Effects of Lactic and Ascorbic Acid Treatments on Proximate Composition and Shelf-Life of Sundried *Oreochromis Niloticus* Fillet

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Abstract

Tilapia Oreochromisniloticus is a perishable commodity and its quality and freshness declines rapidly after death as a result of poor handling lowering its shelf-life and nutritive value which is highly needed by the fast growing world population. This study was therefore carried out to determine the proximate composition and shelf life of sun-dried Nile Tilapia (Oreochromisniloticus) treated with various concentrations of lactic acid and ascorbic acids (3%, 5% and10%) for a duration of six weeks(42 days). Fish samples with average weight of 226.5 ±84.8g, and average total length of 24.7±3.6 cm were obtained from the University of Eldoret fish farm; they were de-scaled, eviscerated, washed and rinsed in a large amount of water. Muscle sample of fish were divided into groups. Group A. (1, 2, 3) were treated with 3%, 5% and10 %, of lactic acid respectively. Group B. (4,5, and 6) were treated with 3%, 5% and 10% ascorbic acid respectively and Group C, without any treatment (control group). Triplicate samples were used for each treatment. The fillets were then soaked in treatments for 30 minutes then dried under the sun in an artificial cage made from locally available materials. After 1 day, 5 days, 2 weeks, 4 weeks and 6 weeks of drying, moisture content, crude protein and crude lipid levels were determined. Data obtained was then analyzed using MINITAB statistical software. The results revealed a significant reduction in moisture content in all the samples from 77.9±1.65% of fresh fillet to 2.6±0.98% of the control. This was as a result of solar drying effect. It was also observed that crude protein and lipids increased in treated samples but reduced in control samples which were attributed to the ability of the preservatives to retard deterioration of protein and lipids in O. niloticusfillets. There was no significant difference ($p>0.05$) in proximate composition of samples treated with different concentrations of lactic acid. This was also evident in samples treated with different concentrations of ascorbic acid. Results in the study also revealed that effects of equal concentrations of lactic and ascorbic acids on proximate composition of Nile O. niloticus had no significant difference. However, means of crude lipids and protein at the end of the study, registered a significant difference ($p<0.05$) between lactic and ascorbic acid, ascorbic acid with overall high crude protein and lipids. It is concluded that solar drying resulted to more stable Nile Tilapia fillets, with higher proteins and lipids. A treatment of 10 % ascorbic acid combined with open hygienic solar drying is therefore recommended so as to reduce post-harvest losses, increase shelf life of Tilapia products and make available high nutritive products with little health side effects, to the ever growing human populations.

Key Words: Lactic, Ascorbic Acid, Proximate Composition, Shelf-Life, *Oreochromis Niloticus* Fillet

INTRODUCTION

Background of the Study

According to the FAO (2005), the world's total tilapia aquaculture production in 2000 was 1.27 million metric tons and contributed about 3.6% of global total aquaculture production. The top five producing countries during 2000 are China, Egypt, Thailand, Philippines and Indonesia, each accounting for 49.7, 12.4, 7.8, 7.3 and 6.7%, respectively, of world's total aquaculture production of tilapia.Worldwide harvest of farmed tilapia has now surpassed 800,000 metric tons, and tilapia is second only to species carps as the most widely farmed freshwater fish in the world (FAO 2012). Nile Tilapia *(Oreochromisniloticus)* was one of the first fish species cultured (Thomas, 1999). Illustrations from Egyptian tombs suggest that Nile tilapia were cultured more than 3,000 years ago (Thomas & Micheal, 1999).

Fish especially Tilapia is gaining an increased importance in the diets of people worldwide due to its role in providing significant amount of important dietary factors in nutritional and proteins including high levels of essential amino acids linoleic, linolenic, lysine, methionine, lipid soluble vitamins A and D, microelements like Iron, Calcium, Copper, Zinc among others and highly unsaturated fatty acids (Connel, 1990). Also, fish is a cheap source of animal protein, which gives it advantage over pork or beef (Auborg, 2008). According to Pillay (2005), Tilapia is the generic name of a group of cichlids endemic to Africa. The important aquaculture genera in Kenya are; Oreochromis, Sarotherodon and Tilapia.

Spoilage of fish food products is due to chemical, enzymatic or microbial activities. According to Baird-Parker (2000), Chemical deterioration which include lipid oxidation and autolysis and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fishery products every year .One-fourth of the world's food supply (Huisin't Veld, 1996) and 30% of landed fish (Amos, 2007) are lost through microbial activity, poor handling and chemical degradation and non-conformity to Hazard Analysis Critical Control Point (H.A.C.C.P). This is a threat to the national food reserves and thus to national food security. With the ever growing world population and the need to store and transport the food from one place to another, food preservation becomes necessary in order to increase its shelf life and maintain its nutritional value, texture and flavor. Different types of preservation methods such as smoking, freezing, chilling, brining, and canning are reported to extend the self-life of sea foods and meat products (Berkel *et al*., 2004). However, low temperature storage and modern techniques for controlling water activity, enzymatic, oxidative and microbial spoilage are expensive and not readily available with some methods like smoking reporting carcinogenic effects (Akinola *et al*., 2006; Berkel *et al*., 2004).

Akinola *et al*. (2006) reported that despite the rudimentary nature of process of traditional methods, lack of control over the drying rate, sometimes results to under-drying or over-drying; expose the fish to unexpected winds, dust, dirt, insect infestation, and contaminants such as flies. Dried fish are usually considered shelf stable, therefore, often stored and distributed unrefrigerated and the resulting product is easily transported to market or from one country to another. The characteristic of dried fish that makes them

shelf stable is their low water activity ($_a$w) and thus prevention of growth of many spoilage microorganisms (Berkel *et al.,* 2004).These methods still remain predominant in Kenya. To reduce post-harvest losses and to improve the quality of fish and fishery products, traditional processing technology must however be improved upon .This includes use of organic acids as additives and preservatives which are accepted by ISO, CAC,WTO,SPS and TBT, from which Kenyan standards are practically adopted.

There has been an increased case of terminal diseases to the human race mainly because of chemical food additives and preservatives which have carcinogenic attributes and consumption of food with lower than required nutrient composition topped by unhealthy lifestyles (WHO2007). These diseases are fatal and expensive for the state and individuals to treat. Large amounts of state and family resources are expended on treatment of such diseases, when there are effective, healthier and cheaper alternatives for food preservation these can be greatly avoided.

MATERIALS AND METHODS

Collection of Fish Sample

Fish samples weighing 226.5g±84.8, and average total length of 24.7±3.6 used for this study were collected from the University of Eldoret fish farm. They were first weighed and their lengths taken. They were rinsed in distilled water to remove any adhering contaminants and drained under folds of filter paper. The fish sample were then dissected with a knife and the scales, intestines, guts and bones removed, then thoroughly washed in large amount water. The head was discarded and fillets weighing 10g obtained from the body muscles.

Sample Treatment

The fillet samples were randomly chosen and divided into 3 groups. Group A. (1, 2, 3) were subjected to 1%, 3%, 5%, of lactic acid respectively. Group B.(4,5,6) treated with 1%, 3% and 5% ascorbic acid respectively and Group C, without any treatment- the control group.

A fresh sample, with no kind of treatment was then taken for proximate composition analysis. Each treatment had triplicates. The fillets were then soaked in treatments for 30 minutes then dried under the sun in a drying chamber constructed from locally available materials. A sample from each group was taken for proximate analysis, after a day, 5 days, 2 weeks, 4 weeks and 6 weeks of drying.

Proximate Analysis

Moisture Content Analysis. Moisture content of fish fillets was determined according to AOAC (2002). The samples were ground and placed in dried empty crucibles. Weight of the crucible plus the sample was taken before heating.These were then placed in an oven at 105 ˚C. Weights of crucible plus content were taken at time interval of 30 minutes until constant weights were obtained.

Percentage moisture content was then calculated as percentage with the formular:

$$X = \frac{(P_1-P_2)100}{m}$$

Where; P_1 is the weight of the weighing bottle along with the sample before drying(g)

P_2 is the weight of the weighing bottle along with the sample after drying(g)

M is the weight if fish sample *(g)*

Protein analysis. Protein content of fish fillets was also determined according to AOAC (2002) - calorimetric method. This method is based on estimation of the nitrogen content (N) of the sample to be analyzed and subsequent conversion of the results into protein content by multiplying with the coefficient 6.25.Where, 0.3g of sample was ground, weighed and transferred into Kjeldahl flask. To the flask, 2.5ml of digestion mixture was added. Digestion continued for an hour at 110^0C which was raised to 330^0C till all solutions in the digestion tubes were colorless. Digested samples were then cooled for 10-20 minutes.

Calorimetry was done to the samples to obtain absorbance. This value then compared with the standards in MS excel and N value calculated as,

% N= Concentration* Dilusion factor/Weight of

sample Protein value= %N *6.25

Lipids Analysis

Crude lipid was estimated by soxhlet method. The fat was extracted from the samples using petroleum ether in the soxhlet apparatus, which consists of a condenser having a globular internal thimble, extractor and receiving flask which are connected through ground closed joints.0.3 g of sample was finely ground and then weighed. Weight of an empty dry flask was taken. Extractor then connected to the condenser and finally to the receiving flask. Extraction proceeded for 12 hours. The flask along with fat was dried at 50-60^0C cooled and weighed.

The fat content was calculated as percentage;

$$X= \frac{(c-c_1) \quad 100}{M}$$

Where; c is the weight of the flask along with the fat,(g)
$c_1$is the weight of empty flask (g)
m is the sample weight of fish material(g)

Statistical Analyses

Nutritional composition analysis was replicated three times (n = 3). Results presented as mean values of each determination ± SD. Descriptive statistics and analysis of variance was performed by one-way ANOVA procedures by use of MINITAB statistical software. *Post-hoc* comparisons were conducted using Duncan's Multiple Range test and the significance defined at $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

Proximate composition of fresh tilapia fillet determined before treatment in preservatives is shown in Table 1.

Table 1. Mean percent composition ± SE of fresh tilapia fillet at the start of study

Composition	% Mean Content ±SD
Moisture	77.9±1.65
Protein	13.65±2.40
Lipid	13.8±4.01

Lactic Acid

Moisture content. Moisture content in samples treated with different concentrations of lactic acid recorded different decrease rates over the 6 weeks. This decrease however is not statistically different $F = 0.24$, $p = 0.787$ ($p>0.05$).Towards the 4th week to end of study, all the samples attained constantly reducing moisture content and later stabilized. This is revealed by the flattening of the line graphs towards the 6th week of drying, in Fig. 1.

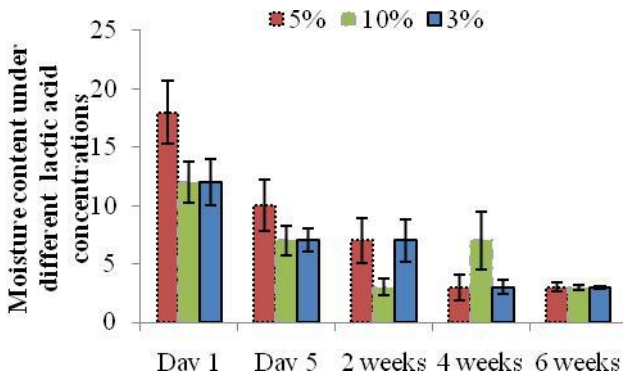


Figure 1. Changes in moisture content in different lactic acid treatments over 6 weeks of drying

Protein. An increase in protein content was seen in the different lactic acid treatments, over the study period with 3% subjects recording higher values throughout in the order, 3% >5% >10%.This difference in increase was however insignificant (F = 2.5, p=0.124)

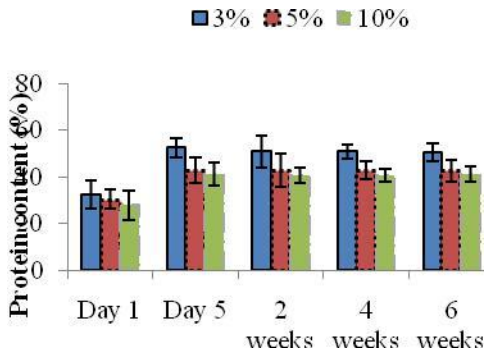


Figure 2. Crude protein variations over 6 weeks of sun drying in samples treated in different lactic acid concentrations

Lipids. Crude lipid content in the treatments increased between day one and day five of sampling after which a sharp decrease was recorded in treatments under 3% and 5%, attributed to absorption of moisture and sampling variation and errors. These later picked up towards the 4th week and flattened toward the end. This is related to the inverse relation between moisture and lipids. The increase in lipid is matched by a corresponding decrease in moisture at same time of sampling. In spite the different levels within treatment, they remain statistically similar as (F = 0.31,p=0.741), in Fig.3.

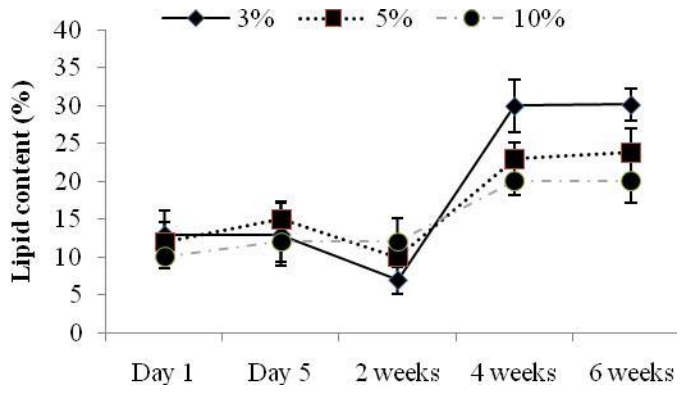


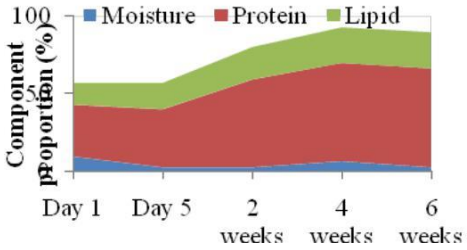
Figure 3. Trend in crude lipid content in fillet samples treated with different lactic acid concentrations over 6 weeks of drying

Ascorbic Acid

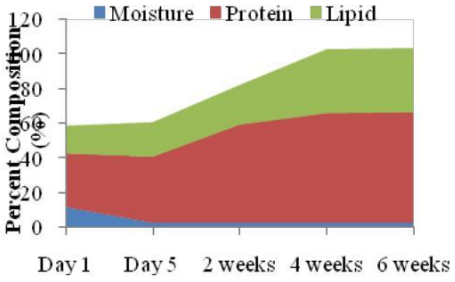
Samples treated by different concentrations of ascorbic acid responded differently in proximate composition over the period of study. In all the treatments, crude protein occupied highest proportion and moisture the lowest. An inverse relation between moisture and crude lipid and moisture and crude protein is evident in Fig 4, (a,b,c).The differences were statistically insignificant($p>0.05$), for all concentrations as depicted in Table 3. In all these however, 10% produced the highest levels in crude protein and lipid as compared to 3%, 5% and the control.

Table 2. Mean ± SE for the proximate components of Nile Tilapia preserved in Ascorbic Acid under different concentrations

	3%	5%	10%	F	P
Moisture	5.2 ± 1.43^{b}	4.8 ± 1.8^{b}	5.6 ± 1.78^{b}	0.06	0.945
Protein	50.75 ± 6.5^{b}	50.42 ± 6.7^{b}	52.55 ± 7.1^{b}	0.03	0.972
Lipids	19.69 ± 1.8^{a}	26.62 ± 4.4^{b}	30.23 ± 5.41^{b}	1.66	0.231



4(a)



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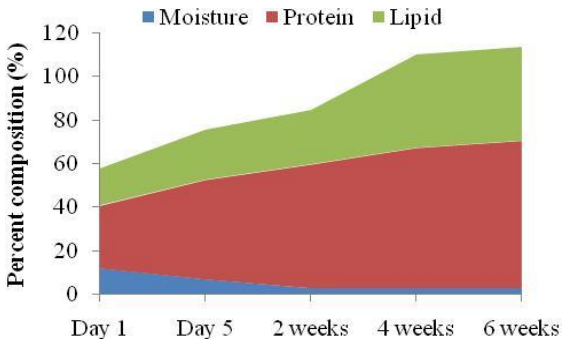

4(c)

Figure 4 a,b &c. Proximate composition and their trends in Nile Tilapia fillets treated in 3%(a), 5%(b),10%(c) of ascorbic acid over drying period

Control

The untreated sample showed a significant decrease in the three proximate constituents, over the six weeks of drying as in Fig 5, as compared to treated samples which showed an increase in both crude lipid and protein.

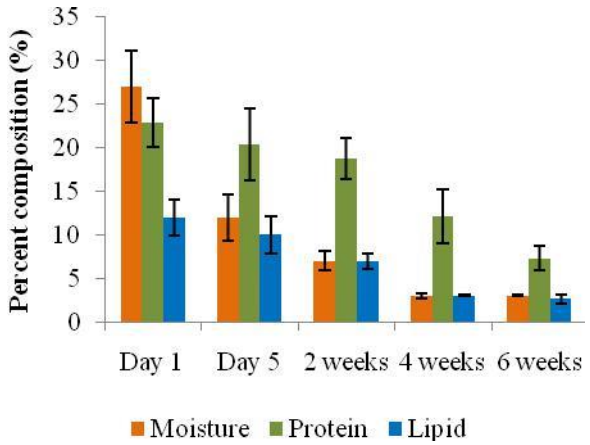

Figure 5. Mean Proximate composition in untreated samples of Nile Tilapia fillets during the 5 sampling sessions

ANOVA

One- way ANOVA and Turkey *Post- hoc* tests done on the merged means after six weeks of study, revealed a significant difference between crude lipids and protein between the two preservatives and the control (p= 0.004 and p=0.000) respectively. Moisture on the other hand, did not show any significant difference between the two preservatives and also with the control.

Table 3. Mean percent composition ± SE of mean after six weeks of study

	Lactic Acid	Ascorbic acid	Control	F	p
Moisture	7.00 ± 1.94^{b}	5.18 ± 1.56^{b}	10.4 ± 4.47^{c}	0.81	0.469
Protein	42.00 ± 2.96^{D}	51.24 ± 6.69^{c}	16.29 ± 2.86^{a}	15.93	0.000*
Lipids	16.8 ± 3.21^{b}	25.6 ± 3.87^{c}	6.9 ± 1.86^{a}	9.11	0.004*

Different superscript letters show means that vary from each other where * shows significant variation between the treatments.

DISCUSSIONS

Moisture Content

It is generally understood that microorganisms need water in an available form to grow in food products and cause spoilage (Huss, 2000). This reason therefore puts moisture at the top of the list as far as proximate composition and shelf life of Nile Tilapia products are concerned. Thus, the control of moisture content in foods is one of the oldest and widely exploited preservation strategies. In this study, the moisture content of raw tilapia in fresh basis was 77.9%. This value agreed with Connel (1990) who found that the moisture content of fresh farmed *Oreochromisniloticus* fish was (60% - 80 %). Moisture content in fish varies within species and between species due to changes in seasons, age, size, sex, feed composition among others (Huss, 2002).The general reduction in moisture observed in all the samples is attributed to the drying mechanism, which involves temperature from solar energy raising the fillet temperature and bringing about sweating which is released to the outside by action of wind (Akintola, 2006).

The insignificant differences in moisture content ($p > 0.05$) observed within and between treatments agree with earlier works of Arekemase *et al*. (2012) who observed a constant decrease in moisture of Tilapia during the 8 weeks of drying. This therefore means concentration and type of preservative has no effects on moisture of tilapia, but method of drying has, as echoed by Ufodike *et al.* (1989). Effectiveness of sun drying, however, varies with; thickness of the fish or fillet, wind velocity, relative humidity, temperature, time of exposure and fish species.

Protein Content

Protein forms a fundamental aspect in human diet especially as body building blocks and energy reserves (WHO, 2007).They are a combination of various amino acids. Nutritionists therefore recommend fish as most preferred source due to the high levels-15%- 28% as compared to poultry-18%-20%,and the fact that fish protein contains all the essential amino acids just like other sources but lysine and methionine are higher (Connel, 1990).This combination of amino acids is highly suited to man‘s nutrition requirements. According to FAO (2005), in quality aspects protein content is one of the physiochemical attribute used to ascertain fish quality and determine shelf life.

Fish protein however undergoes degradation resulting from endogenous and exogenous proteolytic enzymes (Engrang, 2001).These enzymes are found in all tissues in the muscle, and are protein in nature. Their significant activities in fish muscles are of importance in protein turnover (Huss, 2000). FAO (2005) established that after death, the biological function of these enzymes is lost and they resolve to muscles hydrolyzing them by cleaving the peptide bonds and ushering in rigo-mortis, first step of deterioration process. Activities of these enzymes have reported to depend majorly on pH and temperature of surrounding environment, as documented by Huss (2000; 2002). Thus by manipulation of surrounding temperature and pH, protein degradation can be retarded or stopped.

With regard to the data shown in Table 1, the protein content was 13.65 % in fresh fish. This agrees with the findings of Connel (1990), who reported that flesh from healthy farmed fish contained (15-28%) protein. In this study, there was constant increase in protein in all the treatments. Fillet preserved in 10% ascorbic acid recorded the highest crude protein content and this was in accordance with the findings of Arekemase (2012) . The increase in protein levels when compared with the raw tilapia, suggests that protein nitrogen was not lost during drying and thus increase in crude protein level because drying resulted in concentrating crude protein components in tilapia (Arekemase, 2012). And this concentration effect is basically as a result of loss of moisture by the dried fish as explained by Akinola *et al.* (2006) that, the percentage of total protein, lipid and ash contents of dried tilapia increased due to water loss during drying. This could also be due to effects of the organic acids in reducing pH to less than 5.Both lactic and ascorbic have pH lower than 5 but ascorbic has pH of 3 and 5 for lactic acid. For this reason, ascorbic acid proves effective in maintaining proteins in Tilapia fillets.

The protein content of the untreated fish samples decreased from 13.65% to 7.3%.This study shows that storage time causes decrease in the protein content of tilapia (Fig.5) which agreed with earlier work of Ufodike *et al.* (1989) who reported a decrease in crude protein of unpreserved *Oreochromisniloticus* dried under the sun. These workers attributed the decrease to hydrolysis of protein during the process of autolysis by proteolytic enzymes.

The higher crude protein content in fillets preserved in lactic and ascorbic acids is important from a dietary point of view, as this will reduce malnutrition in the county.

Reduction in the percentage crude protein of the control samples, during the period of storage could be due to degradation of the initial crude protein to more volatile products such as Total Volatile Bases (TVB), Hydrogen sulphide and Ammonia by endogenous enzymes. Changes observed in protein content during storage may also have been due to leaching out of some extractable soluble protein fractions (Huss 2002).

Fat Content

Fish is known for a long time to be an important part of human nutrition, because of is importance in brain development, its role in protecting against heart diseases, maintenance of good sight, skin and nervous system (WHO, 2007). All these are attributed to the high content of lipids in fish. According to Frankel (1989), Fatty acids that are more dominant in fish muscles are the long-chained polyunsaturated Fatty Acids (PUFA) especially the Omega-3 fatty acids.

Due to the unsaturated nature of these fatty acids, they become easily oxidized by species of oxygen, thus becoming susceptible to oxidation, during processing and storage (Badii *et al*., 2002). In principle, lipid oxidation is a process where free radicals steal electrons from the lipids in cell membrane, resulting in cell damage and rancidity as defined by Huss (2000).Lipid oxidation is an important index in denoting quality of fish products. This is so because oxidized lipids not only spoil smell and taste of product but generates

a lot of harmful biological effects to humans. Oxidation products like peroxides, aldehydes have reported carcinogenic effects, according to WHO (2007).

Oxidation in lipids results to lower lipid levels as these are reduced to products like; peroxides, aldehydes, ketones among others. Oxidation can be enzymatic, autoxidation or photoxidation depending with the initiator, but autoxidation caused by species of oxygen, accounts for highest fish lipids losses (Salaudeen *et al.*, 2010).

The three stages reaction involves the PUFA reacting with the initiator to form radicals, then these radicals reacting with other fatty acids to propagate a chain reaction and finally the high concentration radicals reacts with each other to form a non-radical compound.

However, according to Badii *et al*. (2002) there are substances that have been used to arrest lipid autoxidation in fish and these are called antioxidants. They do this by breaking the reaction chain and getting oxidized themselves. Some of these include organic acids like acetic, lactic and ascorbic acid and aromatic amines like butyl-hydroxynisole and butyl-hydroxytoulene. These aromatic amines have however been reported carcinogenic despite their wide usage according to the United States Food and Drug Authority. Cherrington (1991), reported better results with use of ascorbic acid in prevention of lipid oxidation in various fish species and attributed this to the fact that ascorbic acid is a bi-acid and thus reacts rapidly with oxidants making it an outstanding donor antioxidant.

As illustrated in Table 1, it is clear that fat content of fresh tilapia was 13.8% .This was also in agreement with Connel (1990) who found out that fresh tilapia contains 2% -13%. There was significant difference in fat contents among lactic and ascorbic acids. This variation might be due to different antioxidation effect of the two preservatives, with ascorbic acid proving to be more effective in retarding lipid oxidation by registering higher levels of crude fat in all the sampling days. The differences between the concentrations was not significant ($p>0.05$) in both the preservatives (Table 2 and Fig 5,6,7).

There was low fat content recorded in the control sample, which reduced over the drying period. This agreed with earlier work of Underland *et al*. (2005)who reported as low as 1.7% lipid after 8 weeks of drying under the sun. This decrease might be due to loss of fat with excluded fluids with osmotic effect as a result of sun drying and also due to oxidation and hydrolysis of lipid which results to products such as peroxides, aldehydes, ketones and free fatty acids. All these depicts quality deterioration (FAO 2005). Amos (2007) also agreed that lipid hydrolysis results in the formation of free fatty acids which are not nutritionally significant.

After the drying process, the percent of fat increased significantly due to loss of moisture and an increase in the dry matter content per unit of weight following sample dehydration. This increase was evident in both the preservatives though ascorbic acid recorded high levels by the end of the sampling period.

The inverse relationship observed between moisture and lipids and also with protein, has also been reported by Ufodike *et al.* (1989) as well as Arekemase *et al.* (2012). Huss

(2002) reported that moisture content was low when other constituents (lipid, protein and carbohydrate) were high in Labeorohita. This is as a result of reduced moisture through dehydration which leads to concentration in dry matter.

CONCLUSIONS

This study establishes that processing and storage significantly affected the proximate composition of Nile Tilapia. The most important is the general reduction effect on the moisture content of the control samples, which is an index of perishability.

Aside the effect of the two antioxidants which increased protein and fat loss upon storage, sun drying maintained and concentrated the crude protein content and increased fat content as a result of increased dry matter. It also reduced moisture for microbial growth thus rendering the fillets stable and with extended shelf life.

From the research finding, it is concluded that pretreatment of harvested fish with lactic or ascorbic acids, then followed by drying under clean environments could help extend its shelf life and nutritive value. These findings are in agreement with Ankitola *et al.* (2006) who studied the fermented and dried fish species of Sudan, Labeo, Tilapia and Clarias species and found, the chemical composition of moisture was ranged between 7.1- 9%, protein 55- 65%, fat 11.3-18.2% and ash 12.5 - 22.9%.

The results obtained have also reinforced that although there is a significant difference in effects of lactic and ascorbic acids on proximate composition, their different concentrations have no significant differences.

RECOMMENDATIONS

From the research findings, it can be therefore recommended that, fish farmers and microprocessors should consider pre-treating harvested Nile Tilapia with 10% ascorbic acid for 30 minutes then sun drying for as long as market is established. It is evidenced from the results that this method extends shelf life by retarding spoilage and results to high nutritive fish products. The high post-harvest losses documented in world‘s and national fisheries, will through this method reduce significantly hence aquaculture output reaches where it ought to be hence, boosting its economic contribution. This naturally available and cheap organic preservative will replace the unavailable and expensive commercial ones which possess health threats to humans.

Future detailed studies on effects of these organic acids on their ability to enhance other nutritive and organoleptic attributes of quality like color, texture and flavor ,is recommended as some observations were noted on different color changes during this study. Similar research should be extended to other farmed species to ensure wholesome improvement of aquaculture as a sector.

Processing industries and other vital fisheries stakeholders should device ways of extracting ascorbic acid and other organic preservatives from nature so as to make these even more available and cheaper to local fish farmers.

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BIO-DATA

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