

Eyes	Gill appearance	Gill odor	General appearance	Texture	Score
Clear, bright convex eyes	Bright red, little mucus	Characteristic of species, fresh	good overall appearance, skin lustrous and shiny, no fading	Flesh is firm and resilient and springs back immediately when released	0
Slightly sunken or somewhat dull	Red, some mucus	Neutral. Total absence of odor characteristic odor no longer detectable but off-odors have not developed	Good overall appearance, very slight bleaching of skin	Reasonably firm some loss of resiliency, thumb indentation slowly fills out.	1
Dull and/or cloudy	Pinkish red to brownish, some mucus	Slight to moderate sour odor	Some loss of metallic luster, some bleaching	Moderately firm, thumb indentations may remain in flesh	2
Very dull, sunken and cloudy	Brown, may be covered with mucus	Very sour, strong, or putrid	Bloom gone from skin, color faded and bleached	Excessively soft flesh	3

Moisture

The rate of moisture in Benni fish was measured as about 75.24 to 77.45% and the range of moisture changes was 2.21%. The rate of moisture in Benni fish during different days of maintenance in ice storage did not change significantly (P<0.05) (table2).

Total Lipid

The rate of total lipid in Bennie fish was measured as about 8.16 to 9.42% and the range of changes was about 1.26%. The rate of total fat in Benni fish during different days of maintenance in ice storage changed significantly and according to Duncan's test the rate of total lipid in sample fish was significantly different during all days except the early days (1stand 4th days) and 13th and 14th days(table 2).

Lipid Oxidation

The lipid oxidation of Benni fish during the maintenance in ice storage in different days was identified by measuring the values of peroxide (PV) and TBA index. The rate of peroxide as the early ingredients of oxidation spoilage in Benni fish was measured in 3.73 to7.52 meq kg^{-1}.The rate of peroxide was significantly different during various times except the early days (1st and 4th) and 10th and 20th days (table2).highest rate of peroxide was observed in the 13th day of maintenance in ice and then it decreased.

TBA index was uses as an approach for measuring secondary components of lipid oxidation spoilage and its rate was 0.5 to 6.6 mg MDA kg^{-1} of Benni fish meat. The rate of measured TBA during different days of maintenance in ice was significantly different ($P<0.05$) (table2).The correlation between two indices of PV and TBA as primary and secondary products of fat lipid oxidation during the maintenance in ice was high and significant in Benni fish ($r = 0.802$). Also, there was a negative significant relationship between correlation coefficients of binary combinations of TBA and PV in Benni fish and other indicators of fat spoilage such as total lipid and a positive significant relationship between them and free fatty acids (table 4).

Table 2. Changes in chemical spoilage indicators in benni during storage in ice.

	1	4	7	10	13	20
Moisture	75.24±1.05^{a}	77.45±2.09^{a}	75.51±1.82^{a}	76.91±2.58^{a}	75.36±0.65^{a}	75.73±1.28^{a}
Total Lipid	9.46±0.14^{a}	9.11±0.03^{b}	9.07±0.02^{b}	8.80±0.04^{c}	8.61±0.04^{c}	8.16±0.04^{d}
Peroxide value	3.37±0.02^{a}	4.18±0.03^{a}	5.06±0.09^{b}	6.18±0.19^{c}	7.52±0.22^{d}	6.58±0.21^{c}
Thiobarbituric acid	0.50±0.09^{a}	1.98±0.06^{b}	3.44±0.06^{c}	3.86±0.04^{d}	4.18±0.02^{e}	6.60±0.11^{f}
Hem Iron	4.80±0.03^{a}	4.68±0.01^{ab}	4.73±0.06^{ab}	4.67±0.05^{ab}	4.73±0.07^{ab}	4.62±0.03^{b}
Free fatty acid	2.05±0.01^{a}	4.86±0.01^{b}	2.97±0.01^{c}	3.45±0.01^{d}	4.26±0.20^{e}	6.58±0.04^{f}

$^{a-f}$ Means followed by different letters are significantly different ($P< 0.05$).

Moisture: %, Total Lipid: %, Peroxide value: meq/kg, Thiobarbituric acid: mg MDA/kg of texture, Hem Iron: mg/kg of flesh, Free fatty Acid: % o f oleic acid.

Hydrolytic (Enzymatic) Spoilage

The hydrolytic (enzymatic) spoilage of fat in Benni fish during its maintenance in ice was determined by FFA indicator which was 2.05 to 6.58 (% of oleic acid).The rate of FFA gradually increased during the maintenance in ice so that the highest rate of FFA was related to the 20th day of maintenance. Moreover, the rate of FFA was significantly different in all days of maintenance (table 2). There was a negative significant relationship between correlation coefficients of FFA and other indicators of fat spoilage such as total lipid and a positive significant relationship between them and PV and TBA (table 4).

Table 3 . Sensory evaluation of fish on different days of ice storage.

	1	4	7	10	13	20
Texture	0.00 ^{a}	0.33^{cb}	0.60^{cb}	1.46^{d}	2.53^{ef}	2.86^{ef}
General appearance	0.00 ^{a}	0.20^{ab}	0.60^{cb}	1.20^{d}	2.40^{ef}	2.80^{ef}
Gill appearance	0.00 ^{a}	0.46^{cb}	0.66^{cb}	1.50^{d}	2.46^{ef}	2.73^{ef}
Gill odor	0.00 ^{a}	0.46^{a}	0.93^{c}	1.66^{d}	2.5^{e}	2.93^{f}
Eyes	0.00 ^{a}	0.40^{cb}	0.53^{cb}	1.54^{d}	2.60^{ef}	2.86^{ef}

Excellent= 0 ,Good = 1 ,Acceptable = 2, Reject > 2

$^{a-f}$ Means followed by different letters are significantly different ($P< 0.05$).

Heme Iron

During the maintenance of Benni fish in ice the rate of heme iron reduced from 4.8 to 4.62 mg per 1000 g meat in the maintained samples. The rate of heme iron was significantly different just in the 1st and the 20th days (table2). There was no significant relationship between correlation coefficients of binary combinations and other spoilage indicators such as total lipid, PV, TBA, and free fatty acids (table 4) .

Table 4. A correlation of the chemical spoilage indicators of lipid in benni fish during ice storage.

	Moisture	Total Lipid	Peroxide value	Thiobarbituric acid	Hem Iron	Free fatty acid
Moisture	1.000	- 0.041	- 0.028	- 0.040	- .052	- 0.042
Total Lipid	-	1.000	- 0.814**	- 0.934 **	0.385	- 0.948 **
Peroxide value	-	-	1.000	0.802 **	- 0.229	0.710 **
Thiobarbituric acid	-	-	-	1.000	- 0.497 *	0.938 **
Hem Iron	-	-	-	-	1.000	- 0.486 *
Free fatty acid	-	-	-	-	-	1.000

* Significant difference in the level of 95% (P≤ 0/05)

** Significant difference in the level of 99% (P≤ 0/01)

Moisture : % , Total Lipid : % , Peroxide value :meq/kg ,Thiobarbituric acid :mg MDA/kg of texture , Hem Iron : mg/kg of flesh , Free fatty acid :% o f oleic acid.

Sensory evaluation

During the maintenance of Benni fish in ice, some changes appeared in its appearance. The results indicated a significant difference between all indicators (P< 0.05) (table4).

Discussion

Reduction of fish quality during the cooling off process has been proved through former studies and researchers believe that the most important factor in lowering the quality is the fat changes of the fish (Hwang and Regenstein, 1996). Oxidative and hydrolytic spoilage begin to happen after the fish are caught and as the time passes.

If fat spoilage spreads in the fish, it causes undesirable smell, terrible taste, and reduction of nutritional value of the product. The increase of peroxide rate for the Benni fish maintained in ice and its comparison with the first day samples (3.73-7.52) indicates quick progress of spoilage during the maintenance of the fish in ice and its reduction at the end of period due to participation in single and bimolecular reactions (Erikson, 2001). Similar data were found for other fish species during storage in ice (Erkan and Ozden, 2007) and (Ozogul *et al.*, 2006). However, A lot of researchers such as (Perez-Alonso *et al.*, 2003) and (Dragoev *et al.*,1998) have measured different rates of peroxide as one of the most important and primary indicators of the fish fat spoilage. Nevertheless, hydro peroxide production makes no changes in organoleptic characteristics of the fish. (Ludorff and Meyer, 1973) proposed the following PV scale as a basis for determining the freshness of fish: PV = 0–2 m mol of O_2 per kilogram of 'very good', PV = 2–5 mmol of O_2 per kilogram of 'good'.

An increasing trend was observed in the rate of TBA in fish samples until the 20th day of maintenance (from 0.5 in the first day to 6.6 in the 20th day) and there was a significant difference between the rates of TBA in all maintaining days which indicates the spoilage progress in sample fish. This increase might be due to increase of free iron or other peroxides in the fish meat. Moreover, it could be due to the increasing trend of hydro peroxides (Gomes and Silva, 2003). The rate of thiobarbituric acid about 1-2 mg MDA kg^{-1} of fish meat is considered as acceptable for the consumer due to its terrible smell (Goulas and

Kontominas, 2007) and the rate of 3-4 mg Malondialdehyde per kg of fish meat shows that the quality of the fish meat has decreased (Karakam and Boran, 1996). After the death of the fish, free fatty acids highly increase due to fat hydrolyzing enzymes (Sankar and Raghunath, 1995). However, according to available reports the rate of FFA does not directly lower the quality (Ben-Gigirey *et al*., 1999) yet, these free fatty acids could participate in fat oxidation process (Dragoev *et al*., 1998).

In this research, the increase of fat oxidation, the development of undesirable smell, the acceleration in spoilage, the decrease of product quality, and the denaturation of protein were clearly resulted from the increase of fat oxidation by FFA. This could be approved of due to a high positive relationship between FFA, TAB, and PV. Therefore, the measurement of free fatty acids could be considered as an indicator for expressing the effect of lipolytic enzymes on fish fat and other meat products (Sankar and Raghunath, 1995). Similar results were obtained in studies which were conducted on the fish maintained in ice such as Mackerel (Aubourg, 2001), Sardines (Aubourg *et al*., 2002), and *Ariomma Indica* (Sankar& Raghunath, 1995). Metal ions such as iron, copper, and cobalt accelerate the formation of free radicals by facilitating the electron transfer and they play an important role in fat oxidation as peroxide factor (Dragoev *et al*., 1998). During the fish maintenance, due to the change of globins' protein, its control over iron is low and thus the rate of heme iron will decrease and the rate of non-heme iron will increase. As the heme iron decreases and non-heme iron increases, fat oxidation spoilage increases. This point confirmed by the negative significant relationship which exists between heme iron and fat oxidation indicators, that is PV and TBA (Hoke *et al*., 2000). Sensory evaluation is an appropriate method for estimating the durability of fish maintenance period (Tang *et al*., 2001) and has been evaluated as a compliment to chemical indicator sin most previous studies.

Sensory evaluation is a good method to estimate the shelf-life of fish during storage (Tang *et al*., 2001) and in the majority of previous research has been as a complementary method for the evaluation of chemical assessments. Lipid spoilage causes the formation of compounds with low molecular weight (El-Sebaiy *et al*., 1987) and proteins degradation (Vidya and Srikar, 1996). In addition, fat spoilage and changes in the composition Trimethylamine Oxide and proteins degradation cause terrible smell which is considered as one of the main factors in sensory evaluations (Ben-Gigirey *et al*., 1999). In this research, sensory evaluation, indicating a trend of corruption in the fish samples. Analysis of sensory data proved significant changes during ice storage. Similar results were obtained in studies which were conducted on the fish maintained in ice such as black-skipjack (*Euthynnus lineatus*) (Mazorra-Manzano *et al*., 2000)

General Conclusion

According to the results of the research, ice is appropriate for short maintenance of the fishes and the best shelf life of Benni fish in ice was set between 7 to 10 days.

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