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EFFECT OF FREEZING PERIOD ON CHEMICAL COMPOSITION, MICROBIAL LOAD AND SOME PATHOGENIC BACTERIA OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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ABSTRACT: This study evaluated the effects of freezing periods on nutritional values, microbial loads, and some pathogenic bacteria in Nile Tilapia (*Oreochromis niloticus*). 8 kg. Samples were collected from El-mawourda Fish Market in Khartoum State, for four experimental treatments. The treatments were conducted on the period 0 day, 7 days, 15 days and 21 days. It is noticed that the freezing period effect on the nutritional value, microbial load and some pathogenic bacteria of Nile tilapia (*Oreochromis niloticus*) revealed that the lowest average nutritional assessment of Nile tilapia (*Oreochromis niloticus*) observed for protein 20% at the third period of freezing (21 days), and minerals were calcium, phosphorus, magnesium, iron, sodium and potassium (5.6 – 2.7%, 4.8 – 2.9%, 2.6 – 2.9%, 1.1 – 0.71%, 4.5 – 2.8%) respectively. The result of this study clarified that the freezing period has a considerable consequence ($p \leq 0.05$) on the chemical composition, microbial load and same pathogenic bacteria (*E. coli* and *Salmonella*) of Nile tilapia (*Oreochromis niloticus*).

KEYWORDS: *Oreochromis niloticus*, freezing period, nutritional value, microbial load

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a commercially significant freshwater aquaculture species throughout Asian Countries (e.g. Thailand, Vietnam, Indonesia, and China). The trade of Tilapia products are growing rapidly, with the major importing regions include the United States, Russia and the European countries. Fishery products made from Tilapia are important export food of those countries (Liu *et al.,* 2015). In Thailand, the whole frozen Nile tilapia exported amounted to 4,900 tons with cash value averaged 300 million baht during January to October in 2016 (Noorit, 2016). Frozen Nile Tilapia is widely used as a raw material in various food products. Consumers highly expect that the frozen Nile Tilapia cloud be applied to maintain the fish qualities after thawing and processing (Cyprian *et al.*, 2013).The demanding high food quality of the consumers is progressively increasing and the product qualities are expected to be maintained during production, storage and consumption. However, fresh fishery products are highly perishable. The deterioration is caused by microbiological, chemical and enzymatic activities that are induced by storage temperature (Huss 1995; Simpson *et al.*, 2003; Cyprian *et al.*, 2013). Freezing process is the effective and most economical method to prolong fishery products freshness and flavour. Nevertheless, a low temperature used in the freezing process could affect the fish muscle properties resulting in quality changes in the final product. Moisture migration during freeze thawing cycle can be exhibited by the development of ice crystal and possibility of effect on protein denaturation and aggregation (Lee *et al.*, 2016). Thus the determination of frozen-thawed and fresh fish qualities is important in food industries, particularly for skinless fish fillets (Diop *et al.*, 2016). Among the fishery production in 2008, fresh product supply (56 million tons) ranked first, followed by frozen product supply (29 million tons). Assured the next rank of processes was canning and drying, followed by smoking.

As a result of freezing fish and fisheries, quality is preserved, shelf-life is increased, surplus fisheries are delivered to high utilization markets, and fish can be sold at higher prices for longer periods of time (Karami *et al.*, 2013). It has some disadvantages, however, including loss of quality, weight loss, fat oxidation (especially in fatty fish) and high freezing costs. Numerous studies have been carried out on the fatty acid composition and chemical and microbial characteristics of different fish species, such as tilapia. Traditionally, freezing and frozen storage have been used to preserve fish's sensory and nutritional properties. Due to their unsaturated lipid composition and prooxidant molecules, fish species are prone to rancidity and quality loss (Richards and Hultin, 2002). As a result of lipid oxidation compounds in frozen conditions, protein denaturation and nutritional loss are facilitated (Rostamzad *et al.,* 2011). The filleting of many fish species is done to meet consumer requirements and to add value to the product, but this depends very much on the type of market. Generally, filleting is used to facilitate culinary preparations and to facilitate presentation (Antonio *et al.*, 2011). Many efforts are being made to prolong shelf life by employing antioxidants to prevent lipid oxidation. In recent years, efforts have been made to replace synthetic antioxidants with natural antioxidants because of the potential adverse effects of synthetic antioxidants as well as the potential benefits that natural antioxidants may provide (Sarkardei and Howel, 2008). There are various spices, herbs, and plants that are used to enhance the flavour or colour of food stuffs. Aside from their antimicrobial properties, these materials are also antioxidants (Baydar *et al.*, 2004). Natural antioxidants were used as pre-treatment for fish fillets to inhibit lipid oxidation (Aubourg *et al.*, 2004).

Having a fast growth rate, an easy cultivation process, as well as a high feed utilization rate, Tilapia has been widely cultivated throughout the world as a freshwater fish species (El-Sayed, 2006). The world market offers a wide variety of tilapia products. There is a variety of options for fresh and frozen Tilapia fillets, including skin-on and skin-off, deep-skinned, individually quick frozen, smoked, and sashimi grade, as well as carbon monoxide and ozone dipped. As a result of these processes, a variety of interesting by-products have emerged, including leather for clothing and accessories, gelatin for time-released medicines, and flower ornaments created from dried and coloured fish scales. Previously, quality changes have been reported only for a single freeze cycle, but little has been reported regarding repeated freeze cycles. Recent works mainly focused on the effects of super chilling and microbiological assessments on Tilapia (Hamid and Hamid, 2014; Sarkar *et al.*, 2020; Melo-Bolívar *et al.*, 2022). In the present study, we examined the importance of *Tilapia* processing for the country. A study was conducted to investigate microbial load, nutritional quality chemical indices, and some bacteria pathogenic to Nile tilapia fillets after frozen storage for 3 weeks.

MATERIALS AND METHODS

Collection of Fish Samples: Nile Tilapia was obtained from a local fish market (El-mawourda Fish Market) located at Omdurman City, Khartoum State, by an average weight 335±61g. The fish are kept in boxes on ice for one hour until they are delivered to the laboratory. Subsequently, the fish were gutted, dressed, filled by hand, and washed with tap water. The weight of each fillet was 137 g. Afterward, Fillets were alienated into four groups; for the first group (control) the chemical composition and minerals were analyzed in the Central Veterinary Research Laboratory. For the second, third and fourth groups (G1, G2 and G3), fillets were packaged individually in polyethylene bags and kept at ±18°C in a freezer. In order to sample raw fillets (initial material), we divided each group into three subgroups. The second group (G1), third (G2) and forth (G3) microbial load and identification of some bacteria, chemical composition and minerals analysis was done after seven days, forty days and twenty one days, respectively, in the microbiology laboratory of College of Animal Production Science and Technology. The chemical composition and minerals were analyzed in the Central Veterinary Research Laboratory, soba - Khartoum, Sudan.

Chemical composition: moisture, dry mater, ash, crude protein and crude fat content (ether extraction) of the fish samples were determined in according to the AOAC standard method 934.01, 942.05, 954.01 and 991.36, respectively (AOAC, 2000). Nitrogen free extracts (NFE) was estimated by subtracting the total of moisture, crude protein, crude lipid and ash from 100.

Determination of mineral contents: [sodium (Na), potassium (K), iron (Fe), zinc (Zn), Magnesium (Mg) and calcium (Ca)] were extracted using dry ashing method no. 942.05 (AOAC, 2005). Samples were ashed at 550°C to a constant weight, dissolved with distilled water and few drops of concentrated HCl were added. Sodium and potassium elements were determined using a flame photometer (Model PFP7). Calcium, Fe, Zn, and Cu were calculated by Atomic Absorption Spectrophotometer (Pekin-Elmar, 3110, USA).

Microbiological evaluation: The total bacterial count (TBC) was determined using the method described by Harrigan and Margaret (1976). In accordance with Swanson *et al.* (1992), psychrophilic bacterial counts (PsBC) were detected.

Data analysis: Data collected during this experiment were analyzed using one-way analysis of variance (ANOVA). The treatment means were analyzed by Turkey's Honest at 5% significance level. SPSS software version 16 was used for analysis according AOAC, 1980.

RESULTS AND DISCUSION

As a major source of animal protein in the tropics, fish has long been considered good for human health, as it contains protein and other essential nutrients (Andrew, 2001). Many countries around the world produce a large amount of farmed tilapias, contributing significantly to global seafood supplies.

Temperature and storage time are the two most important factors affecting fish excellence and shelf life (Whittle, 1997). It is accepted that freezing fish is an successful method of preserving it, despite some disadvantages.

Table 1. The effect of frozen storages at different periods on the chemical composition of Nile tilapia (*Oreochromis niloticus*).

Parameters Treatments	Muture%	DM%	Ash%	CP%	EE%	NFE%
0 Day	73.0±1.00^{a}	27.0±1.00^{a}	5.4±0.10^{b}	31.0±0.33^{a}	7.2±0.06^{a}	30.4±0.99^{a}
One week	71.0±1.00^{b}	29.0±1.00^{bc}	6.0±0.10^{c}	29.3±0.31^{b}	6.9±0.10^{b}	29.7±1.07^{b}
Two week	71.0±1.00^{c}	29.0±1.00^{bc}	6.7±0.15^{a}	25. 4±0.44^{c}	6.1±0.10^{c}	33.4±1.01^{c}
Three week	69.0±1.00^{d}	31.0±1.00^{d}	6.2±0.15^{d}	20.01±0.09^{d}	5.2±0.15^{d}	37.6±0.98^{d}
Sig.	*	*	*	*	*	*

N = 4, *: Significant (P < 0.05)

^{abc} Means by different superscripts at the same column differ significantly by Least Significant Difference (LSD) test at P < 0.05

Results showed that amounts of nutrients, elements and bacterial pathogens (protein, fat, moisture, Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg) and Iron (Fe), and *E. coli spp*. and *Salmonella spp*.) in Nile tilapia (*Oreochromis niloticus*) reduced in freezing method. As shown in Tables 1, 2 and 3, processing and freezing altered Nile tilapia (*Oreochromis niloticus*) fat and protein percentages. There is evidence to suggest that fishes' vital nutrients are greatly affected by storage methods (Apendi *et al.*, 1974 and Ojewola *et al.*, 2003). All test samples may have had different compositions and gross energy contents due to the different processing methods and storage conditions, according to the latter authors. Even though freezing meat and fish is common practice, it has several advantages including extended storage, minimal deterioration of product, color, flavor, and texture, as well as the ability to preserve quality (Obuz and Dikeman, 2003). The percentage of protein and fat in *Oreochromis niloticus* was minimized by frozen storage in this study. Researchers have also observed a reduction in protein, ash, moisture, and fat with frozen storage (Omotosho and Olu, 1995; Kamal *et al,* 1996; Arannilewa *et al.*, 2005).

The results of the study showed a high important effect (p≤ 0.05) of freezing on the mineral content of Nile tilapia, ranging between (5.6 - 2.7%, 4.8 - 2.9%, 2.6 - 2.9%, 1.1 - 0.71%, 4.5 - 2.8%, 4.9 - 3.0 %) for calcium, phosphorous, magnesium, iron, sodium and potassium, respectively. Generally the mineral content decreased with the increase in the freezing time, the magnesium decreased slightly and then increased during the freezing period. The results of this study are compatible with what was mentioned before. An investigation was conducted to determine how the freezing period affected the chemical composition and bacterial content of Nile tilapia. The results of mineral analysis in fresh samples were the control 0 day for the studied species. The content of iron, calcium, phosphorous, magnesium, zinc and iodine was 2.50, 6.35, 1.25, 1.23, 0.395 and 5.95), respectively. After 10 days, the content of iron, calcium, phosphorous, magnesium, zinc and iodine was 2.25, 6, 1.05, 1.19, 0.335 and 5.65), respectively. After twenty days, the content of iron, calcium, phosphorous, magnesium, zinc and iodine was 5.95, 5.70, 0.950, 1.11, 0.285 and 4.95, respectively. At 21 days the content of iron, calcium, phosphorous, magnesium, zinc and iodine was 2.00, 5.10, 0.850, 1.0, 0.275 and 4.65) respectively.

Table 2. The consequence of frozen storages on the minerals of Nile tilapia (*Oreochromis niloticus*) at different periods.

Parameters Treatments	Ca%	P%	Mg%	Fe%	Na%	K%
0 Day	5.6±0.07[a]	4.8±0.08[a]	2.6±0.05[b]	1.1±0.01[a]	4.5±0.06[a]	4.9±0.04[a]
One week	4.9±0.03[b]	3.9±0.07[b]	2.0±0.02[c]	0.95±0.05[b]	3.8±0.04[b]	4.1±0.05[b]
Two week	3.9±0.06[c]	3.3±0.03[c]	1.7±0.06[d]	0.84±0.02[c]	3.1±0.08[c]	3.8±0.07[c]
Three week	2.7±0.07[d]	2.9±0.06[d]	2.9±0.06[a]	0.71±0.03[d]	2.8±0.04[d]	3.0±0.06[d]
Sig.	*	*	*	*	*	*

N = 4, *: Significant (P < 0.05)

[abc] Means by diverse superscripts at the similar column vary considerably by Least Significant Difference (LSD) test at P < 0.05

Table 3. The consequence of frozen storages on some bacterial pathogens of Nile tilapia (*Oreochromis niloticus*) at different periods.

Detected *E. coli* spp. and *salmonella* spp. (log CFU/ml)		
Storage period (week)	***E. coli***	***Salmonella***
0	5.16±0.10[a]	4.87±0.35[a]
1	4.90±0.26[ab]	4.47±0.46[a]
2	4.61±0.31[b]	4.34±0.36[b]
3	Not detected	Not detected
Overall	**3.67±2.23**	**3.42±2.09**
Significant	*	*

*: Significant (P < 0.05)

[abc] Means with diverse superscripts at the similar column vary considerably by Least Significant Difference (LSD) test at P < 0.05

The results of the study showed a high considerable effect, (p≤ 0.05) of freezing on the microbial load, as microbes decrease with the increase of the freezing period, as mentioned by (Liston, 1980). Generally, freezing inhibits the activity of many types of bacteria, but in some cases the number of them continues to increase during the freeing storage period. This result is in agreement with (Tzikas, 2007) who reported that the microbiological load in fish muscle is related to seasonal variation, harvesting conditions and storage. Also this result is in agreement with the findings of (Roopma *et al*, 2012) who reported that the Freezing of fish creates unfavourable environmental conditions which slow down the bacterial growth and biochemical decomposition of fish muscle, thereby increasing the shelf life of meat fish. Also the results of present study showed that the microorganisms (*E. coli* and *Salmonella*) a total inhabit in the third week of the experiment (0, 7, 15 and 21day) where the *E. coli* 5.16 ± 0.10, 4.90 ± 0.26, 4.61 ± 0.31and 0±0, and *Salmonella* 4.87 ± 0.35, 4.47 ± 0.46, 4.34 ± 0.36, and 0±0 respectively. Several studies reported that L. monocytogenes can grow on different refrigerated seafood products during refrigerated storage. This result is not agreement with (Harrison, 1991; Altuntas *et al*, 2012).

CONCLUSIONS

Freezing is one of the means of preserving food and fish products. The study showed that freezing affected the nutritional value of the meat of Nile tilapia (*Oreochromis niloticus*) throughout the freezing period, when the moisture decreased from 73% to 69%, protein from 31% to 20%) and fat from 7.2% to 5.2%. There was a high containing of ash, dry matter and protein-free nitrogen also Minerals calcium, phosphorous, magnesium, iron, sodium and potassium were also affected by a decrease in their content. The results of the study showed a clear consequence of freezing on the microbial load of *Oreochromis niloticus*, where the total count of *E. coli* and *Salmonella* decreased from 5.16 to zero and 4.87 to zero, respectively.

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