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## Effect of Drying Methods and Pre- Treatments on Shelf Life and Microbial Quality of Fish (*Oreochromis Niloticus*, *Pylodictis Olivaris* and *Cyprinus Carpio*) Species Commonly used in Gambella Region

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# Effect of Drying Methods and Pre-Treatments on Shelf Life and Microbial Quality of Fish (*Oreochromis Niloticus*, *Pylodictis Olivaris* and *Cyprinus Carpio*) Species Commonly used in Gambella Region

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**Abstract-** A study was conducted to assess the effect of drying methods and pre-treatments on shelf-life and microbial quality of dried fish. The experiment was conducted in factorial arrangement of 2×3×2 with two drying methods (sun and oven drying,) three fish species (tilapia, cat fish and carp) and two preservatives treatment (garlic and ginger juice) laid out in Completely Randomized Design (CRD). Fresh fillets were analyzed for their microbiological quality. Drying reduced the moisture contents making it safe for long term storage. The dried fillets were stored at ambient condition and the samples were analyzed for microbial status every twenty days starting from the end of drying operation. Fresh fillet and untreated dry fillet were used as control. In the fresh fillets, a high load of aerobic bacteria of 5.11 log<sub>10</sub> cfu/g was observed on carp, and *E.coli* was detected in all three species whereas no *Salmonella* spp. was detected at all. Regarding freshly dried fillets, high load of aerobic bacteria (5.82 log<sub>10</sub> cfu/g) was observed in untreated tilapia whereas the initial load of moulds was <1 log<sub>10</sub> cfu/g in all freshly dried fillets. After sixty days of storage, the load of aerobic bacteria and moulds was 8.03 and 7.92 log<sub>10</sub> cfu/g, respectively, in untreated sundried carp, tilapia and cat fish fillets, higher than that in treated samples.

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## I. INTRODUCTION

Different species of fish had benefits for the world as food for human consumption (Mdegela *et al.*, 2010). The global traditional and improved fish processing technologies in aquatic food production had more than double since 1970, with a total of approximately 93.2 million metric tons in 1997 (Akinneye *et al.*, 2007).

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The drying of different species of fish was one of world's oldest known preservation methods, and dried fish had a storage life. Freshwater fish should be preserved to assure best possible consumption quality, provide a proper form of semi-processed final product, assure safety of products, and reduce wastes to the barest possible extent. To keep the quality and safety of fish, it was essential to minimize water activity in fish in appreciable quantities in good condition until its use is required (FAO, 1990). Akinola *et al.* (2006) reported that different types of preservation methods like; drying, smoking, freezing, chilling and brining were used for prolonging shelf-life of fish products. The techniques of preservation also important for thousands of tons of fish wasted annually through poor handling and unhygienic treatment as well as absence of improved technologies for processing and preservation. Fish is highly susceptible to deterioration without any preservatives or processing measures (Clucas and Sctcliffe, 1987 and Okonta and Ekelemu, 2005).

The common methods of fish preservation in Ethiopia include drying, salting, smoking, and their combinations (Okorely and Kwartan, 2006). Sun drying was one of the traditional methods employed to preserve fish in Gambella region. It had been observed as the most convenient and cheapest form of preservation (Eyo, 1986).

Major problems with traditional sun drying were loss of quality due to contamination and infestation by insects, and inappropriate drying rates (Sablani *et al.*, 2002). These made change in nutritional and organoleptic characters of traditionally processed fish (Afolabi *et al.*, 1984). Akinola *et al.* (2006) reported that in the process of traditional methods, lack of control over the drying rate, sometimes results in over-drying or under-drying, and expose the fish to unexpected winds, dust, dirt, insect infestation, and contaminants such as flies. Because of fish dried slowly and un-hygienically in direct sunlight in the absence of moving air, possibility of contamination was obvious in dried fishes (Siukumar *et al.*, 1995). Therefore, the main objective of

the present study was to examine the effect of drying methods on shelf-life and pretreatments on microbial quality of three fish species commonly used in Gambella region.

## II. MATERIALS AND METHODS

### a) Experimental Location

Three experimental fish species namely tilapia (*Oreochromis niloticus*), flathead cat fish (*Pylodictis olivaris*) and carp (*Cyprinus carpio*) were collected from Alwero reservoir, Abobo District, Anywaa zone, Gambella regional state south west Ethiopia, where drying and pre-treatments were conducted. Microbiological analyses of fresh and dried samples including analysis of bacterial count (aerobic plate count and enumeration of Enterbacteriaceae) and detection of pathogens (*Escherichia coli* and *Salmonella* spp.) were conducted in veterinary microbiology laboratory of the same University.

### b) Experimental Materials

**Fish:** The experimental materials included three fish species namely, tilapia (*Oreochromis niloticus*), cat fish

(*Pylodictis olivaris*), and common carp (*Cyprinus carpio*). These were obtained from the Alwero-reservoir which is located in Abobo District, Anywaa zone, Gambella regional state, the Southwest part of Ethiopia. After the fish were caught, selection of right quality fish was done based on age and type followed by descaling and deboning. Total of 14.69 kg fillets of the three fish species with 4.19 kg of tilapia, 5.51 kg from cat fish and 4.99 kg from carp were collected for this study.

**Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*):** This was obtained from the local markets at Gambella town. A total of 12 kg ginger and 12kg of garlic were cleaned, washed, and stored in a refrigerator.

### c) Experimental Design and Treatment Planning

The experiment of this study was laid out in a factorial arrangement of 2 x 2 x 3 in a completely randomized design (CRD) with three replications. These were three species of fish (tilapia, cat fish and common carp) with two types of preservatives (garlic, and ginger) and two methods of drying (oven drying and sun drying). The controls are fresh and dried fish fillets with no treatment.

Table 1 : Experimental planning

| Methods     | Species |       |          |       |             |       |
|-------------|---------|-------|----------|-------|-------------|-------|
|             | Tilapia |       | Cat fish |       | Common carp |       |
|             | Ga      | Gi    | Ga       | Gi    | Ga          | Gi    |
| Sun drying  | SGaT    | SGiT  | SGaC     | SGiC  | SGaCr       | SGiCr |
| Oven drying | OGaT    | OGiT  | OGaC     | OGiC  | OGaCr       | OGiCr |
| Control     | Fresh   | Dried | fresh    | Dried | fresh       | Dried |

Where: S (sun drying), O (oven drying), T (tilapia), C (cat fish), Cr (common carp), Ga (garlic), and Gi (ginger).

### d) Sample Preparation

**Fish fillets preparation:** The process of fish slices preparation was carried out immediately after sufficient experimental fish was obtained. The descaled and deboned fish was split into fillets or cuts (slices).

**Ginger juice preparation:** The cleaned ginger was chopped and minced before being used for the treatment. Ginger juice was prepared based on FAO (1990 and 2010) for traditional fish drying of Qwanta.

**Garlic juice preparation:** The clean garlic was chopped and minced (Sallam *et al.*, 1995).

**Pre-drying treatment of fish fillets:** The slices of fish samples about (1000 g) of each fish type were submerged in 1000 ml (1:1 w/v) of ginger or garlic juice in flat bowl of 2000 ml capacity (Suleiman, 2010 and Wilson, 1981).

### e) Data Collection about Microbial quality

Microbiological analyses were done to assess aerobic plate count (APC), Enterbacteriaceae count, and presence of the pathogens such as *Escherichia coli* and *Salmonella* spp:

The microbiological analyses were done on fresh fish fillets as well as on dried ones at the beginning

of the experiment. Similar microbiological analyses were conducted on dried fillets of fish within twenty days up to the storage period of sixty days.

### f) Bacterial count

Detection of presence of the pathogens *Escherichia coli* and *Salmonella* spp. was done by taking samples from the dilution level 1:10. Aerobic plate (APC) and Enterbaterceae counts, however, were done by taking samples from both 10<sup>-5</sup> and 10<sup>-6</sup> dilution levels. The total numbers of moulds were counted by taking scraps from the colony counted under APC. Aerobic plate count, enumeration of Enterbacteriaceae, and counting of molds were conduced according to the standards.

### g) Detection of pathogens

#### i. Detection of *Escherichia coli*

Detection of *Escherichia coli* was done according to ISO (2006) method 4831. Samples were taken from 1:10 (10<sup>-1</sup>) dilution level and presence of *Escherichia coli* was done by conducting three incubation steps transferring 1 ml representative from Lauryl Triptose (LT) broth.

ii. *Detection of Salmonella spp.*

In the detection on *Salmonella Spp* 5 incubations were done sequentially. Firstly samples were pre-enriched by incubating at 37°C for 48h. Secondly samples were incubated at 41°C for 24 h (ISO, 2002, method 6579). Thirdly samples were incubated at 37°C for 24 h (ISO, 2002, method, method 6579). Fourthly samples were incubated at 37°C for 24 h (ISO, 2002, method, method 6579) and fifthly about 10% of typical colonies grown on Nutrient agar were transferred and plated onto appropriately marked duplicate plates of Urea agar medium (Lab M Limited, UK). Samples were then incubated at 37°C for 24 h. Finally, smooth colonies (colorless, translucent or pale colonies) that were 2-4 mm in diameter were considered as a positive test for the presence of *Salmonella spp.* In the samples analyzed (ISO, 2002, method 6579) (Libby, 1975; Maeda *et al.*, 1997).

h) *Experimental design and statistical analysis*

Statistical analysis conducted on all data collected after the conversion from CFU to log form to test for significance difference among treatment means. Analysis of variance was performed by one-way ANOVA procedures with statistical software (version SAS 9.1) and means were evaluated at the P<0.05 level of significance using fisher's LSD and Duncan's new multiple range test (AOAC, 2000).

Table 2 : Occurrence of *Escherichia coli* and *Salmonella spp.* in raw fish

| Species of fish | Pathogens               |                        |
|-----------------|-------------------------|------------------------|
|                 | <i>Escherichia coli</i> | <i>Salmonella spp.</i> |
| Tilapia         | +ve                     | -ve                    |
| Cat fish        | +ve                     | -ve                    |
| Common carp     | +ve                     | -ve                    |

Where, -ve shows the absence of the pathogens and +ve shows the presence of the pathogens.

According to Spencer and Clifford (2000), *E. coli* was associated with animal products especially aquatic animals due to water pollution. Mendel (1999) stated that *E.coli* was an infectious pathogenic bacterium originally found in intestines of humans and other animals. This may explain the detection of the pathogen *E. coli* in the fresh fillets. According to Haberg *et al.* (1994), *Salmonella spp* is also most commonly associated with contaminated animal products (Salmon, 1885). It is pathogenic infectious bacteria that caused due to the lack of cleaning or presence of debris in water body that contaminate the fish. However, Salmon (1885) stated that animal products were with lower degree of contamination under appropriate cleaning and sanitation. This may explain the absence of the pathogen *Salmonella spp* in fresh fillets.

III. RESULTS AND DISCUSSION

The present study was conducted to evaluate the effect of sun drying, oven drying and two types of preservatives (garlic and ginger) juices on microbial quality and shelf-life of tilapia (*Oreochromis niloticus*), cat fish (*Pylodictis olivaris*), and common carp (*Cyprinus carpio*) fish fillets. The selected pathogens (*E. coli* and *S. salmonella*) and enumeration of aerobic total count, entro bacteriae and moulds were observed.

Results of this study clearly revealed that microbial growth was increasing through the storage period. Standards guidelines often use much lower bacterial population as indices of acceptability. In a recent European study by consumers, fish was assumed "not to be in a good enough condition to be stored for long" when total plate count were 10<sup>6</sup> cfu/g (EU, 1995). Enumeration of Enterbacteriaceae, aerobic plate counting, total mould estimation and detection of pathogens were conducted under this study to estimate microbial load both in fresh and dried stored fillets of the common three species of fish.

a) *Detection of pathogens in raw fish*

Table 2 shows detection of pathogens namely *Escherichia coli* and *Salmonella* species in fresh fillets of the three species used in this study. It is found that *E. Coli* were detected whereas *Salmonella* species was not in the fresh fillets of the three species.

b) *Detection of pathogens in dried fillets of fish*

Occurrence of *Escherichia coli* and *Salmonella spp.* in treated and untreated fish fillets over the storage period of 60 days are presented in Table 3.

Table 3 : Detection of pathogens on differently treated dried fish stored for sixty days

| MD | Treatment | Spp. | Pathogens            |                  |                  |                  |                        |                  |                  |                  |     |
|----|-----------|------|----------------------|------------------|------------------|------------------|------------------------|------------------|------------------|------------------|-----|
|    |           |      | <i>E.coli</i>        |                  |                  |                  | <i>Salmonella spp.</i> |                  |                  |                  |     |
|    |           |      | Storage period(days) |                  |                  |                  |                        |                  |                  |                  |     |
|    |           |      | 0                    | 20 <sup>th</sup> | 40 <sup>th</sup> | 60 <sup>th</sup> | 0                      | 20 <sup>th</sup> | 40 <sup>th</sup> | 60 <sup>th</sup> |     |
| SD | control   | T    | +ve                  | +ve              | +ve              | +ve              | +ve                    | +ve              | +ve              | +ve              | +ve |
|    |           | C    | +ve                  | +ve              | +ve              | +ve              | -ve                    | -ve              | +ve              | +ve              |     |
|    |           | Cr   | +ve                  | +ve              | +ve              | +ve              | +ve                    | +ve              | +ve              | +ve              |     |
|    | Garlic    | T    | -ve                  | -ve              | +ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | C    | -ve                  | -ve              | -ve              | +ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | Cr   | -ve                  | -ve              | -ve              | -ve              | -ve                    | +ve              | -ve              | -ve              | -ve |
|    | Ginger    | T    | +ve                  | +ve              | +ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | C    | -ve                  | -ve              | -ve              | +ve              | -ve                    | -ve              | +ve              | +ve              |     |
|    |           | Cr   | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
| OD | control   | T    | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | +ve |
|    |           | C    | -ve                  | -ve              | -ve              | +ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | Cr   | -ve                  | -ve              | -ve              | +ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    | Garlic    | T    | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | C    | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | Cr   | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    | Ginger    | T    | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |
|    |           | C    | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | +ve |
|    |           | Cr   | -ve                  | -ve              | -ve              | -ve              | -ve                    | -ve              | -ve              | -ve              | -ve |

Where, spp.= (species), MD= method of drying, SD= sun drying, OD= oven drying, -ve= shows the absence of the pathogens and +ve= shows the presence of the pathogens, T (tilapia), C (cat fish), Cr (carp), 0=Zero month storage period was the storage before the dried fish fillets were stored under ambient condition.

*Escherichia coli* were detected in all untreated sundried fillets of the three fish species from zero days to the 60 days storage periods (Table 3). However, *Salmonella spp.* was detected only in two of the untreated (tilapia and carp) sun dried fillets from zero days to the 20<sup>th</sup> day storage but it was detected in all untreated sundried fillets of the three fish species from 40<sup>th</sup> days to the 60<sup>th</sup> day's storage periods. Untreated sun dried fillets of the three fish species were highly susceptible to *E. coli* and *Salmonella spp* throughout the storage period of 60 days than treated fillets. This result showed that preservatives i.e. garlic and ginger play inhibitory effect on the drying of the fish fillets of the three experimental species. Since, garlic and ginger categorized under bacteria growth inhibitors, then according to Zaika (1988) inhibitors may reduce the levels of microbial growth in foods. The absence of *Escherichia coli* and *Salmonella spp.* in most of the treated dried fillets of three species throughout the storage periods (0-60 days) could necessarily be due to the inhibitory effects of garlic and ginger treatments.

Dried fillets may cross-contaminated during storage/ through handling techniques.

c) Enumerations of microorganisms in fresh fish

The Aerobic plate and *Enterbacteriaceae* of the respective fresh fillets of the three fish species were indicated in Table 4.

Table 4 : Enterbacteriaceae and Aerobic plate counts in raw fish

| Experimental fish species | Type of Bacterial count(log <sub>10</sub> cfu/g) |                         |
|---------------------------|--|-------------------------|
|                           | Aerobic plate count (APC)                        | Enterbacteriaceae count |
| Fresh tilapia             | <1 log <sub>10</sub>                             | <1 log <sub>10</sub>    |
| Fresh flat head cat fish  | 5.09±0.04 <sup>a</sup>                           | 5.00±0.02 <sup>a</sup>  |
| Fresh common carp         | 5.11±0.03 <sup>ba</sup>                          | <1 log <sub>10</sub>    |
| CV                        | 1.90   | 3.03                    |
| LSD                       | 0.19   | 0.29                    |

Where, <(less than), log<sub>10</sub> (logarism in base ten), CFU=colony forming units, CV=coefficient of variances, LSD=least significant differences.

Very few APC and Enterbacteriaceae count were found in fresh tilapia that reported as <1 log 10 cfu/g. However, mean value of APC of 5.09 log<sub>10</sub> cfu/g and mean Enterbacteriaceae count of 5.00 log<sub>10</sub> cfu/g were found in fresh cat fish. The mean APC of 5.11 log<sub>10</sub> cfu/g was found in fresh carp whereas very few Enterbacteriaceae count was found in fresh fillets of the same spp which resulted as < 1 log 10 cfu/g in the same table 4. The low mean values of APC and Enterbacteriaceae count observed in the fresh fish were attributed to dilution levels (10<sup>-5</sup> and 10<sup>-6</sup>) from which samples were used for plating. According to Maurine and James (2001), suitable colony counting range is 25-250.

When plates of all dilutions have no colonies it is reported as less than 25 colonies estimated count. Existence of APC and Enterbacteriaceae counts in fresh fillets of fish species may be due to the handling and cross-contaminations, through the process of deboning and filleting the fish.

An estimation of the total number of microorganisms: named Aerobic plate count and Enterbacteriaceae enumerations of freshly caught fish were laid within (10<sup>2</sup>-10<sup>6</sup> cfu/g) of an acceptability index in standards, guidelines and specifications (EU, 1995). Therefore, the results of the total Aerobic plate count and Enterbacteriaceae in present study agreed with this standard acceptability index. Similar results about these micro-flora were also observed in reports of Huss *et al.*, (1997) and Gram and Dalgaard, (2002) in which the number of microbials fitted the standards of load in shelf-life of fish (10<sup>7</sup>-10<sup>8</sup>). According to the Parallel Food Testing in the European Union (EU, 1995), freshly caught fish should contain a diverse micro-flora APC of 10<sup>2</sup> -10<sup>6</sup> cfu/g. These values are usual on whole fish and cut fillets.

d) Aerobic plate counts in dried fillets of fish

The total aerobic plate counts within the interval of twenty days in sixty days stored products are presented in Table 5. In that the minimum aerobic plate counts recorded in sample, the safe the products are illustrated within the Table 5. As compared to the Aerobic plate counts in the fresh fillets reported in Table

5, an increase in aerobic plate count (APC) was observed in the treated and untreated fillets over the storage periods (0-60<sup>th</sup> days).

The maximum and minimum APC of zero day storage were recorded in untreated sundried tilapia and treated tilapia with 5.82 and 5.11 log<sub>10</sub> cfu/g, respectively. After the storage of 20 days the fillets were with the minimum 5.51 (untreated oven dried tilapia) and maximum 6.53 (untreated sundried carp) log<sub>10</sub> cfu/g. Similarly the maximum (7.11) and the minimum (6.05) log<sub>10</sub> cfu/g after 40<sup>th</sup> day storage were recorded in untreated sundried carp and garlic treated oven dried cat fish fillets. At the end of the 60<sup>th</sup> month storage the lowest (7.01) and the highest (8.03 log<sub>10</sub> cfu/g) APC were recorded in garlic treated oven dried cat fish and untreated sundried carp, respectively.



Table 5 : Aerobic plate count of dried fillets stored for sixty days

| Method of drying | Treatment | Spp. | Aerobic plate count(log <sub>10</sub> cfu/g) |                         |                           |                            |
|------------------|-----------|------|--|-------------------------|---------------------------|----------------------------|
|                  |           |      | Storage period (days)                        |                         |                           |                            |
|                  |           |      | 0  | 20 <sup>th</sup>        | 40 <sup>th</sup>          | 60 <sup>th</sup>           |
| Sun drying       | Control   | T    | 5.82±0.06 <sup>c</sup>                       | 6.46±0.12 <sup>c</sup>  | 7.00±0.06 <sup>e</sup>    | 8.00±0.03 <sup>f</sup>     |
|                  |           | C    | 5.75±0.04 <sup>c</sup>                       | 6.37±0.06 <sup>c</sup>  | 7.09±0.07 <sup>e</sup>    | 8.01±0.05 <sup>f</sup>     |
|                  |           | Cr   | 5.80±0.03 <sup>c</sup>                       | 6.53±0.23 <sup>c</sup>  | 7.11±0.06 <sup>e</sup>    | 8.03±0.03 <sup>f</sup>     |
|                  | Garlic    | T    | 5.43±0.05 <sup>b</sup>                       | 5.81±0.06 <sup>b</sup>  | 6.21±0.02 <sup>cdb</sup>  | 7.16±0.02 <sup>dec</sup>   |
|                  |           | C    | 5.20±0.03 <sup>a</sup>                       | 5.72±0.08 <sup>ab</sup> | 6.19±0.04 <sup>cdb</sup>  | 7.14±0.04 <sup>dec</sup>   |
|                  |           | Cr   | 5.28±0.06 <sup>ab</sup>                      | 5.58±0.06 <sup>ab</sup> | 6.11±0.06 <sup>cab</sup>  | 7.07±0.06 <sup>abc</sup>   |
|                  | Ginger    | T    | 5.25±0.01 <sup>ab</sup>                      | 5.61±0.09 <sup>ab</sup> | 6.09±0.03 <sup>ab</sup>   | 7.05±0.03 <sup>ab</sup>    |
|                  |           | C    | 5.20±0.12 <sup>a</sup>                       | 5.68±0.10 <sup>ab</sup> | 6.12±0.04 <sup>cadb</sup> | 7.08±0.04 <sup>adbc</sup>  |
|                  |           | Cr   | 5.20±0.03 <sup>a</sup>                       | 5.66±0.03 <sup>ab</sup> | 6.11±0.03 <sup>cab</sup>  | 7.06±0.03 <sup>abc</sup>   |
| Oven drying      | Control   | T    | 5.14±0.05 <sup>a</sup>                       | 5.51±0.06 <sup>a</sup>  | 6.23±0.06 <sup>cd</sup>   | 7.18±0.06 <sup>de</sup>    |
|                  |           | C    | 5.20±0.06 <sup>a</sup>                       | 5.70±0.06 <sup>ab</sup> | 6.10±0.03 <sup>ab</sup>   | 7.05±0.03 <sup>abc</sup>   |
|                  |           | Cr   | 5.14±0.01 <sup>a</sup>                       | 5.54±0.10 <sup>ab</sup> | 6.11±0.05 <sup>cab</sup>  | 7.06±0.05 <sup>abc</sup>   |
|                  | Garlic    | T    | 5.11±0.12 <sup>a</sup>                       | 5.57±0.10 <sup>ab</sup> | 6.24±0.03 <sup>d</sup>    | 7.19±0.03 <sup>e</sup>     |
|                  |           | C    | 5.14±0.10 <sup>a</sup>                       | 5.55±0.15 <sup>ab</sup> | 6.05±0.03 <sup>a</sup>    | 7.01±0.03 <sup>a</sup>     |
|                  |           | Cr   | 5.12±0.06 <sup>a</sup>                       | 5.55±0.02 <sup>ab</sup> | 6.14±0.03 <sup>cadb</sup> | 7.10±0.03 <sup>adbec</sup> |
|                  | Ginger    | T    | 5.17±0.01 <sup>a</sup>                       | 5.75±0.06 <sup>ab</sup> | 6.11±0.03 <sup>cadb</sup> | 7.07±0.3 <sup>abc</sup>    |
|                  |           | C    | 5.16±0.01 <sup>a</sup>                       | 5.58±0.06 <sup>ab</sup> | 6.20±0.03 <sup>cdb</sup>  | 7.16±0.03 <sup>dec</sup>   |
|                  |           | Cr   | 5.12±0.10 <sup>a</sup>                       | 5.59±0.12 <sup>ab</sup> | 6.17±0.04 <sup>cadb</sup> | 7.13±0.04 <sup>dec</sup>   |
| CV               |           | 2.06 | 2.94   | 1.21                    | 0.92                      |                            |
| LSD              |           | 0.18 | 0.28   | 0.13                    | 0.11                      |                            |

Where, LSD= list significant difference, CV=coefficient of variation, Spp.=species, T=tilapia, C=cat fish, Cr=common carp, 0-60= Zero day up to sixty days storage period, values are mean ±SE and mean values followed by the same letter in a column are not significantly different at 5% level of significance.

All the fillets of fish had APC>5.0 log<sub>10</sub> cfu/g. No significant differences (P>0.05) in APC were observed between the untreated sundried fish fillets of the three species throughout the storage periods (0-60<sup>th</sup> days). However, significant differences (P<0.05) were recorded between the untreated sundried fillets of the three species and all treated and untreated oven dried fillets of the respective species throughout the storage period of 60 days.

These significant variations between the untreated sundried fillets and all treated fillets showed that garlic and ginger have inhibitory effects on growth of these microorganisms through drying of the fish fillets of the three experimental fish species. The minimum number of APC in untreated oven dried fillets of the three species throughout the storage periods were due to the inhibitory effect of oven drying by reducing the water activity of the fillets lower than sun drying. All the treated fillets of fish reach at 10<sup>6</sup> cfu/g after the 40<sup>th</sup> day storage and it can be concluded that it is not appropriate to stored for such a long period.

The untreated sundried fillets reach points of sensory rejection at 40<sup>th</sup> days storage period were good evidence among the reasons of this thesis work. This was based on a recent European study by consumers, in which fish was assumed “not to be in a good enough condition to be stored for long” when CMT were 10<sup>6</sup> cfu/g (Parallel Food Testing in the European Union: Fish, 1995). After the 60<sup>th</sup> day storage period the load of APC reached 10<sup>7</sup>-10<sup>8</sup> cfu/g. At the point of sensory rejection, the APC of fish products could typically be 10<sup>7</sup>-10<sup>8</sup> cfu/g (EU, 1995).Therefore, this result showed that differently treated fillets have a shelf-life of less than three two months storage period. Nevertheless, standard guidelines and specifications often use much lower CMT as indices of acceptability.

#### e) Enterbacteriaceae count on dried fillets of fish stored for sixty days

All of the fillets from three fish species had Enterbacteriaceae count (EC) of >5.0 log<sub>10</sub> cfu/g (Table 6). The maximum and minimum Enterbacteriaceae

count of zero day storage were recorded in untreated sundried tilapia and oven dried ginger treated tilapia with 5.43 and 5.01 log<sub>10</sub> cfu/g respectively. After the storage of 20 days the fillets were with the minimum Enterbacteriaceae count of 5.64 (garlic treated oven dried cat fish) and maximum Enterbacteriaceae count of 6.04 (untreated sundried tilapia) log<sub>10</sub> cfu/g. Similarly

the highest (6.78) and the lowest E. count (6.45) log<sub>10</sub> cfu/g after 40th day storage were recorded in untreated sundried carp and ginger treated oven dried tilapia fish fillets. At the end of 60th days storage the lowest (7.01) and highest (7.38 log<sub>10</sub> cfu/g) E. count were recorded in ginger treated oven dried tilapia and untreated sundried tilapia respectively.

Table 6 : Enterbacteriaceae count on dried fillets stored for sixty days

| Method of drying | Treatment   | Spp.    | Enterbacteriaceae count(log <sub>10</sub> cfu/g) |                          |                          |                         |                         |
|------------------|-------------|---------|--|--------------------------|--------------------------|-------------------------|-------------------------|
|                  |             |         | Storage period (days)                            |                          |                          |                         |                         |
|                  |             |         | 0  | 20 <sup>th</sup>         | 40 <sup>th</sup>         | 60 <sup>th</sup>        |                         |
| Sun drying       | control     | T       | 5.43±0.04 <sup>b</sup>                           | 6.04±0.02 <sup>d</sup>   | 6.72±0.07 <sup>c</sup>   | 7.38±0.03 <sup>c</sup>  |                         |
|                  |             | C       | 5.38±0.03 <sup>b</sup>                           | 5.99±0.06 <sup>d</sup>   | 6.77±0.03 <sup>c</sup>   | 7.35±0.05 <sup>c</sup>  |                         |
|                  |             | Cr      | 5.39±0.02 <sup>b</sup>                           | 6.01±0.06 <sup>d</sup>   | 6.78±0.05 <sup>c</sup>   | 7.37±0.02 <sup>c</sup>  |                         |
|                  | Garlic      | T       | 5.02±0.05 <sup>a</sup>                           | 5.67±0.06 <sup>bca</sup> | 6.48±0.05 <sup>ab</sup>  | 7.04±0.05 <sup>ab</sup> |                         |
|                  |             | C       | 5.10±0.08 <sup>a</sup>                           | 5.76±0.02 <sup>bca</sup> | 6.51±0.03 <sup>ab</sup>  | 7.07±0.03 <sup>ab</sup> |                         |
|                  |             | Cr      | 5.04±0.06 <sup>a</sup>                           | 5.71±0.05 <sup>bca</sup> | 6.49±0.06 <sup>ab</sup>  | 7.05±0.06 <sup>ab</sup> |                         |
|                  | Ginger      | T       | 5.15±0.07 <sup>a</sup>                           | 5.79±0.03 <sup>c</sup>   | 6.57±0.03 <sup>b</sup>   | 7.13±0.03 <sup>b</sup>  |                         |
|                  |             | C       | 5.02±0.09 <sup>a</sup>                           | 5.75±0.02 <sup>bca</sup> | 6.52±0.01 <sup>ab</sup>  | 7.08±0.01 <sup>ab</sup> |                         |
|                  |             | Cr      | 5.10±0.05 <sup>a</sup>                           | 5.77±0.02 <sup>bc</sup>  | 6.48±0.05 <sup>ab</sup>  | 7.04±0.05 <sup>ab</sup> |                         |
|                  | Oven drying | control | T  | 5.11±0.06 <sup>a</sup>   | 5.77±0.06 <sup>bc</sup>  | 6.53±0.01 <sup>ab</sup> | 7.09±0.01 <sup>ab</sup> |
|                  |             |         | C  | 5.04±0.12 <sup>a</sup>   | 5.66±0.06 <sup>ba</sup>  | 6.47±0.05 <sup>ab</sup> | 7.03±0.05 <sup>ab</sup> |
|                  |             |         | Cr   | 5.04±0.08 <sup>a</sup>   | 5.68±0.01 <sup>bca</sup> | 6.46±0.02 <sup>ab</sup> | 7.02±0.02 <sup>ab</sup> |
| Garlic           |             | T       | 5.14±0.03 <sup>a</sup>                           | 5.72±0.03 <sup>bca</sup> | 6.53±0.03 <sup>ab</sup>  | 7.09±0.03 <sup>ab</sup> |                         |
|                  |             | C       | 5.02±0.03 <sup>a</sup>                           | 5.64±0.03 <sup>a</sup>   | 6.49±0.03 <sup>ab</sup>  | 7.05±0.06 <sup>ab</sup> |                         |
|                  |             | Cr      | 5.09±0.04 <sup>a</sup>                           | 5.74±0.09 <sup>bca</sup> | 6.48±0.03 <sup>ab</sup>  | 7.04±0.03 <sup>ab</sup> |                         |
| Ginger           |             | T       | 5.01±0.02 <sup>a</sup>                           | 5.67±0.03 <sup>bca</sup> | 6.45±0.02 <sup>a</sup>   | 7.01±0.02 <sup>a</sup>  |                         |
|                  |             | C       | 5.02±0.05 <sup>a</sup>                           | 5.70±0.05 <sup>bca</sup> | 6.48±0.05 <sup>ab</sup>  | 7.04±0.05 <sup>ab</sup> |                         |
|                  |             | Cr      | 5.02±0.09 <sup>a</sup>                           | 5.75±0.02 <sup>bca</sup> | 6.47±0.01 <sup>ab</sup>  | 7.03±0.01 <sup>ab</sup> |                         |
| CV               |             |         | 2.07   | 1.35                     | 1.08                     | 0.91                    |                         |
| LSD              |             |         | 0.18   | 0.13                     | 0.12                     | 0.11                    |                         |

Where, LSD= list significant difference, CV=coefficient of variation, Spp.=species, T=tilapia, C=cat fish, Cr=common carp and the values are in mean ±SE.

No significant (P>0.05) differences in Enterbacteriaceae counts were observed among the untreated sundried fish fillets of the three species through out of storage periods of sixty days. Significant differences (P<0.05) were observed between the untreated sundried fillets of the three species and the rest dried fillets of fish species through the storage period of 60 days. No (P>0.05) variations in Enterbacteriaceae counts were observed among the untreated sundried fillets of the three species before storage. Significant (P<0.05) variations between the treated and untreated sundried fillets showed that pre-treatment technology in fish preservation have necessarily inhibitory effects on the number of Enterbacteriaceae count.

The low number of Enterbacteriaceae count in untreated oven dried fillets in relation to the untreated sundried fillets of the three species throughout the storage periods were due to the inhibitory effect of oven drying by reducing the moisture of the fillets much lower than sun drying. The result also showed scrupulous hygienic measures during handling and preparation of spice dilutions before applying them on filleted fillets. All the fillets after the 60<sup>th</sup> day storage period should not be stored further. The fish fillets reach at the point of sensory rejection of 10<sup>7</sup>-10<sup>8</sup> cfu/g after the storage of 60 days showed that shelf-life of differently treated fillets should be less than sixty days storage period (Gram and Dalgaard, 2002).



f) *Total load of moulds in dried fish stored for sixty days*

Results in present study indicated that samples treated with garlic and ginger juices were microbiologically stable than the control samples as these had longer shelf-life and were not covered by visible moldy mass of mycelium during 60 days of storage within twenty days interval of tests. There were steady increases in mould counts as storage period progressed in all the treatments. However, treated fillets showed lower mould counts as compared to untreated fillets in their respective drying methods. Therefore, combination of preservatives with sun drying resulted in variations of microbial levels (i.e. moulds). The standard load of APC and fungi index with total moisture (8.76-13.12%) content at sixty days of storage in this study showed the shelf-life of sundried fillets should be less than three months. This is differed from the findings reported by Jallow (1995). According to Jallow (1995), fish at 10-15% moisture content, reportedly had a shelf life of 3-9 months when stored properly. The differences may be due to the influence of environmental factors.

All the recorded results about total moulds before storage were  $< 1 \log_{10}$  cfu/g. The maximum and minimum total moulds of 20 day storage were recorded in untreated sun dried cat fish and garlic treated sundried of the same species with 6.14 and 5.11  $\log_{10}$  cfu/g respectively. Similarly the lowest (5.14) and the highest total moulds (7.11)  $\log_{10}$  cfu/g after 40<sup>th</sup> day storage were recorded in garlic treated oven dried cat fish and untreated sundried tilapia fillets respectively.

By end of 60th day storage the minimum (6.65) and maximum (7.92  $\log_{10}$  cfu/g) total molds were recorded in garlic treated oven dried cat fish and untreated sundried tilapia and cat fish respectively. No significant differences ( $P>0.05$ ) in total mould counts were observed among the untreated sundried fish fillets of the three spp through out of storage periods of 60 days. The observation of large visible mould mass of mycelium from the first twenty days storage under ambient condition obtained in untreated fillets indicated the effectiveness of garlic and ginger as anti-fungal agents which resulted in extended shelf life of treated fillets. Appropriate treatment with garlic and ginger gave lower load of moulds than untreated sundried samples that could extend shelf life of the dried fish. Due to this, the microbial populations (moulds) for all the treatments observed in this study were within the recommended limits for good quality fish product according to ICSMF, (1986). Significant ( $P<0.05$ ) variations in moulds were observed between untreated sundried and the rest dried fillets of experimental fish within 20 days interval until the 60 days storage.

Table 7 : Total count of moulds on dried fillets stored for sixty days

| Method of drying | Treatment | Spp. | Total count of moulds(log cfu/g)<br>Storage period(days) |                         |                          |                          |
|------------------|-----------|------|--|-------------------------|--------------------------|--------------------------|
|                  |           |      | 0  | 20 <sup>th</sup>        | 40 <sup>th</sup>         | 60 <sup>th</sup>         |
| Sun drying       | control   | T    | <1 log <sub>10</sub>                                     | 6.08±0.05 <sup>e</sup>  | 7.11±0.01 <sup>h</sup>   | 7.92±0.01 <sup>f</sup>   |
|                  |           | C    | <1 log <sub>10</sub>                                     | 6.14±0.03 <sup>e</sup>  | 7.06±0.02 <sup>h</sup>   | 7.92±0.04 <sup>f</sup>   |
|                  |           | Cr   | <1 log <sub>10</sub>                                     | 6.05±0.02 <sup>e</sup>  | 7.07±0.04 <sup>h</sup>   | 7.91±0.03 <sup>f</sup>   |
|                  | Garlic    | T    | <1 log <sub>10</sub>                                     | 5.23±0.02 <sup>b</sup>  | 6.08±0.02 <sup>e</sup>   | 7.03±0.03 <sup>e</sup>   |
|                  |           | C    | <1 log <sub>10</sub>                                     | 5.11±0.05 <sup>a</sup>  | 5.96±0.05 <sup>d</sup>   | 6.46±0.05 <sup>a</sup>   |
|                  |           | Cr   | <1 log <sub>10</sub>                                     | 5.32±0.06 <sup>cb</sup> | 6.17±0.06 <sup>fe</sup>  | 6.68±0.06 <sup>cb</sup>  |
|                  | Ginger    | T    | <1 log <sub>10</sub>                                     | 5.41±0.06 <sup>cd</sup> | 6.26±0.06 <sup>g</sup>   | 7.01±0.03 <sup>e</sup>   |
|                  |           | C    | <1 log <sub>10</sub>                                     | 5.32±0.01 <sup>cb</sup> | 6.17±0.01 <sup>fe</sup>  | 6.68±0.01 <sup>de</sup>  |
|                  |           | Cr   | <1 log <sub>10</sub>                                     | 5.45±0.02 <sup>d</sup>  | 6.30±0.02 <sup>g</sup>   | 6.81±0.02 <sup>d</sup>   |
| Oven drying      | control   | T    | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.30±0.03 <sup>c</sup>   | 6.81±0.03 <sup>d</sup>   |
|                  |           | C    | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.26±0.06 <sup>bc</sup>  | 6.77±0.06 <sup>cd</sup>  |
|                  |           | Cr   | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.23±0.03 <sup>bca</sup> | 6.74±0.03 <sup>d</sup>   |
|                  | Garlic    | T    | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.18±0.02 <sup>ba</sup>  | 6.69±0.02 <sup>cb</sup>  |
|                  |           | C    | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.14±0.05 <sup>a</sup>   | 6.65±0.05 <sup>b</sup>   |
|                  |           | Cr   | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.23±0.02 <sup>bca</sup> | 6.74±0.02 <sup>cdb</sup> |
|                  | Ginger    | T    | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.22±0.01 <sup>bca</sup> | 6.73±0.01 <sup>cb</sup>  |
|                  |           | C    | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.15±0.05 <sup>a</sup>   | 6.66±0.05 <sup>b</sup>   |
|                  |           | Cr   | <1 log <sub>10</sub>                                     | <1 log <sub>10</sub>    | 5.17±0.06 <sup>ba</sup>  | 6.68±0.06 <sup>cb</sup>  |
| CV               |           |      | 1.12   | 1.38                    | 1.13                     | 0.93                     |
| LSD              |           |      | 0.09   | 0.11                    | 0.11                     | 0.11                     |

Where, LSD= list significant difference, CV=coefficient of variation, Spp.=species, T=tilapia, C=cat fish, Cr=common carp and the values are mean ±SE in that the mean values followed by the same letter in a column are not significantly different at 5% level of significance.

In this study, no or very less (<1 log<sub>10</sub> cfu/g) number of total moulds were observed in all dried fillets before or zero day storage. These showed that oven drying had a necessary influence on the incidence of total moulds in reducing the moisture level lower than the sun drying. The untreated sun dried fillets attained point of sensory rejection after forty days storage. However, treated fillets were not reached even after sixty days storage periods. This showed effectiveness of the garlic and ginger extracts were antimycotic agents (Magawata and Shina, 2013) with the load of total molds in appropriate suitable range for consumption with 4.57 to 5.23 log<sub>10</sub> cfu/g.

#### IV. CONCLUSION

The experiment was conducted to assess the effect of drying methods and pre-treatments on shelf-life and microbial quality of dried fish. The experiment was conducted in a factorial arrangement of 2×3×2 with two drying methods (sun and oven drying,) three fish species (tilapia, cat fish and carp) and two preservatives

treatment (garlic and ginger juice) laid out in Completely Randomized Design (CRD). Drying reduced the moisture contents making it safe for storage. Fresh fillets were analyzed for their microbiological quality. The dried fillets were stored at ambient condition and the samples were analyzed for microbial status every twenty days starting from the end of drying operation. Fresh fillet and untreated dry fillet were used as control. In the fresh fillets, a high load of aerobic bacteria of 5.11 log<sub>10</sub> cfu/g was observed on carp, and *E.coli* was detected in all three species whereas no *Salmonella* spp. was detected at all. Regarding freshly dried fillets, high load of aerobic bacteria (5.82 log<sub>10</sub> cfu/g) was observed in untreated tilapia whereas the initial load of moulds was (<1 log<sub>10</sub> cfu/g) in all freshly dried fillets. After 60 days of storage, the loads of aerobic bacteria and moulds were 8.03 and 7.92 log<sub>10</sub> cfu/g, respectively, in untreated sundried carp, tilapia and cat fish fillets, higher than that in treated samples. Upgrading the traditional fish processing technology and adoption of sun drying with locally available specific antioxidants and appropriate storage were needed in the region to minimize quality defects

related to dried fish. Detection of pathogens and numerated microbial loads were low enough due to drying with garlic and ginger treatments than untreated fillets. Therefore, garlic and ginger which are easily available at the place should be used to extend the shelf life of dried fillets. Moreover, important measures need to be taken to train local consumers in hygienic practices.

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