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Effects of freezing storage on the biochemical composition in muscles of *Saurida undosquamis* (Richardson, 1848) comparing with imported frozen

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Abstract
The present study was carried out to determined the quality changes of the nutritive value (protein, lipid, moisture, ash, pH) and minerals of fresh *Saurida undosquamis* and to examine the effect of freezing for (7, 14, 21) days respectively at (-20 °c) comparing with imported frozen fish. The chemical analysis of fresh muscles recorded high values of protein %, lipid % and moisture while there was a significant ($P<0.05$) decrease after 21 days of both freezing and imported frozen fish. The total lipid content of all groups of samples remained more or less stable during 21 days of frozen storage and no significant change was noted during freezing. The ash content was slight increase of no significant importance. pH values increases with duration of storage. The maximum values of Mg, Ca and P recorded (1.16±0.03 mg/100g, 9.28±0.50 mg/100g and 3.28±0.41 mg/100g) for fresh samples and decreased with freezing times. A significant difference was observed between fresh fish, freezing samples after 7, 12 days and imported frozen fish for calcium and phosphorus concentration. In conclusion, the freezing of fish influenced the biochemical composition and mineral contents of their muscles also the quality of fish samples during storage revealed the decreasing of the taste with increasing duration of storage.

Keywords: lizardfish, freezing, biochemical, protein, mineral contents, pH

Introduction
Fish is the most important source of protein, which contains all the essential amino acids in their right proportion, other nitrogenous compounds, water, lipids, carbohydrates, minerals and vitamins. Fish diet has high commercial and medicinal values [1]. The main sources of energy reserves in fish are protein and lipid. The relative contributions of lipids content and amino acids to energy production in fish depends on a number of factors such as the species involved of lipids [2,3], environmental conditions [4,5], stage of maturity of the gonads [6,7], nutritional state [8], and age [9]. The concentration of lipid varies considerably in different parts of the body of the fish [10]. *Saurida undosquamis* is one of the main coastal demersal target species of commercial interest in the Eastern Mediterranean particularly in Egypt. It is among the Lessepsian species that had invaded the Red Sea via the Suez Canal and Kosswig (1951) [11] was the first author report this species in Turkish seas. Freezing is a common practice in the meat, fish and other animal protein based industry, because it preserved the quality for an extended time and offers several advantages such as insignificant alterations in the product dimensions and minimum deterioration in products color, flavor and texture [12]. Frozen storage is an important method for processing of fish. However, when seafoods are frozen and stored in frozen state they necessarily lose quality [13]. Loss in quality of frozen stored fish is mainly due to changes in muscle integrity, proteins and lipids [14]. Cellular disintegration during frozen storage can cause acid hydrolysis of lipids to free fatty acids. The changes in fish muscle fibers, proteins, lipids and textural properties during frozen storage have been studied for several decades because of their economic importance, [15-18] also due to this importance Beroumand and Jooyandeh, (2010) [19] found that the consideration of the types of packaging, maintenance of proper storage temperature and freezing properties of different species must be given for great importance on the quality of fish. These mean that fish if necessary should be stored, for a short period of time to retain the taste, and provide both the protein and fat at optimal level.

Fish is also a vitamin economic value but rich in nutritional value are often not and mineral rich food [20, 21]. Minerals have important tasks in metabolism. Levels of heavy metals and minerals in muscle tissues of marine species have been determined by many researchers [22,25] such as Ca, Mg, and P. The aim of the study is to evaluate the nutritive value of protein, lipid, moisture, ash, pH and minerals (magnesium, calcium and phosphorus) in fresh muscle of *Saurida undosquamis* and stored in conditions (-20˚c) for 7, 14 and 21 days comparing with imported frozen muscle and to investigate the quality changes during its frozen storage.

2. Materials and Methods

2.1 Sample Collection

A total of 50 fresh and frozen imported brushtooth lizard fish *Saurida undosquamis* (Richardson, 1848), were examined during period of November 2014 to March of 2015.

They were divided into two groups (25) fresh *Saurida undosquamis* were obtained from Red Sea by local fishermen and (25) imported frozen fish were bought from local fish markets. The collected fresh and imported frozen fish samples were kept immediately in ice on polyethylene bags and transported to the physiology Lab of NIOF, Alexandria to sustain freshness. Fish were cleaned with deionized-distilled water and muscle tissue was taken from the dorsal part of fish. All samples were kept in cold storage at -20ºc until biochemical analysis. Fresh muscle was frozen at a temperature -20°c for different times (7, 14 and 21 days). Samples were analyzed for the biochemical composition of the dried tissues.

2.2 Biochemical analysis

The total protein content was determined using Lowry *et al*. (1951) [26] method. 10 mg of sample, 1ml NaOH was added for protein extraction in water bath for 30 minutes. Thereafter, it was cooled at room temperature and neutralized with 1 ml HCL. The extracted sample was centrifuged at 2000 rpm for 10 minutes and 1 ml of the sample was further diluted with distilled water (1/9 v/v). From the diluted sample, 1 ml was taken and treated with 2.5 ml of mixed reagent (carbonate-tartrate-copper) and 0.5 ml of Folin's reagent. After 30 minutes, sample absorbency was read at 750 nm using spectrophotometer. The total lipid content was estimated using Folch *et al*. (1957) [27] method. 10 mg of dried sample was homogenized in 10 ml of chloroform methanol mixture (2/1 v/v). The homogenate was centrifuged at 2000 rpm then dry in an oven and the weight was taken for estimate. Moisture content was determined by placing an accurately weighed known amount of ground sample in a pre-weighted porcelain crucible in an electric oven at 105ºc for about 24 hours until constant weight was obtained. The different between the wet and dry weights gave the moisture content. The Ash content was determined by burning oven-dried sample in a muffle furnace at 550ºc for 6 hours. Then the crucibles were cooled in desiccators. The average in percentage of each sample of the remaining materials was taken as ash [28]. pH was measured by using digital pH meter (HANNA) [29].

Mineral contents concentrations magnesium, phosphorous and calcium were measured in the fish muscles according to the method reported by [30]. Five grams dry weight of fish's muscles was placed into a digestion tube. Five ml of conc. HNO_3 and conc.5 ml of $HClO_4$ were added to the samples, respectively. The reaction was slowed down by placing the tubes in a hot plate and heated up to 60ºc for 30 min. After that the tubes were allowed to cool down, 10 ml of conc. HNO_3 was added for each sample and again digested. Cooled and diluted the sample with deionized water and the H_2O_2 additions were repeated until the samples were clear. Finally filtered and diluted with deionized water to50 ml in volumetric flasks. Concentrations of minerals were measured using Atomic Absorption Spectrophotometer (Perkin Elmer) 2280.

2.3 Statistical analysis

Statistical analysis was carried out using analysis of variance (ANOVA) available in MS-Excel 2007 to evaluate the effect of freezing on muscles during storage (7, 14 and 21) days at-20ºc. Means were compared using Duncan's test (1955) [31]. All data were expressed as means standard error (M±SE) and the significance level was set at $P<0.05$.

3. Results and Discussion

Fish is an important part of a healthy diet because they are considered to be an excellent source of high value protein and essential nutrients. The mean (±SE) of protein, lipid, moisture, ash, pH for fresh and imported frozen *Saurida undesquamis* muscle are presented in Table (1) compared with fresh muscle that was frozen for 7, 14 and 21days at (-20°c).

Table 1: Biochemical composition (%) fresh and frozen muscles of *Saurida undsquamis*

Proximate composition	Fresh Fish	Fish frozen 7 days	Fish frozen 14 days	Fish frozen 21 days	Imported frozen fish
Protein %	25.48^{a}±1.32	23.12^{a}±1.99	20.64^{ab}±1.15	13.46^{b}±1.32	22.46^{a}±0.87
Lipid%	3.53^{a}±0.18	3.20^{a}±0.03	3.31^{a}±0.06	3.05^{a}±0.23	3.38^{a}±0.12
Moisture%	71.60^{a}±2.56	70.40^{a}±3.31	68.80^{a}±1.02	66.20^{ab}±1.36	68.80^{b}±0.85
Ash%	0.86^{a}±0.08	0.91^{a}±0.15	0.96^{a}±0.07	1.16^{a}±0.12	1.19^{a}±0.16
pH	6.50^{a}±0.07	6.68^{a}±0.17	6.82^{b}±0.09	6.88^{b}±0.37	6.94^{c}±0.07

Means with different superscripts in the same raw are significantly different at $P<0.05$).

Freezing is a common practice in the meat fish because it preserved the quality for an extended time, minimum deterioration in products color, flavor and texture [32], while there are some disadvantages associated with frozen storage including freezer burn, product dehydration, rancidity, drip loss and product bleaching which can have an overall effect on the quality of the frozen foods [33]. Deterioration during frozen storage is inevitable, and in order to obtain satisfactory results, fish for freezing must be of good quality.

3.1 Total Protein Content

The maximum mean value of total protein content 25.48±1.32% was recorded for fresh muscle while the minimum value was 13.46 ±1.32% after 21 days of freezing. Protein content in imported frozen fish was recorded 24.44±1.80%. According to statistical findings, there were significant ($P<0.05$) differences in the crude protein of both fresh fish and fresh muscle after 14 and 21 days of freezing, also show a significant difference between fresh muscle that was frozen for 7 and 14 days and fresh muscle after 21 days of

freezing. While there were no significant differences (p>0.05) between the fresh fish, imported frozen fish and fresh fish after 7and 14 days of freezing. Protein and lipid are the major nutrients in fish and their levels help to define the nutritional status of the particular organism. Proteins are important for growth and development of body. These results are in agreement with Siddique *et al.*(2011) [34] on *Puntius sp*. Reported significant decrease in protein content during frozen storage at -50°c of 20 days on frozen fish muscle of *Labeo rohita* in *Puntius sp.* for 21 days [18] and fresh and frozen *Tilapia nilotica* muscles for 8 weeks frozen storage [35]. The protein content was decreased due to denaturation and loss in gelatin caused by extended frozen storage also due to proteolysis induced by enzymatic activities of psychotropic microbial growth.

3.2 The total lipid content

The total lipid content slightly decreased during the time of freezing. It was recorded 3.53±0.18%, 3.19±0.03%, 3.13±0.06% and 3.05±0.23% at (fresh muscle fish, 7, 14 and 21 days) respectively. The lipid content of muscle imported frozen was decreased to 3.38±0.12%. There were no significant difference (p>0.05) in the lipid content. Protein and lipid are the major nutrients in fish and their levels help to define the nutritional status of the particular organism. These result comes in agreement with Arannilewa *et al*. (2005) [36] calculated 25.92% decrease in total lipid content in Tilapia after storing it in freezer for 60 days and the similar trend of fat content obtained in mackerel increased during frozen storage [37]. Azzam *et al*. (2012) [38] studied the determination chemical composition (lipid, protein, ash, moisture and glycogen) in four fish species from Tigris River in North of Iraq; Gandotra *et al.* (2012) [18] studied on muscle of *Labeo Rohita* (Ham- Buch). Those workers attributed this loss of lipid occurred mainly due to losses in triglyceride fraction and due to the oxidation rancidity.

3.3 The moisture content

The moisture percentage was found to be 71.6±2.56% in fresh muscle while the value decreased to 68.8±0.85% in imported frozen fish. The fresh muscle samples slightly decreased significantly (P<0.05) 70.4 ± 3.31%, 68.8±1.02% and 66.2±1.36% respectively after 7, 14 and 21 days of freezing at -20ºc. These results are in accordance with Roopma *et al*. (2012) [39] who reported a decrease in total moisture content in muscle samples of *Mystus seenghala* stored at two different low temperature 4°c chilled and -12 °c frozen. They advocated that the more decrease in moisture content was due to evaporation of moisture from meat in chiller whereas the decrease in moisture content was due to sublimation of surface water of meat in the freezer. On contrary to the results of present study, Siddique *et al.* (2011) [34] in *Puntius sp.* found an increasing trend in moisture content meanwhile, Kirschnik *et al.*(2006) [40] observed that moisture content was constant for 14 days in samples of tail meat of the giant river prawn, (*Macrobrachium rosenbergii)* stored without direct contact in ice.

3.4 Ash content

In the present study, the ash content was 0.86 ±0.08% in the fresh samples while increased to 1.19 ±0.16 in imported frozen fish and 1.16±0.12 in fresh fish after 21days of freezing. The ash content has shown slight increase of no significant importance (P>0.05). These results are in agreement with Fouad (2011) [41] who observed that the ash content remained almost the same throughout the 1, 2, 3 weeks of frozen storage of carp. While Roopma *et al.* (2012) [39] and Mariam (2013) [35] both of them observed that the ash content decreased with storage time of *Mystus seenghala* (4±1°c and-12±2°c) and of Tilapia at the end of the eight weeks of freezing. Ash in fish muscle contains nutritionally important minerals. The increase in ash contact was affected with mineral percentage in fish muscle, physiological parameters and nutrition.

3.5 pH value

In addition, pH was found to be 6.5 ±0.07 in fresh muscle and increased significantly (P<0.05) to the value 6.94±0.07 in imported frozen fish. There was comparatively slow increase in pH between fresh muscles during freezing period. It was increased from 6.68±0.17 to 6.88±0.37 respectively. There was significant differences (p<0.05) between fresh fish and imported frozen fish and fresh fish that was frozen for 14 and 21 days. No significant difference between groups of fresh fish that was exposed to the different freezing periods and imported frozen fish. These results are in accordance with Erkan and Ozden (2008) [42] who stated that the increase was due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes. Obemeata *et al.* (2011) [43] observed that the increase in pH was higher in the 4°c stored sample of Tilapia, indicating that biochemical and microbial changes are occurring faster in 4°c stored fish. Pawar *et al.* (2013) [44] showed slightly increased pH in *Catla catla* from 6.50 to 6.79 when stored at chilled temperature (-2 to -4°c). The change in pH of fish muscle is usually good index for quality assessment. The increase in pH is caused by the enzymatic degradation of fish muscle.

3.6 Mineral composition

Fish is a potential source of minerals such as phosphorus, magnesium and calcium. These elements are essential for normal tissue metabolism and for maintenance of health are adequate in fish [45]. The mineral composition in fresh, frozen and imported frozen *Saurida undsquamis* muscle were presented in (table 2). The elemental concentrations of the fishes were expressed in mg/100g. Among the nutrient elements investigated the most abundant was calcium followed by phosphorus and magnesium. The mean values of magnesium decreased during frozen storage. They decreased in imported frozen muscle fish 1.04±0.02 mg/100g, while in fresh muscle was 1.16±0.03 mg/100g. The maximum mean values of calcium and phosphorus were recorded 9.28±0.50 mg/100g and 3.28±0.41 mg/100g for fresh fish while the minimum were recorded 5.72 ±0.35 mg/100g and 2.02±0.16 mg/100g for imported frozen fish respectively. There was not a clear relationship between the Mg concentration and the different freezing period. Significant differences for phosphorus was observed between fresh fish, imported frozen fish and fresh fish that were frozen to 7, 21 days. Calcium concentration decreased significantly (P<0.05) to 7.8±0.39at21 days of freezing.

Table 2: Mineral composition in the muscles of *Saurida undsquamis* (mean ± SE) mg/100g

Minerals	Fresh fish		Fish frozen 7 days		Fish frozen 14 days		Fish frozen 21 days		Imported Frozen fish	
Magnesium	1.16[a]	±0.03	1.07[a]	±0.03	1.14[a]	±0.03	1.03[a]	±0.08	1.04[a]	±0.02
Calcium	9.28[a]	±0.50	7.52[b]	±0.50	7.52[b]	±0.50	7.80[c]	±0.39	5.72[c]	±0.35
Phosphorus	3.28[a]	±0.41	2.34[a]	±0.22	2.62[ab]	±0.17	2.04[b]	±0.21	2.02[b]	±0.16

Means with different superscripts in the same raw are significantly different at P<0.05).

There is a slight change with respect to frozen period in all the mineral evaluated and attributed that to the drip loss and the dehydration associated with frozen storage. These results are agreement with [46, 36]. Calcium and phosphorus are necessary to maintain an optimal bone development, with more of both minerals being required during childhood and growing stages to prevent rickets and osteomalacia [47]. Magnesium is required in the plasma and extra cellular fluid, where it helps in maintaining osmotic equilibrium. It is also required in many enzyme catalyzed reactions.

4. Conclusion

The results of this study showed that the better quality of frozen fish found after 7 days of freezing and the quality of fish is best before frozen storage. The rate of deterioration was accelerated during frozen storage time. The proliferation of bacteria, protein denaturation, lipid hydrolysis and oxidation increase as the storage period increase. The freezing of fresh fish leads to decrease in protein %, lipid %, and moisture % and increase in ash and pH value compared with fresh muscle fish. The increases of pH value for imported frozen *Saurida undosquamis* make more exposure for the decomposition. Recommendation is eating fresh fish which is most benefit for human health.

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