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Effect of Chilled-frozen Storage on the Physico-chemical and Microbiological Quality of Silver Catfish (*Chrysichthys Nigrodigitatus*) from Asejire Lake, Ibadan, Nigeria

OGUNKOYA, TEMITOPE FELICIA, KAREEM, OLADEJI KAZEEM AND TAIWO, OYEBOLA OLUWAFUNKE
Corresponding email: ogunkoyafelicia@gmail.com and *kaykaz2007@yahoo.co.uk*
Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria

Abstract

Fishes are highly perishable and are often discarded or sold at low prices over time due to deterioration. Freezing is a widely accepted preservation technique but fishers often lack freezing equipment on board and at the landing sites. Chilling or icing of fish immediately after the catch has been reported to reduce microbial and enzymatic spoilage before freezing or processing. This study, therefore, investigated microbial and physicochemical changes of frozen and prior chilled *Chrysichthys nigrodigitatus* obtained from Asejire reservoir, Nigeria. A total of 60 freshly caught samples of *Chrysichthys nigrodigitatus* (75-230g) were collected from Asejire dam. The fish were gutted and divided into two equal numbers. The first 30 pieces were frozen immediately after gutting (FRZ) while the remaining were chilled in ice for 24 hours before freezing (CBF). Samples of FRZ and CBF were subjected to sensory, proximate and microbial analysis following standard procedures. Crude protein reduced from 15.43±0.35 in FRZ while it increased from 17.13±0.31 to 17.70±0.25 after 24hrs and 12 weeks, respectively. This could be attributed to the higher moisture content in CBF. The initial and final microbial count increased in both FRZ and CBF $3.777x10^5$±0.35 - $7.5x10^6$±0.30 CFU/g and 17.13±0.31% - 17.70±0.25% respectively. FRZ is fit for consumption and had longer shelf life however CBF had higher protein and fat content at the end of the experiment.

Keywords: Microbial, spoilage, consumption.

Introduction

Fish is a major source of food, income, employment and reaction for both man and livestock all over the world. (Ozigbo *et al.*, 2014). It is one of the major sources of animal protein foods available in Nigeria. The freshwater *Chrysichthys nigrodigitatus* (Silver catfish), constitute one of Nigeria's most dominant fish species in inland waters (Kareem *et al*., 2015). This carnivorous species commonly called Òbòkún in Yoruba language plays a major role in Nigeria's ecology and fisheries, and West Africa at large.

All over the world, people are becoming more aware of food quality and its implication on their health and the image of fishery products fits quite well in the health food trend as it is easy to digest, low in calories and high in protein sources. However, the quality of fish species determines the nutrient values and nutrient content of individual fish species.

One of the major challenges faced globally is how to improve food security to meet up with the world rising operations and ensure

sustainability, studies have shown that food security can be improved by reducing post-harvest losses in different distribution stages involved (FAO, 2010). Post-harvest fish loss refers to fish that is either discarded or sold at a relatively low price because of quality deterioration. Since fish is perishable and prone to spoilage over time, it therefore, requires preservation to extend the shelf life and flesh quality. Studies have indicated that spoilage of fish is a result of high temperature which increases microbial activities in the body of the fish at various stages of distribution (Adelaja *et al.,* 2017). Fish undergoing spoilage has one or more of the following signs; slime formation; discoloration; change in texture; off-odour; off-flavour and gas production (Rawat, 2015). The spoilage of fresh fish after being caught is very rapid and the spoilage process starts with rigor mortis.

It has been widely reported that chilling or icing fish immediately after catching reduces its spoilage (Singh 2011). Inland fish farmers lack freezing equipment on board and at the landing sites so there is a need to store fish in ice to preserve its freshness before freezing or processing. Freezing is a widely accepted preservation technique as chilling does not prevent spoilage but it reduces microbial and enzymatic spoilage (Tawari and Abowei 2011).

The objective of this study is to know the effect of chilled-frozen storage on the quality of *C. nigrodigitatus* by analyzing the proximate composition of chilled and frozen treatment and frozen immediately treatment *C. nigrodigitatus* at the initial and final stage of the experiment, to investigate the physicochemical changes and also evaluate the microbiological quality changes of chilled and frozen treatment and frozen immediately treatment *C. nigrodigitatus*

Materials and Methods

A total of 60 live fish samples of *Chrysichthys nigrodigitatus* were collected from artisanal fishers and middlemen at their landing sites from Asejire Lake in the month of April. These fish samples had a total length ranging from 20cm to 70cm and a total weight ranging from 20g to 150g. The fish was identified using Keys by Olaoseikan and Raji (2013). These specimens were collected in the early hours (0700 and 0900 hrs) and transported to the laboratory.

At the laboratory, fish samples were gutted and washed thoroughly under running tap water before storage. Sample preparation for storage was done within the post-rigor stages to obtain good-quality fish. Thereafter, they were divided into two storage groups representing different treatments:

Treatment FRZ: - Fish are frozen immediately after gutting

Treatment CHI: - Fish chilled in an ice pack for 24 hours immediately after gutting before freezing.

Each treatment consists of 30 fish samples.

Randomly selected samples (3 pieces) from each treatment were analyzed 24 hours after the commencement of the experiment. Thereafter, both treatments were analyzed for microbiological and physicochemical changes at week 4, week 8 and week 12 of the experiment. For each treatment, the microbiological and organoleptic tests had three replicates. The quality and shelf life of Silver catfish were evaluated.

Chemical Analysis: The peroxide value (PV) was determined according to the Chapman and McKay (1949) method. Total volatile Base (TVB) was carried out according to (Malle and Poumeyrol, 1989). Thiobarbituric acid (TBA) was determined by the method of Monez (2001). For proximate analyses, the muscle tissue was analyzed for its proximate compositions (moisture content, crude protein, crude fat, ash and dry matter) under a sterile condition in the laboratory according to AOAC, (2005). Three replicate samples each from both treatments were subjected to initial (After 24 hours) proximate analysis after the resolution of rigor mortis in each treatment using the procedures of the Association of Official Analytical Chemists (AOAC, 2000). This was also repeated in the 12^{th} week of storage.

Microbiological Analysis: Total plate count (TPC) was carried out according to Gunasekaran (1995).

Physical Analysis: The sensory properties of the chilled-frozen stored *C. nigrodigitatus* were evaluated by an eight-man pre-trained panel drawing from Postgraduate students of the Department of Aquaculture and Fisheries Management, University of Ibadan. Assessment of colour, texture, freshness and taste was based on both cooked (by boiling) samples and uncooked (fresh samples from each treatment that was not boiled) fish samples. Questionnaires for the panelists were prepared using the modified 5- point hedonic scale described by Eyo (2001) as follows:

1-1.9, unacceptable; 2.0-2.9, fair; 3.0-3.9, medium; 4.0-4.9, good; 5.0-6.0, very good.

Statistical Analysis: All collected data were stored in Microsoft Excel and Word document format. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 20.0 (IBM Corp., Armonk, New York). One-way analysis of variance (ANOVA) and student's t-test was performed on the means of the values. The significant level was set at 95% ($P < 0.05$).

Results and Discussions

Table 1: Proximate composition (%) changes in chilled-frozen *Chrysichthys nigrodigitatus* muscle tissue

Composition Sampling day	Treatments FRZ	 CBF	Reference limit (USDA, 2010)
Moisture Content			
After 24hrs	$63.56\pm0.88^{a}_{A}$ (62.55 – 64.13)	$65.17\pm2.42^{a}_{A}$ (62.64 – 67.47)	78.00 – 90.00
Week 12	$73.50\pm1.32^{a}_{B}$ (72.50 – 75.00)	$70.42\pm1.42^{a}_{B}$ (69.25 – 72.00)	
Crude protein			
After 24hrs	$15.43\pm0.40^{a}_{A}$ (15.00 – 15.80)	$17.13\pm0.31^{b}_{A}$ (16.80 – 17.40)	15.00 – 28.00
Week 12	$15.33\pm0.35^{a}_{A}$ (14.94 – 15.62)	$17.70\pm0.25^{b}_{A}$ (17.25 – 17.98)	
Crude Fat			
After 24hrs	$8.24\pm0.49^{a}_{B}$ (7.91 – 8.80)	$9.44\pm0.30^{b}_{B}$ (9.12 – 9.70)	15.00 – 18.00
Week 12	$2.04\pm0.10^{a}_{A}$ (1.96 – 2.15)	$5.60\pm0.13^{b}_{A}$ (5.48 – 5.73)	
Ash			
After 24hrs	$2.23\pm0.25^{a}_{A}$ (2.00 – 2.50)	$3.33\pm0.15^{b}_{B}$ (3.20 – 3.50)	-
Week 12	$2.18\pm0.36^{a}_{A}$ (1.83 – 2.50)	$2.15\pm0.22^{a}_{A}$ (2.00 – 2.41)	
Dry Matter			
After 24hrs	$35.51\pm0.36^{b}_{B}$ (35.15 – 35.87)	$29.44\pm0.28^{a}_{A}$ (29.12 – 29.66)	-
Week 12	$26.25\pm0.28^{a}_{A}$ (26.00 – 26.55)	$29.08\pm0.88^{b}_{A}$ (28.25 – 30.00)	

All values are the means ± SD of three replicates. Means across the same row differently superscripted differ significantly ($\alpha_{0.05}$). Values followed by different subscript

capital letters in the same column are significantly different at α=0.05

The table shows there is a significant difference between FRZ and CBF treatments of crude protein, crude fat, ash and dry matter across the treatments.

The variations in the moisture content, crude protein and crude fat of treatments FRZ and CBF after 24 hours and Week 12 are in agreement with the work of Clucas, (1990) which gives the range of the moisture content of a healthy fish as 60-84%, the range of crude protein as 15-24% and the range of crude fat as 0.1% - 22.0%. The increase in the moisture content on day1 and week 12 is also in agreement with the range of 60 -80% given by Daniel (2015) which states that it could be due to the stable water levels in the location where the fish were collected. The percentage of water is also a good indicator of its relative content of energy, protein and lipid (Olagunju *et al.*, 2012). Moisture content in all the species agreed with the observation of Udo (2012), Olagunju *et al.*, (2012) and Mazumder *et al.*, (2008) in several freshwater fish species.

According to (Udo, 2012) *C. nigrodigitatus* can be classified as a high-fat fish which indicates that *C. nigrodigitatus* are better source of lipids in the body when consumed.

Ash is a measure of the mineral content of food items. The range of ash in this study suggests that these species of fish is a good source of minerals such as calcium, potassium, zinc, iron and magnesium. Carbohydrates are sources of instant energy, which can be used in the body's development and growth (Olagunju *et al.*, 2012). Fish generally have very low levels of carbohydrates because glycogen does not contribute much to the reserves in the fish's body tissue (USDA 2010).

Table 2: The changes of PV values, TVB-N and TBA contents of Silver catfish (*Chrysichthys nigrodigitatus*) fish muscle tissue during chilled and frozen storage conditions for 12 weeks

Sampling period	Chemical markers PV (mEq/Kg)		TVB-N (mg/100g)		TBA (mg MA/100g)	
	FRZ	CBF	FRZ	CBF	FRZ	CBF
After 24 hours (Initial)	0.43±0.03[a]	0.44±0.03[a]	4.66±0.14[a]	4.66±0.13[a]	0.16±0.00[a]	0.16±0.00[a]
Week 4	0.42±0.02[a]	0.38±0.02[a]	4.53±0.21[a]	3.87±0.25[a]	0.21±0.00[a]	0.19±0.00[a]
Week 8	0.74±0.03[a]	0.64±0.04[a]	5.93±0.15[a]	6.17±0.15[a]	0.28±0.00[a]	0.27±0.00[a]
Week 12	1.25±0.05[a]	1.55±0.03[a]	7.33±0.15[a]	7.77±0.15[a]	0.47±0.02[a]	0.51±0.01[a]

PV peroxide value; *TVB-N* total volatile bases; *TBA* thiobarbituric acid value; *FRZ* frozen; *CBF* chilled before frozen. All values are the means ± SD of three replicates. Means across the same row differently superscripted differ significantly ($\alpha_{0.05}$).

TBA

From the result shown in Table 3 above, the increment in the value TBA of both treatments is directly proportional to the increase in the duration of storage. From the result, it is observed that After 24hrs of FRZ and CBF has the same mean value of (0.16±0.00[a]) but started increasing as the number of days increased. It shows that Week 12 has the highest mean value from the analysis done and the result also shows that CBF has a higher mean value than FRZ (0.51±0.01[a] and 0.47±0.02[a]). Connell (1995) indicated that rancidity appears in fish when TBA becomes greater than 1-2mg malonaldehyde/kg which contradicts the result of this study. However, the result of this study may be due to species differences depending on lipid content as stated by Al-Shatty (2006). Gamal El-Deen and Shamery (2010) recorded TBA mean value ranging from 0.205-1.21 mgN/100gm on *Lethrinus elongates*, the study affirmed that TBA increases as the storage time increases which agrees with the result of this study. It should be kept in mind that TBA value is a widely used as fish quality indicator and for

oxidative rancidity (Pereira– deAbreu *et al.,* 2010).

TVB

From the result shown in Table 3 above, Week 12 has the highest value in which CBF mean value is greater than FRZ (7.77 ± 0.15^{a} and 7.33 ± 0.15^{a}) respectively. During frozen storage, many reactions occurred between different meat components, for example, fish and poultry meat are susceptible to oxidative reactions due to their high concentrations of oxidation catalysts such as myoglobin and iron (Ashgar *et al.,* 1998). This explains what we found as there is a significant increase in TVN in frozen fish. And this agrees with Hassan *et al.,* (2012) who reported a continuous increase in the level of total volatile nitrogen within the time of storage among fish species.

Scherer *et al.,* (2006) noted that TVBN levels are more suitable for spoilage assessment in marine than in freshwater fish No statutory TVBN limit values have been laid down for freshwater fish. Hassan *et al.,* (2012) reported a continuous increase in the level of total volatile nitrogen within the time of storage among fish species. The result of this study also disagrees with the report by Nayeem *et al.,* (2010) and Desniar *et al.,* (2009) which state the range of 1.12 – 3.12 mg/100g gotten from their study which is still an acceptable product.

Perioxide Value

From the result shown in Table 3 above, the increase of PV in frozen fish in contrast with fresh fish showed the development of rancidity during frozen storage. Initial PV indicates oxidation already occurring during the course of handling and processing. The week 4 has the least mean peroxide value for both treatments (FRZ and CBF) with a significant different Moral-Rama, (1987) Peroxide value is widely employed for determining the formation of hydro-peroxides, which are the primary products of oxidative reactions, the increase of PV in frozen fish in contrast with fresh fish showed the development of rancidity during frozen storage. Lakshmanan (2000) state that if the peroxide value of fish is above 20meq/kg the fish would probably smell and taste rancid although the result of this study gave a lower value to what was stated though it was noted that at the end of week 8 there is already an outset of rancidity in the fish of CBF treatment and by week 12 it was already unacceptable for consumption according to Organoleptic result gotten; the increase could be related to the lipid oxidative deterioration.

Table 3: Change in the microbial count of the fish muscle of *Chrysichthys nigrodigitatus* during chilled and frozen storage conditions for 12 weeks

Sampling day	TVC (CFUs/ml)	
	FRZ	CBF
After 24hrs	$3.77 \times 10^{5}\pm0.35^{a}$	$5.33 \times 10^{4}\pm0.21^{b}$
Week 4	$2.70 \times 10^{4}\pm0.10^{a}$	$7.40 \times 10^{3}\pm0.36^{b}$
Week 8	$7.58 \times 10^{4}\pm0.30^{b}$	$1.30 \times 10^{4}\pm0.20^{a}$
Week 12	$7.50 \times 10^{6}\pm0.30^{a}$	$8.27 \times 10^{6}\pm0.15^{a}$

TVC total viable count; TCC total coliform count; *CBF* chilled before frozen; *TFC* total fungal count; *FRZ* frozen; *CBF* chilled before frozen. All values are the means ± SD of three replicates. Means across the same row differently superscripted differ significantly ($\alpha_{0.05}$).

There TVC the FRZ mean value was higher than CBF on after 24hrs, week 4 and week 8 but

CBF mean value was higher than FRZ on week 12

The total plate count (TVC) of the fish in both treatments (FRZ treatment and CBF treatment). After 24 hours and Week 4 falls within the range of ICMSF (1986) standard which gives the standard bacterial count in fish flesh to be less than 5 x 10^5 (5.7 log10 CFU/g). Week 8 of FRZ and CBF treatment falls in the normal range of fish as stated by Liston (1980). CBF mean value at week 12 doesn't fall within the range of Jeyasekaran *et al.,* (2006) which states that chilled fish shouldn't be more than 10^6 cfu\g for chilled fish. According to the organoleptic done in this study the fish of CBF treatment is considered at a critical point and very unacceptable at 8.27 x 10^6cfu\g compared to (Olafsdottir *et al.,* 1997) who reported that the total viable count of fish products is 10^7 – 10^8 cfu\g at the point of sensory rejection.

Table 4 Sensory evaluation test of experimental fish at different times of storage

FROZEN				
	AFTER 24HRS	WEEK4	WEEK8	WEEK12
TASTE	1.25 ± 0.46^{c}	1.50 ± 0.53^{bc}	1.88 ± 0.35^{ab}	2.12 ± 0.64^{a}
ODOUR	1.75 ± 0.46^{b}	1.75 ± 0.46^{b}	2.12 ± 0.64^{ab}	2.37 ± 0.51^{a}
TEXTURE	1.62 ± 0.51^{b}	1.75 ± 0.46^{b}	2.37 ± 0.51^{a}	2.37 ± 0.51^{a}
APPEARANCE	1.62 ± 0.51^{a}	1.75 ± 0.46^{a}	1.87 ± 0.64^{a}	2.12 ± 0.83^{a}
CHILL BEFRORE FROZEN				
TASTE	1.50 ± 0.53^{c}	1.62 ± 0.51^{c}	3.62 ± 0.51^{b}	4.87 ± 0.35^{a}
ODOUR	1.75 ± 0.46^{d}	2.25 ± 0.46^{c}	3.62 ± 0.51^{b}	4.75 ± 0.46^{a}
TEXTURE	1.50 ± 0.53^{c}	1.62 ± 0.51^{c}	2.50 ± 0.53^{b}	3.87 ± 0.35^{a}
APPEARANCE	1.62 ± 0.51^{c}	1.62 ± 0.51^{c}	3.12 ± 0.64^{b}	4.25 ± 0.46^{a}

All values are the means ± SD of eight replicates. Means across the same row differently superscripted differ significantly ($\alpha_{0.05}$). Values followed by different subscript capital letters in the same column are significantly different at α=0.05

Table 4, shows the result of the sensory evaluation carried out by 8 trained panelists and there were slight changes in the taste, odour, taste, texture and appearance in the FRZ treatment as the sampling time increases, while the CBF treatment has a higher change in the sensory evaluation as the storage days increases, which shows that the fish quality is best at after 24rs in both CBF and FRZ at week 4 while at week12 the sensory evaluation of CBF treatment shows that it is unfit for consumption. This agrees with Aranilewa (2005) that states that deterioration increases as the duration of the storage increases.

Conclusion

At the end of this study, it was established that storage procedures affect the shelf life of frozen fish and it was also established that FRZ fish had the best quality and longer shelf life

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