Bull. Nat. Inst. Oceanogr. & Fish., ARE, 15 (1), 1989: 107 - 118

EFFECT OF ICING AND FROZEN STORAGE ON THE STABILITY AND SOME QUALITY CHARACTERISTICS OF EGYPTIAN EELS (ANGUILLA ANGUILLA) AND CAT-FISH (CLARIAS LAZIRA).

NADIA S. AHMED*; MAHMOUD A. HAMZA**
AND MOHAMED H. ABDEL-AAl**.

* National Institute of Oceanography and Fisheries, Alexandria, Egypt.
**Department of Food Science & Technology, Faculty of Agriculture, Alexandria University, Egypt.

ABSTRACT

The effect of icing and frozen storage on the chemical composition, free fatty acids (FFA) content, thiobarbituric acid (TBA) value, peroxide value (PV), protein fractions and protein electrophoretic patterns of Egyptian eels and cat-fish muscles were investigated. Changes in texture and cooking drip value in relation to other quality characteristics affecting the consumer acceptability were evaluated.

The results indicated that the effect of forzen storage on the chemical composition of both fish species was less pronounced than icing. Myofibrillar proteins constituted the major fraction in both fish species followed by sarcoplasmic ones. A noticeable decrease in these two main protein fractions took place upon icing and freezing. However, the amount of non protein nitrogen and stroma and denatured proteins were increased. The changes in protein electrophoretic patterns after seven days of icing were more obvious than eight months frozen storage for both fish species.

Gradual and continuous increase of levels of FFA, PV, TBA and CDV was found by increasing the duration of cold storage. The changes in texture and other organoleptic properties affecting the quality of stored fish were subjectively studied.

INTRODUCTION

Fish constitute an important source of high quality protein. Eels and cat fish are among of the most popular fish for the Egyptian consumer which served in several delicious dishes. Under cold storage conditions, three types of changes may take place, leading to undesirable changes in fish quality. These changes are due to microbial action, changes to the action of endogeneous enzymes and chemical or physical actions (Connell and Howgate, 1968). However, the extent of deterioration was found to be affected by many other factors such as fish type, species, chemical composition, season of harvest, handling procedures, and storage time and conditions (Dyer et al., 1950; Lee and Dawson, 1973; Josephson et al., 1985 and Hsien and Regenstein, 1989). Dyer (1951) found that the gradual development of toughness, which characterize the prolonged

cold storage of fish meat was related mainly to the denaturation of actomyosin. Connell (1960) and Connell and Howgate (1968) concluded that cod actomyosin became insoluble after eight months storage at -14°C. Ragnarsson and Regenstein (1989) stated that the frozen storage caused textural changes which often decrease the water retention of muscle proteins. They also found that most of the electrophoretic studies on fish muscle proteins after frozen storage did not show any major differences among the muscle proteins, except a general loss of solubility.

Besides, hydrolytic and oxidative rancidity of lipid components were developed in frozen storage, which in turn caused an objectional changes at prolonged fish flavour, especially after cooking (Aman and Smirnova, 1970 a & b). Although, the extent of deterioration occurred in many fish species had been reported in numerous studies, no data are available about the effect of icing and frozen storage on the chemical and other quality characteristics of eels (Anguilla anguilla) and cat-fish (Clarias lazira). Hence, the present investigation was undertaken to study the effect of cold storage conditions on the changes of the gross chemical composition, stability and other quality characteristics of Egyptian eels and cat-fish.

MATERIALS AND METHODS

Live eels and cat-fish ranging from 500 to 750 g were obtained from Lake Edku, EL-Behera Governorate, Egypt. The fish samples were immediately transferred alive into the laboratory after which they were killed. Fish belonging to each species was divided into two parts. The first part was individually placed in perforated wooden boxes as a whole fish and mixed with crushed ice and kept at room temperature (25°C) for seven days. The melted ice was thourcughly compensated when neccessary. The second part was separately packed in polyethylene bags and kept in deep freezer at -18°C and RH 90%, for eight months. Representative fish samples were regularly withdrawn either from the crushed ice daily or from the deep freezer bimonthly. Samples for chemical analysis were first deheaded, eviscerated, filleted, skinned and minced in Braun blendor for 30 second. The experiments were carried out daily and montly on iced and frozen stored samples, respectively.

1- Proximate Chemical Composition and Rancidity:

Moisture and crude protein contents were determined according to the AOAC method (Anon, 1980). Total lipids were determined by the method of Folch et al. (1957). Free fatty acids (FFA) expressed as oleic acid was measured as described by Kwon and Rhee (1986). Thiobarbituric acid value (TBA) was determined according to Yu and Sinnhuber (1967) and reported as mg malonaldehyde per Kg flesh. Peroxide value (PV) was determined by the AOAC method and expressed as milliequivalent of peroxide per Kg oil.

2- Determination of Protein Fractions:

The method outlined by Aman (1983) was followed for fractionating the muscle proteins by solubility. Sarcoplasmic proteins were extracted from the minced fish meat using cold (4°C) phoshpate buffer of pH 7.5 and ionic strength 0.05. Myofibrillar proteins were extracted from the residue using phosphate buffer of pH 7.2 and ionic strength of 0.5. The residue containing the denatured and stroma proteins were calculated by differences. The total nitrogen in each fraction was determined by the Micro-kjeldahl method as described by Anon. (1980).

3- Polyacrylamide Gel Electrophoresis (PAGE)

a- **Sample preparation:** The defatted samples were mixed with distilled water (1:5, w/v) and shaken for one hour. After centrifugation (500 g), the supernatant (water soluble proteins) was used directly for electrophoresis.

b- **Gel preparation:** Discontinuous (disc.) PAGE technique was used in which gel with 1 mm thickness was poured between two glass plates using the "bag technique". After pouring the separating gel (15% polyacrylamide in Tris-HCl buffer pH 8.8), the stacking gel (5% polyacrylamide in Tris-HCl buffer pH 6.3) was poured and the comb was inserted.

c- **Electrophoresis:** Electrophoresis was performed vertically in the apparatus POOMA-PHOR (Labor Muller, D-3540 Hann Munden-West Germany) with Tris-borate buffer pH 8.3. Other experimental conditions concerning gel polymerization, running conditions, staining and destaining steps were given in the instruction laboratory manual by Stegemann et al. (1987).

4- Determination of Cooking Drip Value (CDV):

The method described by Krivchenia and Fennema (1988) with minor modifications was followed. Minced fish meat samples (25 g each) were placed in 50 ml centrifugal tube and immersed in water bath (70°C) for 30 minutes. The tubes were centrifuged for 10 minutes (500 g) and the drained drip after removal of the fat layer was weighed. The CDV values were calculated as follow:

% CDV = Weight of drained fluid/weight of sample x 100

5- Sensory Evaluation

Samples to be evaluated were first filleted and cooked for 20 mins. in aluminum foil trays at 250°C in a gas stove. The cokked samples were served warm and judged by ten panalists using 5 points descriptive scale. A score 5

donated excellent texture comparing to the fresh fish. Score 2.5 means acceptable and below 2.5 indicated that toughness or rancid flavour was pronounced (Kramer and Twigg, 1962).

RESULTS AND DISCUSSION

1- Changes in Chemical Composition

Changes in moisture, crude protein and total lipids contents of both iced and frozen eels and cat-fish flesh throughout the cold storage periods are illustrated in Tables 1 and 2. These results indicated that the cat-fish flesh contained higher levels of moisture and crude protein but lower amount of total lipids than eels. Table 1 shows positive relationship between the moisture content of fish samples and the icing time. The longer the icing time, the higher the moisture content. Gradual daily increase in the

Table 1

Changes in the proximate chemical composition, protein fractions, cooking drip values and average texture score during storage of cat-fish and eels with crushed ice.

Icing time (Days)	Proximate composition (%)			Nitrogen compounds % of total nitrogen					Average texture score
	Moisture	Crude[a] Protein	Total[a] Lipids	S	M	D + St	NPN	CDV %	
Cat-fish									
0	78.7	86.9	6.7	28.8	38.7	14.2	18.3	16.0	5.0
1	78.7	86.9	6.8	27.8	37.1	16.4	18.7	16.0	4.5
2	78.9	86.7	6.7	26.5	35.6	19.9	18.0	17.5	4.2
3	79.1	86.6	6.8	25.9	34.7	20.1	19.3	16.5	4.2
4	79.5	86.4	6.3	24.6	33.2	21.6	20.6	19.0	3.8
5	80.4	86.5	6.2	22.1	32.6	23.9	21.4	19.0	3.1
6	81.7	86.4	6.2	19.3	31.3	26.0	23.4	20.0	2.4
7	81.5	86.4	6.1	16.8	31.0	27.7	24.5	20.0	1.9
Eels									
0	59.9	62.9	31.6	26.7	37.2	20.1	16.5	11.0	5.0
1	60.3	62.9	31.4	26.1	35.6	21.1	17.2	12.3	4.8
2	60.8	62.9	31.4	25.8	34.4	21.1	18.2	12.5	4.5
3	60.7	62.7	31.2	21.4	32.3	26.0	20.3	13.6	4.0
4	61.5	62.8	31.2	20.6	30.2	28.8	20.4	15.0	3.7
5	62.1	62.7	31.1	18.7	28.3	31.6	21.4	17.0	3.2
6	62.5	62.5	31.1	16.8	28.4	32.7	22.1	17.5	2.3
7	62.9	62.4	31.0	15.9	28.0	34.3	21.8	18.0	2.0

a) On dry weight basis.
S = Sarcoplasmic protein.
M = Myofibrillar protein.
NPN = Non-protein nitrogen.
D + St. = Strom and denatured proteins.
Calculated by difference.

Table 2

Changes in the proximate chemical composition, protein fractions, cooking drip values and average texture score during frozen storage of cat-fish and eels with crushed ice.

Frozen Storage time (months)	Proximate Composition (%)			Nitrogenous compounds (% of total nitrogen)				CDV %	Average texture score
	Moisture	Crude[a] Protein	Total[a] lipids	S	M	D + St[#]	NPN		
Cat-fish									
0	78.7	86.9	6.7	28.8	38.7	14.2	18.3	16.0	5.0
2	78.1	86.7	6.7	25.8	30.7	23.4	20.1	20.0	3.8
4	77.8	86.6	6.7	23.3	20.3	34.1	22.3	22.5	3.0
6	77.2	86.7	6.5	24.1	14.5	37.1	24.3	24.0	2.5
8	76.8	86.5	6.4	24.0	12.3	37.9	25.9	26.0	2.1
Eels									
0	59.9	31.6	62.9	26.7	36.7	20.1	16.5	11.0	5.0
2	59.7	31.6	62.7	25.3	30.6	26.5	17.6	12.5	4.1
4	59.6	31.4	62.5	24.2	25.0	32.5	18.3	16.5	3.5
6	59.4	31.5	62.4	22.1	17.7	40.3	19.9	24.5	3.0
8	59.0	31.3	62.1	21.7	16.3	41.8	20.2	26.0	2.3

a) On dry weight basis.
S = Sarcoplasmic protein.
M = Myofibrillar protein.
NPN = Non protein nitrogen.
D + St. = Denatured + Stroma proteins.
Calculated by difference.

moisture content was observed for both species. However, a slight decrease in crude protein and total lipids was noticed for both fish species upon icing. Changes in the proximate chemical composition due to the frozen storage of both examined fish species were less pronounced than icing. Upong freezing, eels and cat-fish lost 0.9 and 2.1% moisture, respectively. This was associated with a slight increase in both of the crude protein and total lipids after eight months frozen storage.

2- Changes in Protein Fractions:

Results in Table 1 also show that the initial values of protein fractions in fresh cat-fish muscles were slightly higher than that recorded for eels flesh, except for stroma and denaturated proteins. The high amount of stroma and denatured proteins in eel muscles may be due to the effect of prolonged frozen storage leading to a more denaturation as reported by King et al.(1962) and Aman and Smirnova, (1970 a & b). Myofibrillar protein constituted the major

fraction in both fish species followed by the sarcoplasmic ones. As the icing storage time increased, a noticeable and sharp decrease in the amounts of sarcoplasmic and myofibrillar protein fractions occurred. This was associated with parallel increase in non protein nitrogen and stroma and denatured proteins.

Table 2 shows that the decline in myofibrillar protein fraction was much greater than sarcoplasmic and other proteins are considered more liable under frozen storage conditions. These results are in agreement with the findings of Love (1958) and Awad et al. (1969).

3- Changes in Cooking Drip Value (CDV):

The CDV values for fresh eels and cat-fish were 11 and 16% respectively (Table 1). Both species exhibited continuous and gradual increase in CDV values, from 16 up to 20% and from 11 up to 18% after seven days of icing for cat-fish and eels, respectively. The percentages of CDV values were similar (26%) for both fish species after eight months frozen storage but higher than those obtained after icing. This may indicate that the ability of proteins to retain water is decreased after frozen storage.

4- Changes in Texture

Scores for Textural changes of cooked fish meat presented in Table 1 show that changes in fish texture took place and increased by increasing the icing periods for both fish species. Eels and cat-fish showed unacceptable texture after five days of icing. Marked deterioration in fish texture of both species were observed after six months frozen storage (Table 2). Similar results were obtained by Awad et al., (1969) who reported that the CDV of white fish increased from 24 up to 48% after four months storage at $-10^{\circ}C$. They also found a linear relationship between protein extractability and textural changes of cold stored white fish.

5- Changes in The Protein Electrophoretic Patterns:

Changes in the electrophoretic patterns of water soluble proteins of eels and cat-fish muscles during icing and frozen storage are shown in Fig. 1. Fish samples were removed either from ice at intervals of 0, 1, 3, 5 and 7 days or from the sharp freezer at 0, 2, 4, 6 and 8 months. The resolved protein banding patterns of fresh eel muscles contained a characteristic strong and sharp band which appeared in the upper part of the gel (i.e. high melecular weight) which degraded into small number of faint bands after seven days of icing (Fig. 1A). Similarly, a dark and sharp band in the same region of the gel which is clearly appeared in the fresh cat-fish completely became weak by increasing the icing. This was associated by the appearance

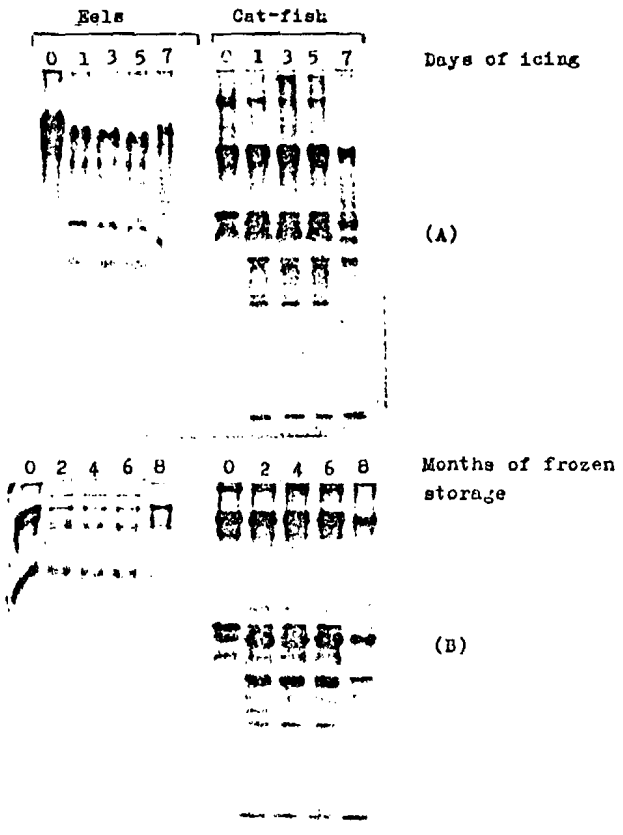

FIG. 1
Effect of icing (A) and Frozen storage (B)
on the water soluble protein electrophoretic patterns
of eels and cat-fish.

Electrophoresis was performed in disc. PAGE in Tris-glycine buffer pH 8.3 for 4 hours, 400 volts and 75 mA at 4°C.

of new bands at the lower part of the gel.

On the other hand, changes occurred in the water-soluble protein electrophoretic patterns of both species after eight months frozen storage at -18°C were not obvious as in case of seven days icing. These changes were restricted to the band intensity and sharpness but not in their number or their travelled distance (Fig. 1B).

Little work was found in the literature on the changes in the protein electrophoretic patterns of these Egyptian fish species due to icing or frozen storage.

6- Changes in FFA, PV, TBA and Fish Flavour:

The percentages of free fatty acids of iced and frozen eels and cat-fish are illustrated in Fig. 2 A & B. Values obtained for either iced or frozen eels are always higher than the corresponding amounts of iced and frozen cat-fish since the maximum amount of FFA was found in eel muscles after six months of frozen storage followed by five days icing. Deng (1978) stated that the longer period of icing storage of fish might prolong the activity of lipases and phospholipases and subsequently increase the free fatty acid production. He also reported that the differences in ratios of unsaturated to saturated lipids, phospholipids to triglycerides and the activities of the lipolytic enzymes could be the reason for the different amounts and rate of FFA production between fish species. The present results are in good accordance with those reported by Deng (1978). However the FFA values reported by Aman and Shehata (1978) for frozen Russian cat-fish were higher than that shown in Fig. 2 B.

Peroxide values showed also gradual increase with the time of cold storage for both fish species being lower in iced fish than the frozen ones (Fig. 2 A, B). The maximum values were obtained after seven days of icing for both species. Eels had higher PV after six months storage at -18°C than those obtained for cat-fish after 8 months. Mc-Donald et al., (1979) stated that microsomes of both light and dark muscles of lean and fatty fish contained enzymes catalyzing lipid peroxidation. These enzymes may also contributed to the oxidative deterioration of frozen fish. TBA test is widely used as a good indicator for developing rancid fish flavour during the cold storage. Data illustrated in Fig. 2-A & B can clearly indicate that both of icing and freezing storage conditions caused continuous and gradual increase in TBA values of eels and cat-fish. Eels had higher TBA values than cat-fish. Also, frozen fish contained higher TBA values than iced ones. However, the panalists were unable to detect any changes in fish flavour below seven mg malonaldehyde per kg of flesh. These findings are important from the technological point of view, suggesting that the minimum threshold value for iced

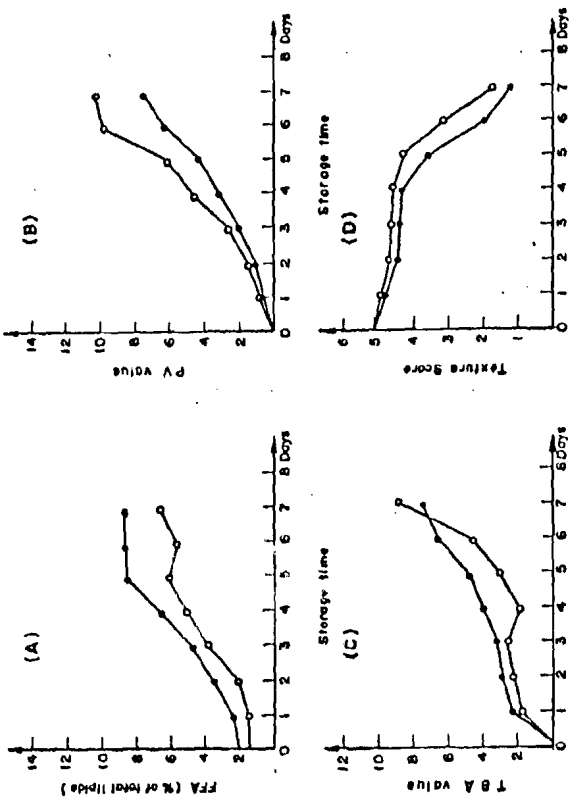

Fig.2a:Changes in free fatty acids content (A), peroxide value (B), thio barbituric acid value (C) and texture score of iced catfish and eels. (o——o Iced catfish, •——• Iced eels).

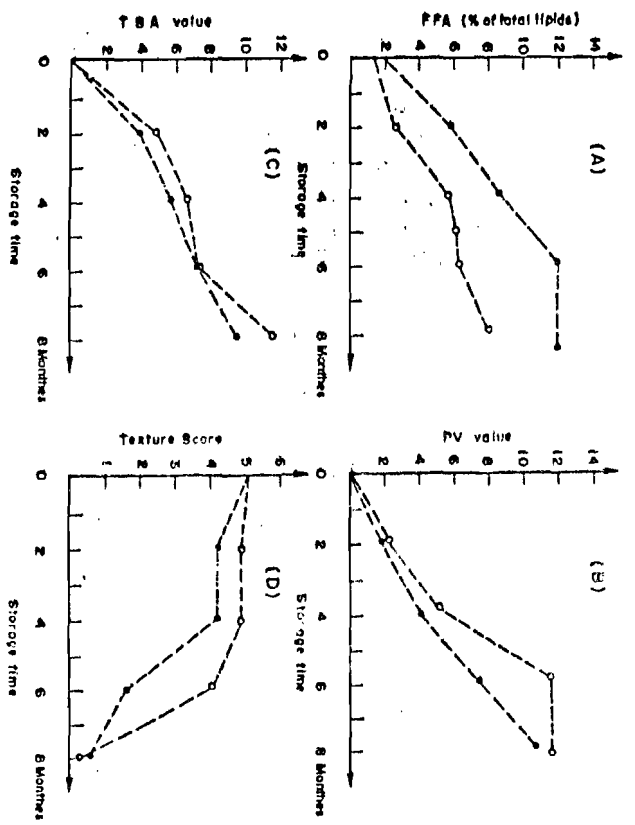

Fig2b:Changes in free fatty acids content (A),peroxide value (B), thio barbituric acid value (C) and texture score of frozen stored catfish and eels.
(o---o Forzen storage catfish , •——• Forzen storage eels).

and frozen stored eels and cat-fish occurred after 6 days of icing or 6 months of freezing. Mendenhall (1972) and Deng et al., (1977) reported that the threshold value of mullet occurred at a level from 4 to 6 mg malonaldehyde per kg flesh.

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