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Chemical and Microbial Quality Assessments of Some Economic Important Artisanal Fresh Water Fish Species (*Tilapia zilli, Clarias gariepinus, Chrysichthys nigrodigitatus* and *S. melanotheron*) in Selected Coastal Markets of Lagos State, Nigeria

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Abstract

Nutritional qualities of some selected fresh water fish species sold for consumption in the Badagry, Epe, Ikorodu and Ojo areas of Lagos state, Nigeria was assessed to ascertain the wholesomeness and health risk associated with consuming the most common sort after fish in these locations. A total of 100 fish samples, Red belly Tilapia *(T. zilli*), African Mud Catfish *(C. gariepinus*), Silver Catfish (*C. nigrodigitatus*) and Black Chin Tilapia (*S. melanotheron*) (25 specimens each) with a weight ranges between 50-180 g, were collected from different locations. The samples were iced and taken to the Biochemistry and Pharmaceutical laboratory sections of the College of Medicine University of Lagos, Idi-Araba, Lagos, Nigeria. The iced samples were maintained in alternate layers of ice until spoilage was noticed. Chemical analysis was conducted using Spectrophotometer, Atomic Absorption Spectrophotometer, Sohxlet Extraction Apparatus, Thiobarbituric Acid (TBA), The Acid Value (AV), Peroxide Value (PV), Iodine value, Free Fatty Acids (FFA) and Saponification value were determined. The result indicates higher TBA (8.79 ± 0.05), (7.59 ± 0.06), (7.46 ± 0.04) and (6.99 ± 0.05) for *T. zilli, C. nigrodigitatus, S. melanotheron and C. gariepinus* respectively in Ojo Lagoon, higher iodine value (9.69 ± 0.01) for *T. zilli* in Ojo Lagoon; (8.75 ± 0.21), (9.35 ± 0.07) and (8.45 ± 0.07) for *C. nigrodigitatus, S. melanotheron* and *C. gariepinus* respectively in Epe Lagoon and the acid values (3.31 ± 0.04), (2.45 ± 0.04) and (3.79 ± 0.04) for *T. zilli, C. nigrodigitatus* and *S. melanotheron* respectively in Badagry Lagoon and (2.25 ± 0.04) in Ojo Lagoon were within the standard limits. Higher mean mould load recorded were (1.22×104), (1.78×103), (1.90×103) for *C. gariepinus, S. melanotheron* and *T. zilli* respectively in Ikorodu Lagoon, while (3.47×103) was recorded for *C. nigrodigitatus* in Badagry Lagoon. The results indicated a notable quality improvement leading to a high quality fish in terms of the taste, colour, odour, shelf life and market value. The high iodine value recorded in this study from different locations suggests that the fish species contain high level of unsaturated oil and as such could reduce the risk of heart diseases, high cholesterol, depression, anxiety, low immunity, cancer, eye disorders and ulcers in humans when consumed. None of the fish samples exceeded the mould load limit for fish thereby exhibiting no risk on public health; therefore the fish are wholesome and safe for human consumption.

Keywords: Chemical analysis; Freshwater fish; Fish quality; Nutritional qualities

Introduction

Fish which contributes 36.6 g per day of net protein utilization in Nigerian homes fall shorts the recommended World Health Organization (WHO) daily requirement of 46 g/day to 52 g/day [1]. The decrease is not due to non-availability of the resource but to non-maximization and sustainability of available water resources. Fish is a major source of food for humans, it provide a significant proportion of the dietary protein intake of a large number of people, most importantly in the developing countries, where it contributes about 14% of all animal protein on global basis [2-4]. More than 50% of the animal protein intake is derived from fish in most Asian countries [4] and it also accounted for between 30% to 80% of the total animal protein consumption of the coastal residents of West Africa [5]. Eyo et al. [3] noted that, fish has an edge over meat in Nigeria because of its relative abundance and low cost. Eyo et al. [3,4] also opined that fish constitutes about 40% of the animal protein intake.

As reported by USDA, Fish is rich in lysine, an amino acid suitable for replacing high carbohydrate diet as well as providing high quality protein in addition to a variety of vitamins and minerals, which include vitamins A and D, phosphorus, magnesium, selenium, and iodine. Its protein is easily digestible and favourably complements dietary protein provided by cereals and legumes that are typically consumed in many developing countries. Fish is also rich in unsaturated fat while the total fat content is relatively low (about 5%). It is high in polyunsaturated fatty acids that are important in lowering blood cholesterol level [2,3,6]. Parelman [7] noted that fish is rich in long chain omega-3 Polyunsaturated Fatty Acids (PUFA), which includes Alpha-Linolenic Acid (ALA, C l8:3) and its longer-chain metabolites: Eicosapentaenoic Acid (EPA, C 20:5) and Docosahexaenoic Acid (DHA, C 22:6). Beneficial health effects of omega-3 PUFA, especially long chain EPA and DHA are well demonstrated, mainly in the prevention

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of cardiovascular diseases. Artisanal fisheries, industrial trawlers, aquaculture and imported frozen fish are the four major sources of fish supply in Nigeria. Production from aquaculture is increasing compared to artisanal sources and supplied between 5%-22% of total domestic fish production between 2000-2007 [8]. According to National Bureau of Statistics, Nigeria's fish production reflected that 5,788,474 tonnes of fish had been produced between 2010 and 2015. Year 2014 recorded the highest tonnes of fish produced with 1,123,011 tonnes; the second highest tonnes of fish produced were recorded in 2015 while the least were recorded in 2010.

Nonetheless, the increase in fish production does not commensurate with the rate of consumption because demand for fish is on the rise as a result of population explosion while the supply is gradually on decline in the country in recent time [9,10]. Aside from the aforementioned, increase in fish production and consumption is being hampered as a result of time between catch, landing and consumption that allows rigor motis to set in. Other factors are inadequate storage facilities, fluctuating electricity supply and inadequate processing equipment's. The study therefore investigated chemical qualities and microbial loads of harvested fish from artisanal fishermen and sold for consumption in four different locations in Lagos State, Nigeria to ascertain the fish wholesomeness and health risk associated with consuming these highly sort after fish in Lagos State.

Materials and Methods

Samples collection

Fish samples were collected from four different locations in Lagos State. These locations are Epe, Badagry, Ojo and Ikorodu coastal markets (Figure 1). The samples were then taken to the laboratory for proper identification and analysis. The samples were identified as *T. zilli, C. gariepinus, C. nigrodigitatus* and *S. melanotheron*. The weight of the collected samples ranged between 50 to 180 g, and these were divided into four batches (Table 1). The batches were iced immediately in crushed ice block and cubed ice, respectively in insulated boxes before taking to the laboratory, at the ratio of 1:1 ice to fish. Water from the melted ice was drained from a tap at the base of the insulated box. The iced samples were maintained in alternate layers of ice until spoilage was noticed. Chemical and microbial analyses were carried out to determine the overall quality of the samples. All the analyses were carried out at Biochemistry Laboratory and Pharmaceutics and Pharmaceutical Technology of College of Medicine University of Lagos, Idi-Araba, Lagos.

Determination of thiobarbituric acid (TBA) value

Spectrophotometric method was used in the determination of the TBA values with keeping time in which 10 g of the minced fish was macerated with 50 mL of distilled water at an interval of 2 minutes, then washed and transferred to a distillation flask with 47.5 mL of water [11]. 2.5 mL of 4N HCl (Antifoam liquid) and 5 glass beads were added. The mixture was distilled at the rate of 5 mL/min, the distillate (5 mL) was taken in a glass stoppered tube and 5 mL of TBA reagent added and heated in boiling water for 35 min. The sample was allowed to cool and absorbance measured against blank at 532 nm. Each absorbance was used to calculate the TBA value [11].

Determination of acid value or free fatty acids (FFA)

25 mL of diethyl either was mixed with 25 mL ethanol and 1 mL of phenolphthalein solution (1%) and neutralized with 0.1M potassium hydroxide. 1 to 10 g of the oil or melted fat extracted from the fish was dissolved in the mixed neutral solvent and titrated with aqueous 0.1M potassium hydroxide shaking constantly until a pink colour which persisted for 15 second was obtained [12].

$$Acid\quad value\quad (mgKOH/g)=\frac{Titre\quad value\quad (ml)\times\quad 5.61}{Weight\quad of\quad sample\quad used}$$

The FFA value is usually calculated as oleic acid, where 1 mL of 0.1M potassium hydroxide=0.0282 g of oleic acid in which case the acid value=2×FFA.

Determination of peroxide value

A 100 mL of round bottomed flask with a ground glass join is attached to a plain reflux tube, long 9 mm internal diameter the upper 15 cm of which are cooled by a water jacket. 10 mL of chloroform and 10 mL of glacial acetic acid was added to the flask and, using a micro gas flame close to the flask, the mixture was boiled to top of the tube where it was condensed by the water jacket. One gram of potassium iodide dissolved in 1.3 mL was poured slowly down the condenser when the mixture was boiling steadily so that the refluxing was not interrupted 0.3 mL water was added to re-dissolve any precipitated iodide. 1 g of the sample was added down the condenser without interrupting the refluxing and condenser water was turned off so that the entire sample was washed into the flask. The mixture was boiled for more 4 min; the flask was removed, and cooled rapidly. 50 mL water was added and the liberated iodine titrated against 0.01M sodium thiosulphate using starch as an indicator [12].

$$Peroxide\quad value=\frac{a-b\times 10}{W}$$

Where a=Titre of sample; b=Titre of blank; W=Weight of sample used

Determination of saponification value

The Saponification Value (SV) of the fish oil was determined following procedures described in AOAC method [12]. Oil sample (1 g) was dissolved in 12.5 mL of 0.5N ethanolic potassium hydroxide. The mixture was refluxed for 30 min until oil droplets disappear and was left to cool to room temperature. Phenolphthalein indicator was then added and the hot soap solution was titrated with 0.5N Hcl until the pink colour disappears. A blank titration was also carried out in the same manner except that no oil was added. Saponification value was calculated using the formula:

$$Saponification\quad value=\frac{(a-b)\times N\times 5.61}{w}$$

Where: a=Volume (mL) of 0.5 mol/L hydrochloric acid consumed in the blank test; b=Volume (mL) of 0.5 mol/L hydrochloric acid consumed in the test; N=Normality of hydrochloric acid, W=Weight of oil sample (g).

Microbial determination

Media preparation: The SDA was prepared according to the manufacturer's direction that is 65 g per litre of distilled water and was sterilized at 121°C for 15 min in the autoclave which was later cooled in the water bath at 45°C [13].

Sample preparation: The fish was blended 10 g of each sample was

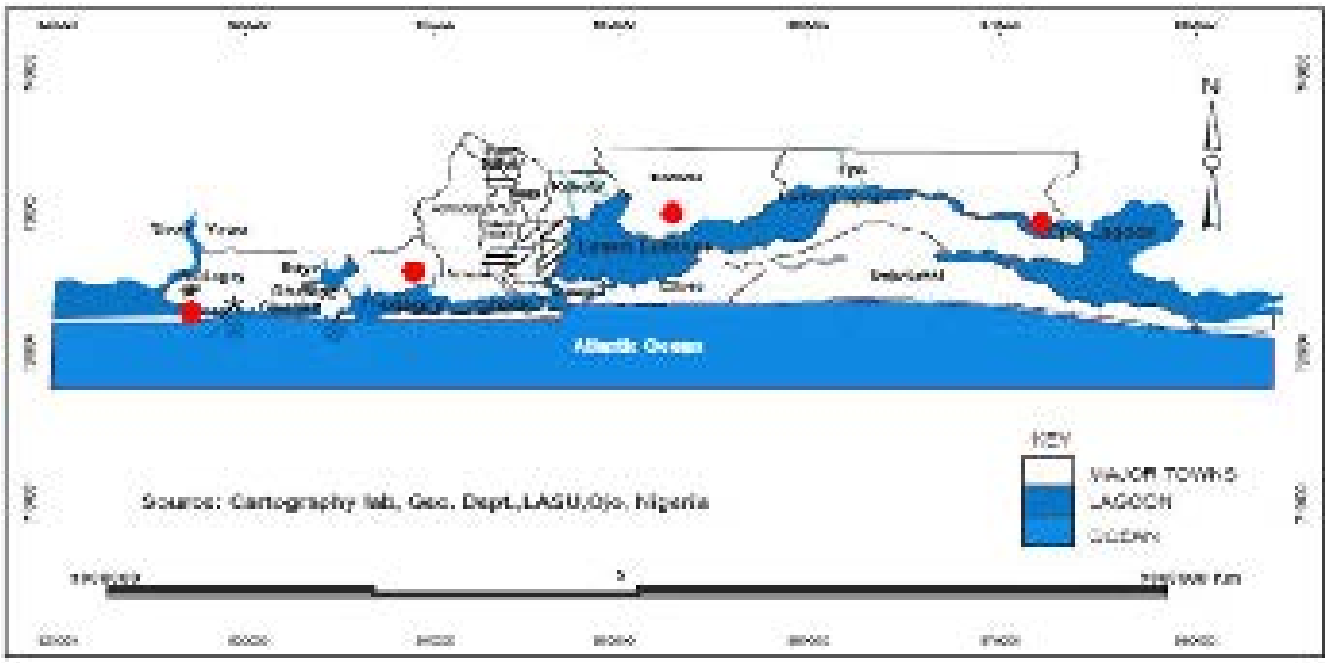

Figure 1: Locations of markets where fish species were collected for the research.

Nutritional qualities	Fish species	Location			
		Epe	Ojo	Badagry	Ikorodu
Thiobarbituric Acid (mg/kg)	*T. zilli*	5.96 ± 0.04^{a}	8.79 ± 0.05^{b}	6.73 ± 0.06^{c}	7.10 ± 0.04^{d}
	C. nigrodigitatus	5.14 ± 0.02^{a}	7.59 ± 0.06^{b}	4.31 ± 0.04^{c}	6.31 ± 0.07^{d}
	S. melanotheron	6.07 ± 0.01^{a}	7.46 ± 0.04^{b}	6.40 ± 0.06^{c}	7.05 ± 0.07^{d}
	C. gariepinus	4.13 ± 0.06^{a}	6.99 ± 0.05^{b}	3.79 ± 0.06^{a}	5.38 ± 0.04^{c}
Acid value (mg/kg)	*T. zilli*	2.04 ± 0.06^{a}	3.30 ± 0.08^{b}	3.31 ± 0.04^{b}	2.99 ± 0.04^{b}
	C. nigrodigitatus	2.29 ± 0.07^{a}	2.02 ± 0.08^{a}	2.45 ± 0.04^{a}	1.97 ± 0.04^{a}
	S. melanotheron	2.34 ± 0.03^{a}	3.11 ± 0.04b	3.79 ± 0.04^{c}	2.68 ± 0.06^{a}
	C. gariepinus	2.21 ± 0.04^{a}	2.25 ± 0.04^{a}	1.95 ± 0.04^{a}	2.06 ± 0.06^{a}
Peroxide value (mEq/1000 g)	*T. zilli*	8.20 ± 0.14^{a}	9.69 ± 0.01^{b}	8.81 ± 0.02^{c}	9.75 ± 0.01^{b}
	C. nigrodigitatus	8.75 ± 0.21^{a}	8.04 ± 0.02^{b}	8.46 ± 0.01^{c}	8.37 ± 0.02^{c}
	S. melanotheron	9.35 ± 0.07^{a}	9.05 ± 0.02^{b}	9.28 ± 0.01^{a}	8.88 ± 0.02^{b}
	C. gariepinus	8.45 ± 0.07^{a}	8.31 ± 0.02^{b}	7.15 ± 0.01^{c}	8.17 ± 0.02^{d}
Iodine value (gI2/100 g)	*T. zilli*	94.92 ± 0.08^{a}	96.19 ± 0.01^{b}	93.48 ± 0.05^{a}	93.10 ± 0.06^{a}
	C. nigrodigitatus	95.67 ± 0.48^{a}	84.77 ± 0.04^{b}	80.88 ± 0.05^{c}	84.40 ± 0.06^{b}
	S. melanotheron	98.05 ± 0.04^{a}	97.40 ± 0.13^{a}	95.77 ± 0.08^{b}	95.23 ± 0.06^{b}
	C. gariepinus	97.22 ± 0.08^{a}	80.13 ± 0.05^{b}	82.63 ± 0.08^{c}	82.49 ± 0.04^{c}
Free fatty acid (%)	*T. zilli*	1.02 ± 0.03^{a}	1.65 ± 0.04^{b}	1.66 ± 0.02^{b}	1.50 ± 0.02^{c}
	C. nigrodigitatus	1.15 ± 0.04^{a}	1.01 ± 0.04^{b}	1.23 ± 0.02^{a}	0.99 ± 0.02^{b}
	S. melanotheron	1.17 ± 0.01^{a}	1.56 ± 0.02^{b}	1.90 ± 0.02^{c}	1.34 ± 0.03^{d}
	C. gariepinus	1.11 ± 0.02^{a}	1.13 ± 0.02^{a}	0.98 ± 0.02^{ab}	1.03 ± 0.03^{a}
Saponification value (mgKOH/g)	*T. zilli*	251.50 ± 3.54^{a}	230.50 ± 3.54^{b}	245.00 ± 4.24^{c}	229.50 ± 2.12^{b}
	C. nigrodigitatus	212.0 ± 14.14^{a}	204.50 ± 2.12^{ab}	213.50 ± 2.12^{a}	219.00 ± 2.83^{a}
	S. melanotheron	218.50 ± 2.12^{a}	244.00 ± 4.24^{b}	210.50 ± 3.54^{c}	254.50 ± 2.12^{d}
	C. gariepinus	200.50 ± 4.95^{a}	194.50 ± 3.54^{a}	177.00 ± 2.83^{b}	198.00 ± 5.66^{a}

Table 1: The results of the chemical analysis of the four fish samples from different locations in Lagos State. Figures in the same horizontal row having the same superscript are not significantly different (p>0.05).

Fish species	Badagry	Ikorodu	Ojo	Epe
C. gariepinus	1.00×101	1.22×104	4.01×103	3.47×103
Chrysichthys nigrodigitatus	3.47×103	8.25×102	3.60×102	4.01×102
Sarotherodon Melanotheron	1.02×103	1.78×103	9.30×102	9.30×102
T. zilli	9.30×102	1.90×103	1.21×103	9.30×102

Table 2: The mean mould load of four selected fish in four different locations.

weighed on analytical balance. It was transferred into 90 mL sterile distilled water, to make 10^{-1} dilution. After adequate shaking, 10 mL was transferred into another 90 mL sterile water to make 10^{-2} dilution. All sterile water has been previously sterilized at 120°C for 15 min in the autoclave [13].

Sample dispensing: 1 mL of 10^{-1} and 10^{-2} dilutions of each sample after thorough shaking was measured and dispensed separately into pre-labelled sterile Petri dish using 1 mL pipette previously sterilized at 180°C for 1 hour using hot air oven and cooled. 20 mL of dissolved Sabouraud Dextrose Agar (SDA) was poured into the Petri dish containing 15 g of each sample. They were gently mixed and allowed to set. The sample preparation and dispensing were done in the ultraviolet safety cabinet. The ultraviolet safety and all materials used were swabbed with 70% absolute ethanol.

Plate incubation: The SDA plates were incubated at 25°C for 5 days.

Plate reading: The total mould count of each Petri dish was recorded [13].

Statistical analysis

All data collected were analyzed for significant differences ($p>0.05$) (ANOVA) on Graph Pad Prism 5. The results were expressed as mean (n=3) ± SD (Standard Deviation) and pictorial representation. Determined differences among treatments were partitioned by the Least Significant Difference (LSD) and the Duncan's New Multiple Range Test (DNMRT) [14].

Results

Microbial assessment

Table 2 indicates the mean mould load of fish in different locations of Lagos State. The highest mean mould load was found in *C. gariepinus* in Ojo Lagoon. Mould load was highest in *C. nigrodigitatus* in Badagry followed by Ikorodu and least in Ojo. Epe and Ojo had the highest mould load in *S. melanotheron* and the least in Badagry. *T. zilli* had highest mould load in both Badagry and Epe and the least in Ojo.

Discussion and Conclusion

Shortly after capture, chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules [15]. Hansen et al. [16] stated that autolytic enzymes reduced textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odors and off-flavors. This indicates that autolytic degradation can limit shelf-life and product quality even with relatively low levels of spoilage organisms. Most of the impact is on textural quality along with the production of hypoxanthine and formaldehyde. The digestive enzymes cause extensive autolysis which results in muscle softening, rupture of the belly wall and drain out of the blood water which contains both protein and oil. Enzymes and other related chemical reactions do not immediately cease their activities in the fish muscle after fish death (captured). Thiobarbituric Acid (TBA) values which is an important quality index for fatty fish [17], obtained for different fish species are shown in Table 1. The values, obtained by distillation method in *T. Zilli* from Epe (5.96 ± 0.04) was significantly different ($p>0.05$) from those from Ojo (8.79 ± 0.05) and Ikorodu (7.10 ± 0.04) while no significant difference ($p<0.05$) was observed in the TBA of *S. melanotheron* from all locations. Significant difference ($p>0.05$) was also observed in the TBA of *C. gariepinus* of Badagry (3.79 ± 0.06) and Ojo (6.99 ± 0.05). Higher TBA values according to Tokur et al. [17] is as a result of heating during distillation and can cause increase in quantities of aldehydes and disrupt certain carbonyl products which is formed by reactions between malonaldehyde and amino acids, pyridines or protein [18].

The Acid Value (AV), a common parameter in the specification of fats and oils is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1 g of fat and it is a measure of the Free Fatty Acids (FFA) present in the fat or oil. Significant difference was observed in the acid values obtained between *C. gariepinus* from all the locations and *T. zilli* from Ojo, Badagry and Ikorodu. No significant difference between Acid values obtained across the sample locations between *C. nigrodigitatus* and *S. melanotheron*. All the acid values obtained for the four fish species ranges between 1.95 mg/KOH in *C. gariepinus* and 3.79 mg/KOH in *S. melanotheron*. These values are within the standard value of less than 5 mg/KOH for fish oil. These obtained values thus, are indications of notable quality improvement thus, lead to a high quality fish oil in terms of the taste, colour, odour, shelf life and market values [19].

Detection of peroxide gives the initial evidence of rancidity in unsaturated fats and oils. It gives a measure of the extent to which an oil sample has undergone primary oxidation, are measured by the PV. The PV as indicated in Table 1 shows a significant difference in *C. gariepinus* from Badagry (7.15 ± 0.01) mEq/1000 g and *T. zilli* from Ojo (9.69 ± 0.01) mEq/1000 g. The decrease could be as a result of secondary oxidation. Consumers of fishery products always require a PV of less than 10 especially in marine oils, but PV may be as low as 2. The PV test is a good way to measure the amount of primary oxidation products in fresh oils. Oils with significant levels of peroxides may still be odourless if secondary oxidation has not begun. If oxidation is more advanced, the PV may be relatively low but the oil will be obviously rancid. Peroxide values of fresh oils were less than 10 mEq/kg; when the peroxide value is between 30 mEq/kg and 40 mEq/kg, a rancid taste is noticeable [20].

The most important application of the iodine value is to determine the amount of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. Iodine value is used to measure unsaturation or the average number of double bonds in fats and oils [21]. Decrease in iodine value shows decrease in the number of double bonds and it indicates oxidation of the oil. The higher the iodine value, the more unsaturated fatty acid bonds are present in a fat [22]. Therefore consumption of fish with lower iodine value could increase risk of cardiovascular disorder in humans. Hence, the high iodine value recorded for in this study from different locations suggests that the fish species contain high level of unsaturated oil and as such could reduce the risk of heart diseases, high cholesterol, depression, anxiety, low immunity, cancer, eye disorders and ulcers in humans when consumed. The level of FFA gives an indication of the oxidative state of the oil. FFA is very susceptible to oxidation leading to the deterioration of the oil taste. The value of Free Fatty Acids (FFA) as indicated in Table 1 ranges between 0.98% ± 0.02% in *C. gariepinus*

from Badagry to 1.66% ± 0.02% in *T. zilli* from the same location. These values according to Bako et al. [23] are below the standard value of 2% to 5% for fish oil.

Saponification value is expressed by potassium hydroxide in mg required to saponify 1 g of fat. It is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. It is an index of average molecular weight of the triacylglycerols in the sample. The SV of in this study varies among species from different locations. It ranges between (177.00 ± 2.83) mg KOH/g in *C. gariepinus* from Badagry to (254.50 ± 2.12) mg KOH/g in *S. melanotheron* from Ikorodu. This value was above the standard value range for fish oil (165-195) mg KOH/g as opined by ISO [24]. Kirk et al. [25] reported that high saponification values are due to the predominantly high proportion of shorter carbon chain lengths of the fatty acids. This assertion was confirmed by Nielson [26], that the smaller the saponification values the longer the average fatty acid chain. If the fatty acids present in the glycerides are low molecular weight (short-chain acids), there will be more glycerides molecules per gram of fat than if the acids are high in molecular weight (long-chain acids). Thus, since each glyceride molecule requires three potassium hydroxide molecules for saponification, fats containing glycerides of low molecular weight correspondingly have higher saponification values [27].

This result suggests that the extraction method according to Lynch et al. [28] can alternatively be used to determine the amount of lipid oxidation in fish muscle instead of the distillation method according to Tarladgis et al. [29]. This procedure also makes it possible to determine lipid oxidation products in large numbers and small quantities of samples. Table 2 shows, the mould load of the selected fish samples from different locations in Lagos, with *C. gariepinus* from Ikorodu having the highest microbial count. The microbial count of *T. zilli* and *T. sarotherodon* is very low. However there were variations in the mould load among the fish species within the same location and same species at different locations. It is found that the mould load of *C. gariepinus* from Ikorodu is the highest from all the *C. gariepinus* collected for this study while that from Badagry is very low. For *C. nigrodigitatus* the mould load of samples from Ikorodu and Ojo are almost the same while that from Epe is slightly higher than that from Badagry.

The *S. melanotheron* and *T. zilli* have similar trend in their mould load irrespective of location [30]. In Badagry market *C. nigrodigitatus* has the highest colony formed per gram from different location in which *C. nigrodigitatus* is considered. The high mould count may be due to microbial activity present in the surrounding environment, which is the water in which the organisms were caught. This may have effect on the shelf life of the organism thereby causing autolysis. The maximum bacterial counts for fresh and frozen fish samples recommended as 5×105 cfu/g [13]. The study revealed that fish samples demonstrated no risk on the public health and are wholesome for consumption. The quality of the fish and fish products largely depends on the interval between the harvesting, storage and processing time. Consumers are therefore advised to patronize these locations for wholesome fish as the demand for fish is on the increase due to population explosion.

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