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The Effect of Un-Stable Freezing on Nutritional Value of Fish (Indian mackerel - *Rastrelliger kanagurta*, Russel) from Yemen Coastal Waters

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Abstract:
This study was designed to investigate the effect of different freezing protocols (stable freezing and un-stable freezing) on the biochemical composition of one of the important edible fish species popular among the consumers in Yemen, *Rastrelliger kanagurta*, collected from some landing site in coastal areas of Al-Hodaidah. The results demonstrated that *R. kanagurta* has a valuable biochemical component. In the fresh samples (group 1), the values of moisture content, lipid, protein, and ash were about 76%, 3.57%, 20.43% and 4.66%, respectively. Investigating the changes could occur in these components in the fish subjected to stable freezing (group 2) and fluctuate freezing (group 3) for 4 weeks were carried out. This study revealed that there were significant changes in moisture and ash in group 2 while the significant changes were in moisture, lipids, and ash in group 3. Significant differences were also observed in the values of protein and pH level within group 3 which divided into 2 sub-groups subjected to alternative freezing and unfreezing periods (sub-group 1 kept in cold; on the lower shelf of the refrigerator and sub-group 2 kept at room temperature overnight during the study period). The present study points out the importance of temperature stability during freezing storage to achieve a better quality of preserved fish.

Keywords: Un-stable freezing, stable Freezing, Nutritional Value, Indian mackerel, Yemen.

INTRODUCTION

As food is an important item in human life, its quality becomes vital for health and condition. Therefore, preference of fish as food is not because of any legacy of the past, but on its own merits, as judged by present standards of nutritional requirements and dietary allowance. In addition to protein, fish flesh also offers minerals, iodine, vitamins and fat (Srivastava, 1999; Ravichandran *et al*., 2012; Smida *et al.,* 2014).

Since, fish is an extremely perishable food and the spoilage begins as soon as the fish caught; most fishes become inedible within twelve hours at tropical temperatures. Hence, it is necessary to be either consumed or preserved as soon as possible after landing in order to prevent the growth of spoilage bacteria (Fellows and Hampton, 1992). In addition to bacteria enzymes and oxygen are the main factors responsible for several physiological, microbial and deterioration causing fish spoilage (Farid *et al.,* 2014). Fish species, storage temperature, time and enzymatic degradation during frozen storage, are factors playing role in the deterioration of fish (Yerlikaya and Gokoglu, 2010). Freezing is one of the practices commonly used in meat storage and preservation for its importance and efficiency (Obuz and Dikeman, 2003; Saulum, 2011). Rodriguez et al. (2007) reported that the deterioration due to lipid oxidation can be delayed by lowering the temperature and using oxygen impermeable containers.

Frozen fish can be stored for a considerable period of time under frozen conditions. However, over time the overall quality of frozen fish can deteriorate. The biochemical components of fish do not remain normal and they necessarily lose quality during storage and preservation processes (Mackie, 1993). The quality loss occurs mainly due to changes in muscle safety protein and lipids (Shenouda, 1980). Ryder et al. (1993) have linked the availability of vital nutrients in fish to the method of storage. Whittle (1997) reported that storage time and temperature are major factors implicated in losing of quality and shelf life of fish. The decrease in the protein content is associated with the increase in storage duration (Gandotra *et al.,* 2012; Aberoumand, 2013). The rate of deterioration accelerates during frozen storage time and proliferation of bacteria, protein denaturation, lipid hydrolysis and oxidation increase as the storage period increase (Mazrouh, 2015). Pourshamasian *et al.* (2012) reported that during frozen storage some of the deterioration still occurs in the stored food, during which the freezing rate and temperature fluctuation are affecting the extent of quality loss.

Indian mackerel (*Rastrelliger kanagurta*, Russel) found in warm shallow waters along the coasts of the Indian and Western Pacific oceans. Its range extends from the Red Sea and East Africa in the west to Indonesia in the east, and from China and the Ryukyu Islands in the north to Australia, Melanesia and Samoa in the south. It has also been reported in the Mediterranean Sea as it entered it through the Suez Canal (FAO Species Catalogue, 1983).

The fish *R. kanagurta* is one of the important and cheap fish foods for Yemeni people. In Yemen, fishes are collected from its coastal areas (Red sea and Gulf of Aden) and transported considerable distances from landing points to different cities under chilling condition. After reaching the markets, fishes are usually stored under freezing conditions. However, because of the unsuitable conditions in Yemen, the electricity current exposed to continuous cut off. Freezing stores in the markets may undergo fluctuating temperature because of electricity problems. This fluctuation in temperature is expected to affect the biochemical components of the flesh of frozen fishes. The present investigation is going to deal with the nutritional value of *R. kanagurta* under stable as well as unstable (fluctuating) freezing conditions comparing with fresh samples. It would also cover a part of lacking data on the biochemical composition of fishes from Yemeni waters.

MATERIALS AND METHODS

Sample collection

Specimens of *R. kanagurta* with different sizes were collected from landing site in coastal areas (Al-Hodaidah). The fish specimens were kept in ice box and then transported, to the capital city (Sana'a) within 24 hours. Fishes were immediately brought to the laboratory in polythene bags along with crushed ice.

Sample preparation

About 50 specimens selected to be used in this investigation, cleaned up to remove any particles or excess water. The specimens were then divided into 15 sets of 3 – 4 fish. Muscles of each set were isolated carefully, mixed together and divided into three samples, each sample kept in airtight plastic bags. The samples were categorized and subjected to three storage protocols.

The three samples of each set were categorized as following: group 1 (fresh) was weighed and dried in an oven for biochemical analysis, group 2 (stable frozen), stored in the freezer at stable temperature of -20°C for 4 weeks. The third group; group 3 (un-stable frozen) was divided into two sub-groups and stored at fluctuating temperature, in which half of the samples were daily, removed from the freezer during night and kept on the lower shelf of the refrigerator (sub-group 1). The other half kept at room temperature overnight (sub-group 2). The two-frozen groups were weighed and dried in the oven after 4 weeks of storage, and then subjected to biochemical composition analysis.

Biochemical analysis

Biochemical components (moisture, protein, lipid, and ash contents) and pH level were analyzed according to standard Association of Official Analytical Chemists (AOAC, 1980) methods.

Moisture content was measured by using hot air oven at 70°C until a constant weight reached. The difference in weight was calculated and expressed as percentage moisture content using following formula

Moisture % = (weight of tissue – dry weight of tissue) x 100 /Weight of tissue.

Protein content was determined by Lowry's method (Lowry *et al.,* 1951) with slight modification and results were expressed in percentage.

Crude lipid content was estimated by Soxhlet method and the amount of lipid was calculated as following:

Lipid % = Weight of lipid / Weight of sample × 100

Ash content was estimated by taking about 3-5 g prepared sample was taken in a pre-weighed porcelain crucible and was placed in muffle furnace at 550ºC for 6 hours. Then the crucibles were cooled in desiccators. After recording the weight of ash, the ash content percentage of the samples could be computed as following

Ash content % = Weight of ash**/** Weight of sample × 100

Determination of pH: One gram sample was homogenized in 10ml of distilled water and the mixture was filtered. The pH of the filtrate was measured using a pH meter.

Statistical Analysis

Data obtained were analyzed using statistical package (SPSS 23) to calculate the mean±SD for each sample. Comparison between fresh and the two groups of frozen samples was done using paired T- test. The difference between the two sub-groups within unstable frozen group was also analyzed using independent T- test. All tests used were examined at 0.05 significance level.

RESULTS

The present study was carried out to understand the influence of the fluctuation in temperature during freezing process on the biochemical composition of *R. kanagurta* fish. The values of all biochemical components (moisture, lipids, protein and ash) and pH level of the three groups in the present investigation are shown in Table (1). The mean values of the components in all the 3 groups are depicted in Figure 1 to show the change occurred in the means of each component.

The values of the Moisture content in the fresh samples were oscillating between 74.1% and 77.54% with a mean value of 76.71%±0.93. The moisture was slightly decreased in the stable frozen samples, which ranged from 72.08% to 75.62% and mean value 75.27%±0.89. The change occurred to lesser limits in the unstable frozen samples, in which the moisture range was fluctuating between 73.21% and 76.56% with mean value 74.20%±0.99. The mean values and ranges of lipids were 3.57%±0.19 (3.22% - 4.1%), 3.51%±0.04 (3.49% - 4.61%) and 3.68%±0.20 (3.43% - 3.60%) for the fresh, stable frozen and unstable frozen samples, respectively.

Results of the protein estimation showed similar change occurred in lipids, which recorded a mean values of 20.43%±5.24 in the fresh samples, 19.43%±5.09 in the stable frozen samples and 20.40%±4.50 in the third group; unstable frozen samples. The mean values of ash were 4.66%±0.80, 5.46%±0.51 and 5.29%±0.79, in fresh, stable frozen and unstable frozen samples, respectively. Results of pH determination were between 6.06 to 6.38 with a mean value of 6.19±0.85 in group 1, 6.04 to 6.46 (6.20±0.85) in group 2 and 6.06 to 6.38 (6.24±0.14) in group 3.

Comparing the means of fresh and stable frozen samples using paired T- test showed significant changes in some studied components after freezing for 4 weeks. High level of significance (P< 0.05) observed in the changes occurring in moisture and ash contents. Changes in other components and pH level were found to be non- significant. Comparison between fresh and un-stable frozen samples using paired T- test appeared as highly significant (P< 0.05) changes in the values of moisture. Changes in lipids and ash values were also significant as it is clear in Table 2.

To determine if there is a difference between 2 sub-groups within unstable frozen samples independent T- test was used. Both protein and pH were significantly different between the 2 sub-groups during this study, while the changes were not significant in the other components as the result shown in Table 3.

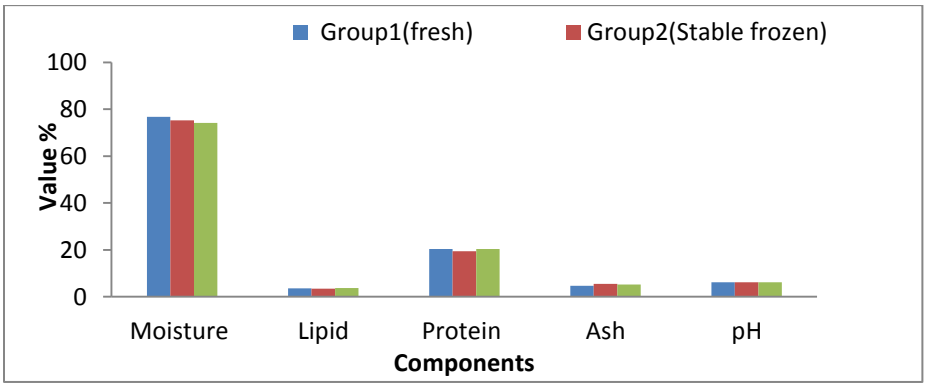

Fig. 1. Biochemical components and pH of *R.kanagurta* in fresh and frozen samples.

Table 1. Mean values of the biochemical compositions and pH level of fresh and frozen samples of *R. kanagurta*.

Sr. No.	Fresh Sample					Frozen Sample (Stable Freezing)					Frozen Sample (Un-stable Freezing)				
	Moisture%	lipids%	Protein%	Ash%	pH	Moisture%	lipids%	Protein%	Ash%	pH	Moisture%	lipids%	Protein%	Ash%	pH
1	74.09	3.60	11.00	5.05	6.12	73.21	3.47	10.30	4.50	6.12	72.08	3.52	14.40	5.22	6.1
2	77.53	3.62	12.00	5.21	6.21	74.45	3.45	11.10	6.19	6.21	75.10	3.63	13.90	4.91	6.1
3	76.49	3.60	19.00	5.02	6.27	75.86	3.43	12.30	5.73	6.27	73.34	3.59	14.20	7.11	6.2
4	76.57	3.59	22.00	4.36	6.06	74.74	3.52	17.00	6.38	6.06	74.62	3.50	15.60	7.05	6.1
5	77.42	4.10	12.90	4.93	6.22	75.79	3.51	16.00	5.11	6.22	75.33	4.61	17.10	5.74	6.3
6	76.59	3.64	16.00	5.06	6.17	75.36	3.56	19.50	5.27	6.17	74.29	3.99	19.90	6.48	6.4
7	77.51	3.49	19.00	4.78	6.21	75.29	3.60	21.00	5.36	6.21	74.88	3.86	20.00	5.07	6.1
8	76.22	3.62	26.00	5.06	6.10	75.56	3.50	23.00	5.38	6.10	73.33	3.76	21.20	5.14	6.0
9	75.60	3.57	23.00	4.81	6.10	74.09	3.51	20.00	5.43	6.10	73.47	3.57	22.10	7.45	6.2
10	77.47	3.45	22.20	4.96	6.20	76.56	3.57	19.90	5.22	6.20	75.20	3.51	21.00	4.96	6.5
11	77.38	3.33	23.10	4.99	6.28	75.90	3.52	22.70	4.90	6.28	75.62	3.51	23.20	3.83	6.4
12	76.83	3.22	26.10	3.73	6.20	76.08	3.49	23.10	6.01	6.20	74.40	3.54	24.90	4.08	6.4
13	77.06	3.62	23.20	2.17	6.30	75.80	3.48	24.30	5.13	6.30	73.90	3.49	25.60	5.42	6.3
14	76.40	3.46	24.20	4.36	6.17	74.51	3.53	25.10	6.09	6.17	73.04	3.52	25.90	4.48	6.2
15	77.54	3.65	26.70	5.37	6.38	75.82	3.49	26.20	5.23	6.38	74.36	3.61	27.00	5.38	6.4
Mean±SD	76.71±0.93	3.57±0.192	20.43±5.237	4.66±0.800	6.1993±0.085	75.27±0.893	3.51±0.045	19.43±5.091	5.46±0.519	6.20±0.085	74.20±0.991	3.68±0.295	20.40±4.503	5.29±0.794	6.24±0.143

Table 2. Comparison Between fresh and frozen samples using paired t- test.

	Comparison between fresh and stable frozen samples			Comparison between fresh and unstable frozen samples		
	Group	Mean± SD	T (sig-2tailed)	Group	Mean± SD	T (sig-2tailed)
Moisture%	fresh frozen	76.71±0.93 75.27±0.89	0.000*	fresh frozen	76.71±0.93 74.20±0.99	0.000*
Lipid %	fresh frozen	3.57±0.19 3.51±0.45	0.258	fresh frozen	3.57±0.19 3.68±0.99	0.046*
Protein%	fresh frozen	20.43±5.24 19.43±5.09	0.204	fresh frozen	20.43±5.24 20.40±4.50	0.975
Ash%	fresh frozen	4.66±0.80 5.46±0.52	0.008*	fresh frozen	4.66±0.80 5.29±0.79	0.037*
pH	fresh frozen	6.20±0.09 6.20±0.09	--	fresh frozen	6.20±0.09 6.24±0.14	0.260

-- There was no difference. * significant

Table 3. Comparison within the sub-groups of Un-stable frozen samples at cold and room temperature using Independent t- test.

	Sub-group	N	Mean± SD	(sig-2tailed)
Moisture%	Sub-group1 Sub-group2	8 7	74.12±1.11 74.28±0.91	0.76
Lipid %	Sub-group1 Sub-group2		3.81±0.37 3.54±0.42	0.074
Protein%	Sub-group1 Sub-group2		17.04±2.96 24.24±2.19	0.000*
Ash%	Sub-group1 Sub-group2		5.59±0.58 4.94±0.90	0.20
pH	Sub-group1 Sub-group2		6.15±0.12 6.33±0.97	0.006*

* significant

DISCUSSION

The present study revealed that the values obtained for all the biochemical components in the fresh samples of *R. kanagurta* were in good quantities. However, they were similar to the results recorded by other investigators (Nisa and Asadullah, 2011; Ravichandran *et al*., 2011; Shaji and Kannan, 2013; Sonavane *et al.,* 2017) in the most components of *R. kanagurta*. Although, ash values were differing from the results of the previously mentioned studies they were close to results recorded by Sumi et al. (2016). On the other hand, greater values of protein and ash were obtained by Qari et al. (2017) in the same species. This difference between present and other studies results in the values of protein and ash may be attributed to the environmental conditions, quality of food that fish eat, or the season during which samples were collected.

As it is shown in the results, the moisture was slightly decreased in the stable frozen samples but, the change occurred to lesser limits in the unstable frozen samples. It is known that when flesh freezes, the water content of the cells turns to ice and when ice gets melt, it flows out of the cells leading to lose a part of the water. Presence of samples either on the lower shelf of the refrigerator or outside at the room temperature in the third group may expose the samples to lose some water. The changes in the moisture content were not quit different between the two frozen groups. However, the limited changes in moisture content were highly significant ($P< 0.05$) in both the cases. The present results indicate that both freezing states either stable (group 1) or unstable (group 2), reduced the amount of moisture in the fish tissue. It was found by Gandotra et al. (2012) that biochemical components including protein, lipid, moisture and ash contents decreased significantly during the 21 days storage period in the muscle of *Labeo Rohita*. Reduction of moisture content in frozen cat fish was also concluded by Akinwumi (2014).

It is worth to mention that the samples were directly stored in the ice before they transferred to polythene bags, this may lead to record a slightly higher moisture values than it was supposed to be.

Lipid is one of the compounds that exposed to deterioration. The rate of deterioration is accelerating during frozen storage time and proliferation of bacteria. Protein denaturation, lipid hydrolysis and oxidation increase as the storage period increase (Mazrouh, 2015). In the present investigation the change occurred in the mean value of lipid content after freezing was not high and was in reverse with moisture values. However, those changes were non-significant in case of stable frozen samples ($P> 0.05$) but were significant in the case of unstable frozen samples ($P< 0.05$).

It seems that the period of freezing was not long enough to make significant changes in lipids in the state of stable freezing, but the freezing un-stability played an important role in the other group. This finding is corresponded with that of Foruzani et al. (2015), which found that freezing have an effect on the chemical composition of fish which can reduce crude protein and fat in the fish stored for 180 days.

It is clear from the mean values that the protein was slightly changed in both the groups but the changes were non-significant ($P> 0.05$) as it is evident from the statistical analysis. This result was in agreement with the statement by Srivastava (1999) that freezing leads to desiccation and denaturation of protein and no actual loss in nutritional value of protein occur. Similar to lipids, the protein was affected by freezing for 4 weeks but this damage was limited. The damage limitation may be attributed to the time of storage even in the case of fluctuated temperature (group 3). This explanation is in agreement with the statement of Yerlikaya and Gokoglu (2010) which confirm that time of freezing affects the ultimate quality of frozen fish.

The results suggest that the period of 4 weeks freezing of *R. kanagurta* might be not enough periods to make dramatic changes in protein values in both cases stable and unstable freezing. Decreasing in protein and fats associated with the increasing in freezing duration was reported by Aberoumand (2013) may support the suggestion added by the present work. On the other hand, significant influence of freezing on the nutritional values of cat fish *C*. *gariepinus* was observed by Akinwumi (2014) which found that freezing and smoking were better efficient preservation methods in terms of retention of the protein value and reduction of moisture content.

On contrary, ash values have increased significantly ($P< 0.05$) in both the groups of frozen samples. Losing water from cells after freezing might have increased the minerals concentration leading to high ash percentage.

Determination of pH level in different samples clearly showed that there were non-significant differences between the fresh and frozen samples, neither stable frozen nor the unstable frozen samples.

Comparison between the two sub-groups within unstable frozen sample indicated a significant difference ($P< 0.05$) between the samples were kept on the lower shelf of the refrigerator (sub-group 1) and that kept at room temperature overnight (sub-group 2) in the values of protein and pH. This may suggests that the freezing conditions during un-stable freezing (alternative periods of freezing and unfreezing) was an important factor affecting the value of protein and pH level. Higher protein value was observed in the sub-group kept at room temperature may attribute to higher temperature which increased the alkali soluble protein and this was in agreement with Qixing et al. (2014).

It is notable that the weather was mild and the room temperature was not high during the present study.

RECOMMENDATION

In order to achieve clear view about the effect of different protocols and durations of freezing it is recommended to perform this study with several freezing temperatures at different durations of experiment.

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CONFLICT OF INTEREST

All the authors have declared that no conflict of interest exists.

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