

Effect of Frozen Storage Time on the Lipid Deterioration and Protein Denaturation During Caspian Sea White Fish (*Rutilus frisi kutum*)

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Abstract: The study was designed to investigate the effect of duration of frozen storage on chemical analysis, lipid damage and extractability of Myofibrillar proteins of Kutum (*Rutilus frisi kutum*). The fish were collected from Anzaly landings in north of Iran and were subjected to 12 months of frozen storage and analyzed at intervals of three month. Protein content ranges from 21.8 ± 0.01 to 19.9 ± 0.01 . Protein decrease with increasing duration of frozen storage; fish samples that were for frozen thirty days; having highest protein content 21.8 ± 0.01 while the least 19.9 ± 0.01 was recorded for fish samples that were frozen for 12 months. Similar results obtained for the fat content where the highest fat content 3.21 ± 0.01 was recorded for the fish samples that were for frozen 30 days and the least value was recorded for those stored for 12 months. The least moisture content was observed for fish samples the was stored for 12 months but, the highest Ash content was observed for fish samples the was stored for 12 month. Lipid damage were measured on the basis of Free Fatty Acids (FFA), Peroxide Value (PV), Thiobarbituric acid index (TBA-i). PV, TBARS and FFA concentration of frozen Caspian Sea white fish stored at -18°C ; the temporal variation of these three variables were statistically significant ($p < 0.001$). SDS-PAGE patterns showed that myosin heavy chain was much more susceptible to hydrolysis than actin.

Key words: Frozen storage, chemical, lipid damage, SDS-PAGE, *kutum*

INTRODUCTION

The *kutum* fish (*Rutilus frisi kutum*) which is distributed abundantly in Caspian Sea in Iran. *Kutum* fish (*Rutilus frisi kutum*) is the most popular fish in Iran with the highest economic value. Fish is one of the most important sources of animal protein available in the topics and has been widely accepted as a good source of protein and other elements for the maintenance of healthy body (Arannilewa *et al.*, 2005). Fish tissue protein is characterized by a very desirable composition of amino acids (Aburg *et al.*, 1999) and also is a rich source of group B vitamins and is rich in vitamins A and D (Zmijewski *et al.*, 2006). Fish muscle is composed of myofibrillar proteins, Sarcoplasmic proteins, connective tissue, stroma proteins, polypeptides, nucleotides and non-protein nitrogen compounds. The major alteration in fish tissue occurs largely in the myofibrillar proteins, the main muscle proteins, comprising myosin ($500\text{-}600 \text{ mg g}^{-1}$) and actin ($150\text{-}250 \text{ mg g}^{-1}$), (Badii and Howell, 2001).

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Freezing is one the best methods, for fish preservation and has been employed increasingly both on shore and fishing vessels (Begona, 1999). However, measurement of sensory, chemical and physical changes have shown deterioration of fish quality continues to some extent during frozen storage (Celik *et al.*, 2005). Although, microbial deterioration of fish muscle can be inhibited by frozen storage, fish proteins undergo a number of changes that affect the flavor and texture of the flesh, (Abourg *et al.*, 1998).

This study therefore is aimed at determining the acceptable storage life of frozen storage and the freezing effects on the proximate analysis, chemical (PV, FFA, TBRAS) composition and protein denaturation of the white fish (*Rutilus frisi kutum*) in Iran.

MATERIALS AND METHODS

Sample Collection

Kutum fish (*Rutilus frisi kutum*) average weight (1000±100 g) used in this study, were caught in 15th of 2006 March from Caspian Sea. After being caught, they were transferred to laboratories, filleted and then frozen at -30°C. Frozen fillets dispatched packed into a box ice to the faculty of Veterinary Medicine, University of Tehran. Analyzes of the frozen *kutum* fish (*Rutilus frisi kutum*) were carried out 1, 3, 6, 9 and 12 months from the beginning of frozen storage.

Determination of Proximate Analysis

Water content was determined by the sample drying technique at temperature of 105°C; crude protein content with the use of the Kjeldahl method with 6.25 multiplier; ash content by sample mineralization at a temperature 550-600°C and fat content was determined with blight and dyer method (Ozogul and Ozogol, 2007).

Chemical Analysis

Chemical parameters (PV, TBRAS, FFA) were studied for frozen *kutum* fish. Thiobarbitic Acid Reactive Substance (TBRAS) were analyzed by the direct method of Vyncke and Peroxide value were determined according method Lee and Free fatty acid were analyzed according Pearson method, (Zmijewski *et al.*, 2006).

SDS-Poly Acryl Amide Gel Electrophoresis (SDS-PAGE)

The protein pattern of *kutum* fish (*Rutilus frisi kutum*) muscle was analyzed by SDS-PAGE according to the method of Laemmli (Bauchart *et al.*, 2007). To prepare the protein sample, 27 mL of 5% (w/v) SDS solution heated to 85°C, were added to the sample (3 g). The mixture was then homogenized using homogenizer (Ependorph, model 5810R, Germany) for 2 min. The sample were centrifuged at 4000 x g for 10 min to remove undissolved debris. Protein concentration was determined according to the method of Warburg. SDS-PAGE gel was made of 10% running gel and 4% stacking gel. After separation were fixed and stained with coomassie Blue R-250.

Statistical Analysis

Data was analyzed statically by the Analysis of variance (Repeated measure) and using SPSS 13.0.

RESULTS AND DISCUSSION

Fish tissue possesses high nutritional value and is therefore a particularly recommended directly component (Abourg *et al.*, 2004). Lowering of fish quality during frozen storage has been

attributed to undesirable changes associated with lipid and proteins (Huidobro and Tejaja, 2004). Changes in lipid occur through hydrolysis and oxidation mechanism. Fish muscle proteins can undergo denaturation during frozen storage due to formation and accretion of ice crystals resulting in dehydration (Simeonidou *et al.*, 1997). Protein aggregation in frozen fish depends on storage temperature, temperature fluctuation, moisture change, storage time and enzymatic degradation (Bauchart *et al.*, 2007).

The proximate composition of the *Kutum* fish that was stored in a freezer compartment of refrigerator for different number of month analysis is presented in Table 1. Protein content ranges from 21.8±0.01 to 19.9±0.01 protein decrease with increasing duration of frozen storage; fish samples that were for frozen thirty days; having highest protein content 21.8±0.01 while the least 19.9±0.01 was recorded for fish samples that were frozen for 12 months. Similar results obtained for the fat content where the highest fat content 3.21±0.01 was recorded for the fish samples that were for frozen thirty days and the least value was recorded for those stored for 12 months. The changes in fat content during frozen storage could be associated with the oxidation of fat. The highest moisture content was observed for fish samples was stored for one month and least moisture content was observed for fish samples the was stored for 12 months but, the highest ash content was observed for fish samples the was stored for 12 months and the least ash content was observed for fish samples the was stored for thirty days.

Table 1: Proximate composition (dry weight basis) of *kutum* fish (*Rutilus frisi kutum*) subjected to different freezing periods (Mean±SE)

Freezing periods (months)	Ash (SE)	Fat (SE)	Protein (SE)	Moisture (SE)
1	1.29±0.013	3.21±0.01	21.8±0.02	75.9±0.04
3	1.30±0.040	2.43±0.01	21.2±0.02	75.2±0.01
6	1.34±0.030	2.52±0.01	20.5±0.05	74.5±0.01
9	1.49±0.030	2.30±0.02	19.9±0.08	73.6±0.04
12	1.83±0.050	1.91±0.04	19.9±0.01	72.3±0.04
p-value	0.008	0.03	0.08	0.03

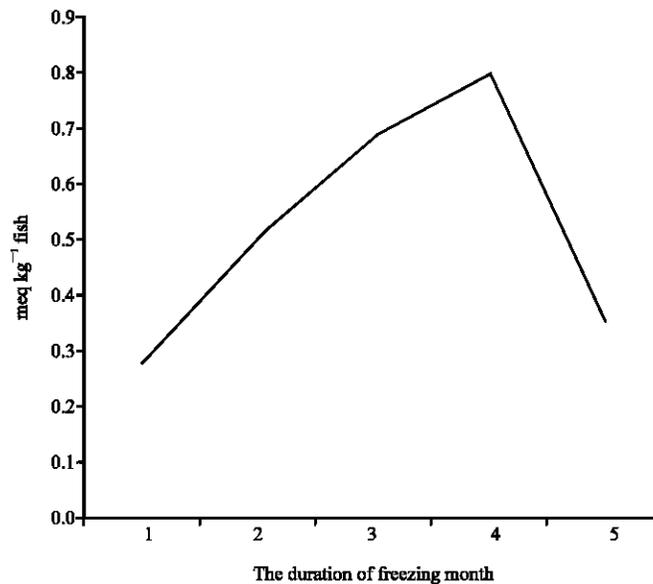


Fig. 1: Peroxide values content in frozen Caspian Sea *kutum* fish stored at -18°C for 12 months

The lowering of quality during frozen storage could also be caused by lipid changes. Oxidative rancidity during frozen storage at -18°C was evaluated by determining the peroxide value (Fig. 1) Peroxide value is widely employed for determining the formation of hydro peroxides, which are primary products of oxidative reaction (Begona, 1999). The concentration of TBRAS has not significant during storage time at -18°C (Fig. 2). FFA, resulting from lipid hydrolysis, may accumulated during frozen storage and accelerate quality deterioration (Saeed and Howell *et al.*, 2002). Although FFA have not been reported to directly cause quality defects. The concentration of FFA increased during storage time at -18°C (Fig. 3). Measurement of chemical changes has shown that deterioration of fish quality continues to some extent during frozen storage (Rodriguez *et al.*, 2007).

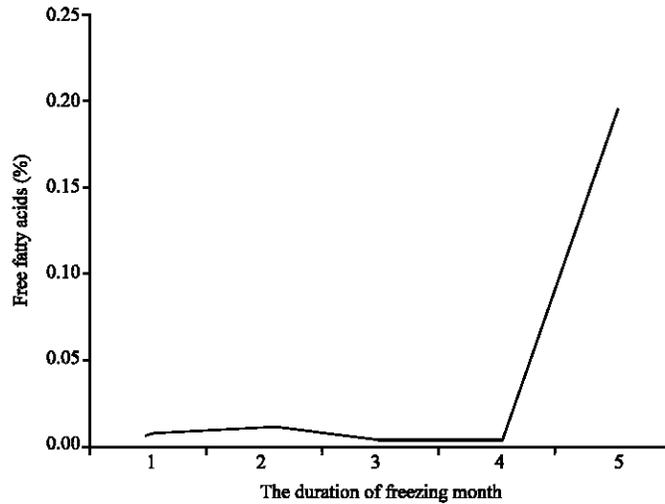


Fig. 2: FFA content in frozen Caspian Sea Kutum fish stored at -18°C for 12 months

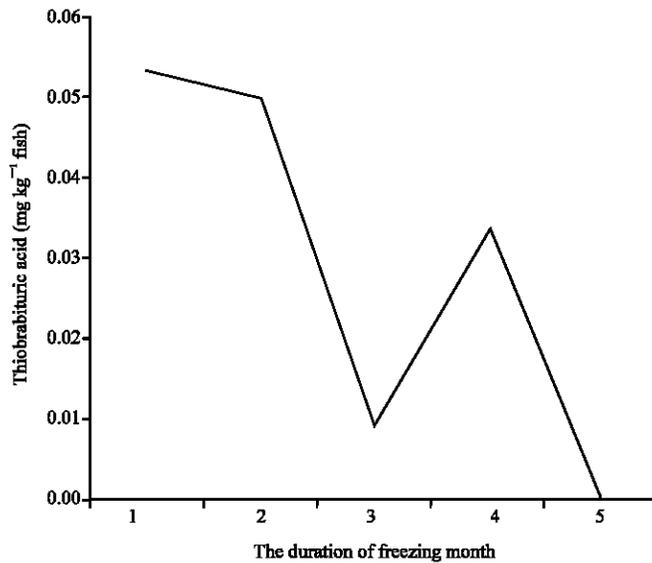


Fig. 3: TBARS content in frozen Caspian Sea *kutum* fish stored at -18°C for 12 months

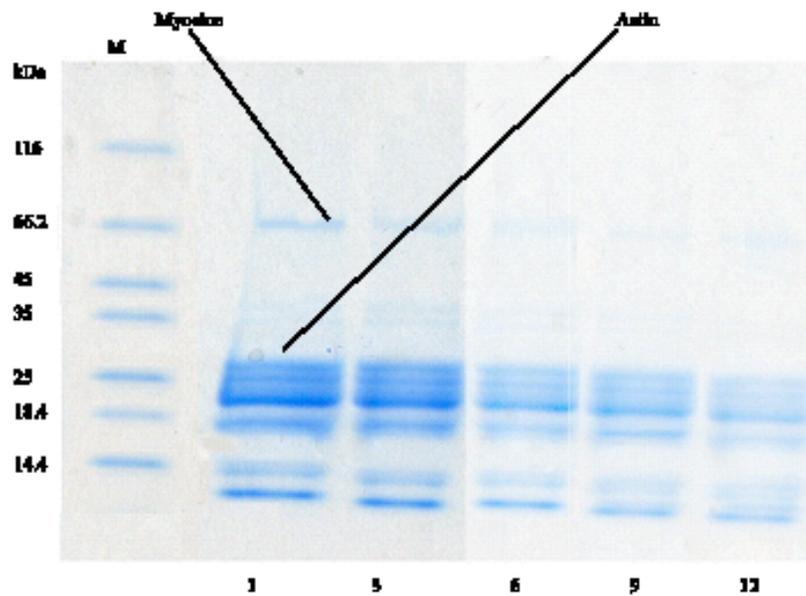


Fig. 4: SDS Pattern of muscle prote in Caspian Sea kutum fish stored at -18°C

The major alteration in fish tissue occurs largely in the myofibrillar proteins. The main muscle proteins comprising myosin and actin. Storage of frozen fish brings about a decrease of extractability of myofibrillar proteins. There is also deterioration of the texture of functional properties on the flesh, (Samra *et al.*, 2002). SDS analyses revealed that intensity of the myosin heavy chain and actin bands was reduced with increasing storage time. SDS-PAGE patterns showed that myosin heavy chain was much more susceptible to hydrolysis than was actin (Fig. 4). The decreased in solubility is accompanied by changes in texture and development of toughness or rubberiness in fish muscle. This result was in agreement with Benjakule *et al.* (2003). Protein aggregation in frozen fish depends on storage temperature, moisture, temperature function, storage time and enzymatic degradation (LeBlank and Leblank, 1992).

The result of the investigation shows that the fish is a good source of protein, fat and the quality of fish decrease in the 12 months frozen storage, deterioration increased as the duration of storage increased.

ACKNOWLEDGMENT

Authors would like to thank central laboratory of Faculty of Veterinary Medicine, University of Tehran.

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