

Quality Evaluation of Stored Tilapia (*Oreochromisniloticus*) Pretreated With Selected Spices

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Abstract

Fish quickly deteriorates immediately after harvest. Nowadays instead of using synthetic preservatives, spices are now being used as flavoring and preservative agents. This study sought to arrest the development of deterioration in fish using different spices. Fresh fish samples were pretreated with selected spices before oven drying and stored at ambient temperature. The samples were labeled according to the spice used as ginger (A), garlic (B), clove (C), and mixed-spices (D). The untreated fish served as control (E). The physicochemical, microbial and sensory properties of the fish samples were evaluated after 2 months of storage. The results showed that the fish samples were high in protein (46.41 - 53.12%). The moisture content was lower in the treated fish samples (7.85 to 9.02%) than in the control, E (10.12%). The lipid quality-indices indicated that the treated samples had lower Free Fatty Acids (5.50-6.34%), Trimethyl amine (9.10-11.34mg/100g) and Peroxide value (3.70-4.12 mEq/kg) than the control, E. The mineral analysis indicated that highest iron (0.58 mg/kg), manganese (0.40 mg/kg), lead (0.08 mg/kg) and potassium (3.16 mg/kg) contents were respectively recorded in fish samples B, E, D and C. The bacterial $(9.2x10^2 \text{cfu/g})$ and fungal $(7.3 \times 10^{1} \text{cfu/g})$ counts were highest in the control sample, E. Coliform Count was <3 cfu/g in all the samples except in the control, E which had 14 cfu/g. Sample D was the most acceptable (6.5) while sample C (4.40) was the least acceptable to sensory panelists. This study demonstrated that pre-treating fish sample with spices prior to processing significantly (p < 0.05) reduced fish deterioration during storage; and that blends of different spices was more effective than a single spice. The application will increase shelf life and utilization of fish.

Keywords: Tilapia, spice preservatives, quality characteristics

Introduction

Nigerians are large consumers of fish and it remains one of the main produce consumed as a rich source of animal proteins. It is relatively cheap and highly acceptable with little or no religious bias, which gives it an advantage over pork and beef (Onwuka, 2014;Alfa, 2014).

Fish species have protein digestibility between 16-20% (Fagbenro et al, 2005). Fishes contain components ranging from water, protein, fat. ash and carbohydrate. Other important nutrients like vitamins and bacteriological characteristics of fishes tend to vary with species, feeding habits. seasonality (Archana et al, 2009). Sea foods are a good source of important minerals and an excellent source of iodine in particular. Canned fish with the bones, such as sardines. salmon are also excellent sources of calcium and phosphorus (Bakkali, 2008).

Fish also contain a specific type of fat, omega-3 fatty acids that may reduce the risk of developing heart disease and other medical problems. Fish intake has also been linked to a lower risk of stroke, depression, and mental decline with age. Fish consumption is also important for pregnant women. mothers who are breastfeeding, and women of childbearing age. because it supplies DHA, a specific omega-3 fatty acid that is beneficial for the brain development of infants. (Onwuka, 2014; Ndife, 2016).

Fish is highly susceptible to deterioration immediately after harvest by a metabolic process which causes fish to be undesirable or unacceptable for human consumption resulting in negative changes in the chemical characteristics and sensory quality of the fish (Viuda-Martos, 2010). This will lead to nutritional and economic losses. The processing and preservation of fresh fish seeks this development to arrest (Onwuka, 2014; Alfa, 2014).

Studies on continuous use of synthetic preservatives have proven that many of them health hazard constitute to consumers. This brings about the need to use natural preservatives (Viuda-Martos, 2010: Ndife. 2016). Plant preservatives most of which are herbs and spices are used as flavoring agents and due preservatives to their antimicrobial antioxidant and properties (Archana et al., 2009). These spices are used in the form of powder, extracts or essential oils in food applications (Bakkali, Ndife. 2008: 2016). Unlike synthetic compounds. natural preservatives obtained from spices are rich in phenolic compounds and they can enhance the overall quality of food by decreasing lipid oxidation and microbial growth (Viuda-Martos, 2010). The major aim of this research was to investigate the effect of some indigenous spices as natural preservatives singly and in combination on oven dried Tilapia for longer shelf life.

Materials and Methods

Material Collection and Preparation

The fish and selected dry spices were obtained from Wudil local market Kano, in Kano state, Nigeria. The different spices were processed into fine powders, as shown in Figure 1.And the fish samples were processed as outlined in Figure 2.



Fig.1: Flow chart for the Production of Spice Powder



Fig. 2: Flow chart for Production of Oven Dried Fish.

Processing of Fish Samples

The fish samples were scaled, gutted, cut into fillets (200 g each) and washed with potable water. The fish fillets were divided into five samples. One of the five lots was coated with 50 g of Ginger powder and labeled sample A, the second sample was coated with 50g Garlic powder (sample B), the third sample was coated with 50 g of Cloves powder (sample C) while the fourth sample D was however coated with spice mixture of Ginger, Cloves and Garlic powders of same quantities (16.67 g), leaving the fifth sample as a control without any spice coating

(sample E). All samples were then dried in hot-air oven at 80 °C for 7 hours, sealed in polythene, to avoid contamination and stored at room temperature (35-40 °C) for 2 months before analysis.

Methods of Analysis

Determination of Proximate

Composition of fish samples

The proximate composition of the fish samples which include moisture, ash, fat, and protein contents was determined using the methods of Onwuka (2018).

Carbohydrate content was determined by difference.

Physicochemical Analysis

The pH of the samples was recorded using a digital pH meter. Trimethylamine The (TMA). Peroxide Value (PV) and Free Acids (FFA) Fatty were determined using the methods of AOAC (2005). Trimethyleamine (TMA) was determined by mixing Magnesium oxide with distilled water containing 2% boric acid into which 2drops of methyl red indicator was added. This was titrated against 0.1 N H₂SO₄ and the expressed in mg/100g.The Peroxide value (mEq/kg) of the sample was determined by titrating potassium iodide solution with 0.002 M Thiosulphate using starch as indicator. And the % Free Fatty Acid was determined by mixing the fish sample with 25 ml alcohol which 1% phenolphthale into inindicator has been added and titrated against 0.1MNaOH solution.

Mineral Analysis

This was determined according to the method described by AOAC, (2005). The sample (10 g) was weighed and then ashed in a muffle furnace at 500 °C for 2 h. After ashing, 10cm³ of 6M Nitric acid (HNO₃) was added and agitated until a uniform solution was obtained. This was filtered and distilled water (10 ml) added. The elemental contents of the digests were determined using Atomic Absorption Spectrophotometer (AAS).

Microbiological Analysis

The method APHA (1992) was adopted. Briefly; A tenfold dilution of the fish samples was made; from this, further dilutions were made. One 1ml of each dilution mixed with was appropriate sterile molten media at 45 °C: Plate count agar (PCA) for total bacteria; Potato dextroseagar (PDA) for fungi; MacConkey agar for coliforms. Incubation period was 48 h at 37°C for bacteria and 25°C, 72 h for fungi. The microbial counts were expressed as colony forming unit per gram of sample (cfu/g).

Sensory Evaluation

The fish samples were subjected to sensory evaluation on appearance, texture, taste, aroma and overall acceptability using a 9 point hedonic scale, with 1 as dislike extremely and 9 as like extremely. Twenty (20) sensory panelists used were semi trained consisting of 15 students and 5 laboratory staff from the Department of Food Science and Technology of Michael Okpara University of Agriculture, Umudike (Iwe, 2002).

Statistical analysis

Analytical determinations of the fish samples were made in

triplicates. Data were presented as mean \pm standard error. The fish samples were statistically analyzed using SPSS (Statistical Package for the social sciences) version 16 for PC windows. Sample means were compared using one way analysis of variance (ANOVA) at 5% level of significance

Results and Discussion

Proximate Composition of Fish Samples

The results from proximate composition of fish samples (Table 1) show that the fish had high contents of protein (46.41 53.12%). Sample B had the highest protein (53.12 %) while sample E was the least in protein content (46.41%). There was significant difference (p<0.05) between the five samples. However the protein contents were lower than 64.10 and 76.53 % which was reported for oven dried tilapia by Dagne et al. (2016) and Ogbonnaya (2009).

Sample	Protein	Moisture	Ash	Fat	Carbohydrate
А	48.69±0.7 ^y	9.02±169 ^x	1.53±0.29 ^y	21.45±0.42 ^y	19.31±0.60 ^x
В	50.87 ± 0.79^{y}	8.82±0.02 ^x	2.85±0.19 ^x	25.87±0.31 ^x	11.59±0.94 ^y
С	53.12±0.88 ^y	7.85±0.15 ^y	3.37 ± 0.09^{y}	23.55±0.07 ^x	12.11±1.01 ^y
D	49.78±0.74 ^x	8.54±0.14 ^x	2.92±0.05 ^x	23.35±0.07 ^x	15.41±0.64 ^x
Е	46.41±0.66 ^x	10.12±0.16 ^x	1.45 ± 0.15^{y}	24.40±056 ^x	17.62±0.08 ^x

 Table 1: Proximate Composition of Fish Samples (%)

*Means within a column with different letters are significantly different at p < 0.05; A-Ginger treatment, B -Garlic treatment, C - Clove treatment, D - Mixed spice treatment and E - Control.

There was significant difference (p<0.05) between the five samples. However the protein contents were lower than 64.10 and 76.53% which was reported for oven dried tilapia by Dagne *et.al.* (2016) and Ogbonnaya, (2009).This could be due to variations in processing conditions.

The moisture content ranged from 7.85 to 9.02% for the pretreated fish samples. The control sample E had the highest moisture content of 10.12%. There were significant

differences in the moisture values of the samples. Sample E had the highest moisture content of 10.12% among the fish samples. The moisture values of the fish samples (7.85 - 10.12%) were lower than 17.13% reported by Ogbonnaya (2009) for tilapia dried in electric oven.

There was significant difference (p<0.05) in ash content of the pretreated samples which ranged from 1.53 to 3.37 % with sample C having the highest value of 3.37 % while sample E have lowest value of 1.45 %. This is lower than 6.67 % reported by Dagne *et al.* (2016) for oven dried tilapia.

In the case of fat content of the fish (21.45-25.87%) sample B had the highest value of 25.875 % while sample D had the lowest fat content (23.35 %). This is higher than the range (6.37-7.01 %) reported by Dagne *et al.* (2016) and close to 20.25% reported by Ogbonnaya (2009).

Percentage carbohydrate was highest in sample A (19.31%), closely followed by control sample E (17.62%). The nutritional value of freshwater fish was reported by Zhang *et al.*(2016) to differ between species, sexes, sizes, season and geographical localities.

Chemical Properties

The chemical properties of pretreated oven dried fish are shown on Table 2. These are the of the breakdown measure products from biochemical and microbes deterioration of the fish samples (Ozpolat and Duman, 2017). The Free Fatty Acid (FFA) values of the fish samples ranged from 4.93 to 6.91%. Sample C (4.93 %) had the lowest FFA content and the control.

Sample E had the highest (6.91 %). Lower FFA of 1.00 to 1.23 % was reported by Adeyeye et al. (2016) in silver catfish after convective Kiln smoking. Fresh fish has low FFA which increase with storage (Holley and Patel, 2005). However for these fish samples, it is an their indication of level of deterioration. Autolytic deterioration and microbes are factors which catalyse lipolytic activites and release FFA from glycerides and phospholipids.

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Sample	FFA(%)	TMA (mg/100g)	Peroxide (mEq/Kg)	P ^H
А	5.500±0.19 ^y	11.34±0.19 ^x	$3.92{\pm}0.05^{y}$	7.32±0.20 ^x
В	6.34±0.20 ^x	10.43±0.39 ^y	$3.70{\pm}0.09^{y}$	7.18±0.31 ^x
С	4.93±0.20 ^y	10.85 ± 0.27^{y}	4.12±0.05 ^x	7.10±0.23 ^x
D	5.36±0.10 ^y	9.10±0.36 ^y	$3.80{\pm}0.08^{\text{y}}$	7.24±0.11 ^x
Е	6.91±0.19 ^x	17.08±0.34 ^x	4.72±0.05 ^x	8.05 ± 0.23^{y}

 Table 2: Chemical properties and pH of fish samples

*Means within a column with different letters are significantly different at p < 0.05: A-Ginger treatment, B-Garlic treatment, C-Clove treatment, D-Spice-mix treatment and E-Control; FFA-Free Fatty Acid, TMA-Trimethylamine

The Trimethylamine (TMA) values of the pretreated fish samples which ranged from 9.10 to 11.34 % were lower than that of the untreated control sample E(17.08). These results are higher than the range of 2.11 to 2.69mg/100g Adeveve reported by et al. (2016). The formation of TMA is related to many factors such as differences in species, bacterial growth, processing methods and storage conditions (Rodríguez-Vaquero et al., 2013).

The peroxide value (PV) followed similar trend; the PV was higher in the control (4.72 mEq/Kg) than in the pretreated fish samples (3.70-4.12 mEq/kg). Among the pretreated fish, sample C had the highest PV of 4.72 mEq/Kg. The peroxide value is used to measure

Mineral content

The result in Table 3 obtained for mineral analysis of the fish samples showed Iron values of 0.39 to 0.58 mg/100g for pretreated the primary lipid oxidation products of hydroperoxides (Rodríguez-Vaquero *et a*l., 2013; Adeyeye *et al.*, 2016).

The pH values of the pretreated fish samples which ranged from 7.10 to 7.32 were lower than that for untreated sample E (8.05). pH has a preservative influence on foods. The pH of the samples was derived from the also acid constituents of the spices used. The difference in pH values during the storage period may be attributed to the accumulation of ammonia and organic amine compounds and bacterial growth (Ozpolat and Duman, 2017). Low pH has been reported to be detrimental to the growth of prevalent proteolytic microbes in fish (Ezeama, 2007).

fish samples and 0.29 mg/100g for sample E (control). The higher Iron values obtained for pretreated fish samples could have been derived from the spices used. Garlic pretreated sample had the highest Iron content (0.58 mg/100g). The quantity of minerals in foods does not necessarily correspond to the amount absorbed by the human intestine or its bioavailability to the organism (Adefemi *et al.*, 2010; Onwuka, 2014). However, regulating mechanisms exist to control iron absorption which vary according to the concentration of hemoglobin and body reserves of iron (Onwuka, 2014).

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Sample	Fe	Mn	Pb	Κ	Cr
А	0.39°±0.01	0.30 ^b ±0.02	0.04±0.01x	2.30±0.02 ^x	0.06±0.02 ^y
В	$0.58^{a}\pm0.02$	0.20 ^a ±0.01	0.04 ± 0.02	2.18±0.01 ^x	0.13±0.02 ^x
			х		
С	0.39°±0.02	$0.30^{b}\pm0.02$	0.03±0.01 ^y	3.16±0.02 ^y	0.12±0.0 ^x
D	$0.48^{b}\pm0.03$	0.40ª±0.02	0.08 ± 0.02	2.00±0.01 ^x	0.12±0.01 ^x
			х		
Е	0.29±0.02	0.10 ^c ±0.01 ^y	0.03±0.01 ^y	2.27 ± 0.02^{x}	0.06±0.02 ^y
		1.1 11.00		1.01 1 11	

Table 3: Mineral content of fish samples (mg/100g)

*Means within a row with different letters are significantly different at p < 0.05: A-Ginger treatment, B-Garlic treatment, C-Clove treatment, D-Spice-mix treatment and E-Control.

The Manganese values of pretreated fish samples (0.2-0.4 mg/100g) were higher than the manganese content of the control E mg/100g). (0.1)The highest manganese content was in spicemix pretreated sample D (0.4 mg/100g). The same trend was observed for lead and chromium contents of the fish samples. Lead and chromium in sample E were 0.03 and 0.06 mg/100grespectively. High levels of Potassium (2.00 - 3.16 mg/100g)and iron in fish samples could be attributed to the fact that these metals are naturally abundant in the soil water (Fagbenro et al., 2005: Adefemi and Awokunmi. 2010). However samples C (3.16 100/g) and A (2.30 mg/100g) had the highest Potassium. The fish samples are good source of both micro and macro minerals. The mineral elemental contents of fish species is a function of the availability of these elements in their local environment, diet absorption capability and as well as their preferential accumulation (Fagbenro *et al.*, 2005; Adefemi and Awokunmi, 2010; Alfa *et al.*, 2014).

Microbial content

The results of the microbial contents of the fish samples are presented in Table 4. The microbial load of fish samples ranged from 2.4×10^2 to 6.1×10^2 cfu/g, these were lower

than 9.2×10^2 cfu/g recorded in the untreated fish sample. At low moisture levels (less 10 %) deteriorative enzymatic and activities of bacteria and yeast are halted (Hollev and Patel, 2005). lowest The microbial count $(2.4 \times 10^2 \text{ cfu/g})$ was observed in

sample D which was treated with a mix of the spices. Rodríguezvaquero *et al.* (2013) reported the use of wine phenolic compounds as antibacterial agent to extend the shelf life of fish.

T	Table 4. Wher oblar quanty of fish samples (cru/g)					
Sample Tot		Total Bacteria	Total Fungi	Coliform Count		
		Count	count			
		1 0	1 0			
	А	$6.1^{b}x10^{2}$	$3.0^{b}x10^{2}$	<3		
	В	$3.1^{c}x10^{2}$	$2.1^{c} x 10^{2}$	<3		
	С	$3.3^{c}x10^{2}$	$2.4^{\circ}x10^{2}$	<3		
	D	$2.4^{d}x10^{2}$	$1.0^{d}x10^{2}$	<3		
	Е	$9.2^{a}x10^{2}$	$7.3^{a}x10^{2}$	14		

 Table 4:Microbial quality of fish samples(cfu/g)

*Means within a column with different letters are significantly different at p < 0.05: A-Ginger treatment, B-Garlic treatment, C-Clove treatment, D-Spice-mix treatment and E-Control.

The total fungal counts of the fish samples also followed the same trend as the microbial load. The fungal counts of the pretreated fish samples ranged from 1.0×10^2 to 3.0x10² cfu/g. Significant difference (p<0.05) in microbial content exist between the samples. Sample E (control) had the highest fungal 7.3x10² cfu/g.The count of moisture content is an indicator of the susceptibility of a product to undergo microbial spoilage (Ezeama, 2007; Kalemba and Kunicka. 2003). The higher moisture content of sample E (control) could have been

responsible for the high fungal growth.

The coliform count was < 3 cfu/gfor all the pretreated fish samples except for sample E which had higher value of 14cfu/g. The low coliform count is an indication of the handling and hygienic protocol followed during processing and storage of the fish samples. The results of the microbial load showed that the spice-mix used in sample D was more effective in reducing the microbial load and activities in the fish sample. Several researchers have reported on the antibacterial and antifungal properties derived from essential oils extracted from different spices

(Gould, 1996; Bakkali *et al.*, 2008; Archana *et al.*, 2009; Kalemba and Kunicka, 2003). Also at low moisture content (<10 %) deteriorative activities of biochemical reactions, bacterial

Sensory quality

The results of the sensory assessment of the fish samples are presented on table 5. The panelist scores on appearance for the pretreated fish samples (5.26-6.53) were higher than scores for the control sample E (5.13). Sample B

and fungal growth were prevented (Holley and Patel, 2005; Ezeama, 2007). The microbial counts were below 1.0×10^6 the upper acceptability limit for fresh marine species (APHA, 1992).

had the lowest appearance score (5.26). Food colour is a quality indicator of the appearance of foods. It also helps to determine the degree of processing or spoilage level (Holley and Patel, 2005; Onwuka, 2014).

Table 5: Sensory evaluation of fish samples.

Sample	Appearance	Taste	Aroma	Texture	Acceptance
А	6.33 ^a ±1.11	5.25 ^b ±1.74	5.86 ^b ±1.24	5.73 ^b ±1.66	5.60 ^b ±1.50
В	$5.26^{b}\pm1.59$	4.26°±1.59	5.53 ^b ±1.55	5.33°±1.54	5.46 ^b ±1.35
С	$5.46^{b}\pm1.12$	4.66°±1.71	$5.31^{b}\pm 1.03$	5.13°±1.06	5.13°±0.99
D	6.53 ^a ±0.63	6.53 ^a ±0.51	6.13 ^a ±0.74	6.26 ^a ±0.59	6.40ª±0.50
E	5.13°±1.06	5.20 ^b ±1.14	5.06°±1.16	4.86 ^d ±0.59	4.40 ^d ±1.35

*Means within a column with different letters are significantly different at p< 0.05; A-Ginger treatment, B-Garlic treatment, C-Clove treatment, D-Spice-mix treatment and E-Control.

The flavor of the fish samples were evaluated in terms of the taste and aroma. Samples B (4.26) and C (4.66) had the lowest taste scores. The control sample E was scored (5.20) better than the pretreated samples except for sample E (6.53) for taste. For the aroma of the fish samples; control sample E (5.06) had lower aroma scores when compared with the pretreated fish samples (5.06-6.13). Sample D had the highest aroma score of 6.13. The spice pretreatments had a profound influence on the flavor of fish samples. The aromatic constituents of the spices had an influence in the flavour properties of the pretreated fish samples (Archana *et al.*, 2009; Zhang, 2016).

Pretreated fish samples had better texture scores (5.13-6.26) than the control sample (4.86). Sample D with pretreatment-mix had the highest score (6.26). There was no significant difference between (p>0.05) all samples. The extent of dried fish texture, toughness, and dryness is greatly influenced by loss of moisture during drying and protein denaturation effects (Joram and Kapute, 2016).

In terms of overall acceptability with mixed-spice sample D, pretreatments was most preferred (6.40) while the control sample E was the least (4.40). The results of the sensory assessment indicated that shelf-life of fish flesh was affected the pretreatments. Similar results were seen in different studies of the positive effects of plant extracts thyme, oregano, clove and rosemary) on fish (Dagne et al., 2016; Zhang et al., 2016; Ozpolat and Duman, 2017). Sensory evaluation is based on the perception of the sense organs to accepting the food products (Joram and Kapute, 2016).

Conclusion and Recommendations

In conclusion, this research showed the selected spices as effective natural preservatives with positive impact on the chemical and microbial quality parameters. The spice-mix of Ginger, Garlic and Clove (E) was the most effective pretreatment in the preservation of stored fish samples. This was closely followed by 100% Garlic **(B)** and 100% Ginger (A) pretreatments. The spices were found to also improve the sensory quality of the stored fish. The use of spices as natural preservatives is hereby recommended. More so it will be of useful interest if the shelf life of the fish samples is determined using the most effective preservatives on raw fish.

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