World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:5, No:12, 2011

Comparison of Different Techniques for Processing and Preserving fish *Rastrineobola argentea* from Lake Victoria, Kenya

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***Abstract*—**This study was set to determine the antimicrobial activities of brine salting, chlorinated solution, and oil frying treatments on enteric bacteria and fungi in *Rastrineobola argentea* fish from fish landing beaches within L. Victoria basin of western Kenya. Statistical differences in effectiveness of the different treatment methods was determined by single factor ANOVA, and paired two-tail t-Test was performed to compare the differences in moisture contents before and after storage. Oil fried fish recorded the lowest microbial loads, sodium chloride at 10% concentration was the second most effective and chlorinated solution even at 150ppm was the least effective against the bacteria and fungi in fish. Moisture contents of the control and treated fish were significantly lower after storage. These results show that oil frying of fish should be adopted for processing and preserving *Rastrineobola argentea* which is the most abundant and affordable fish species from Lake Victoria.

***Keywords*—**Fish landing beaches, Lake Victoria, oil frying, preservatives.

I. Introduction

FRESH fish is highly perishable, especially in tropical temperatures and need to be preserved for long term storage. Due to perishable nature of fish, traditional methods of preservation have been developed over the years which include salting, drying and smoking [1]. Salting which is cheap and easily available has been used in many areas of the world for years to preserve fish and fishery products [2]-[3]-[4]-[1]. The preservative effect of salting is mainly due to the decrease in water activity and thus prevention of growth of many spoilage microorganisms along with the formation of a more membranous surface which further inhibits the growth of microorganisms [5]-[6]. Moreover, chloride ions are toxic to some microorganisms [7].

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Salting has no adverse effects on the value of the fish protein and bacterial growth can be significantly retarded by the presence of sufficient quantities of common salt (sodium chloride). At salt concentrations of 10% in the fish, the activity of most bacteria that cause spoilage is inhibited [8]-[9]. When fish is placed in a brine solution, the salt penetrates the fish and water is extracted from the tissues by osmosis. Since fresh fish contains 70-80% water, the amount of brine used must be adjusted accordingly, the higher the salt contents in the fish, the longer the shelf life [10]. Traditional methods involve rubbing salt on the flesh of the fish or making alternate layers on fish which causes the problem of un-uniform application of salt. Brining takes care of this problem, which involves immersing the fish into pre-prepared solution of salt; the advantage is that salt concentration can be more easily controlled and salt penetration is more uniform [6].

Chlorinated solutions can be used as a method of sanitizing or eliminating bacteria and moulds from fish and sanitizing agents such as sodium hypochlorite have generally been proven effective in reducing overall microbial populations as well as numbers of specific bacterial pathogens on fish and other food products [11]-[12]. Chlorinated solutions have been used for sanitizing fish products especially during processing [13]. The inhibitory or lethal activity of chlorine depends on the amount of free available chlorine in the solution in the form of hypochlorous acid present in bleach and chlorine solutions that comes in contact with microbial cells. Free chlorine disinfects by chemically disrupting bacterial cell walls and membranes through oxidation of a chemical group known as the thiol group [14].In the Lake Victoria basin, and other tropical countries, oil frying is commonly used for processing and preserving Nile tilapia (*Oreochromis niloticus*) [15] but not Dagaa (*Rastrineobola argentea*), which is instead commonly processed by sun-drying on sometimes contaminated ground [16], which does not protect the fish from flies, chicken and dogs, and this increases the chance of microbial contamination of fish. Given that the fish is laid on the ground, water accumulates around it instead of drying away, and the resultant inadequate drying also makes it susceptible to microbial contamination, and post harvest losses [16]. Our recent studies have also shown that water and fish from Lake Victoria are contaminated with enteric bacteria [17] which call for processing and preservation methods which can reduce or eliminate microbial contamination of fish for long term storage or shelving in commercial outlets and safety for human consumption.

This study reports a comparison of salting, treatment with chlorinated solution, and oil frying for processing and preserving *Rastreniobola argentea*, which is the most abundant and affordable fish in the Lake Victoria basin.

II. MATERIALS AND METHODS

A. Samples Collection, Processing and Treatments

For this study, fresh *Rastrineobola argentea* fish samples originally from fish landing beaches of Luanda Kotieno, Usenge, Denda Island and Oyamo, all in Bondo county, Nyanza province within L. Victoria basin of western Kenya, were randomly bought from fish transporters and mongers in Kisumu Municipality, western Kenya, after determining their source of origin and the approximate time of loading at the beaches. Approximately two kilograms (1000-1200 pieces of fresh *Rastrineobola argentea*) fish originally from each beach, i.e., Luanda Kotieno, Usenge, Denda Island or Oyamo, was bought and immediately placed in sterile clearly labeled plastic bags and transported in cooler boxes with ice packs within two (2) hours of collection to Kenya Marine and Fisheries Research Institute (KMFRI), Kisumu, for processing and treatments by washing in distilled water then brine salting or sanitizing in chlorinated solution before sun-drying for six hours in open field in raised racks, or washing then sun-drying in raised racks before frying in edible vegetable oil.

For brine salting or sanitizing in chlorinated solution, washed fresh fish was immersed in 10% sodium chloride (100g in 1 litre distilled water), or 150ppm (3.9ml of 3.85% commercial sodium hypochlorite in 1 litre distilled water), respectively, for two hours before sun-drying in raised racks. Control (untreated fish) and fish to be fried were also washed and then immersed in distilled water for two hours, then similarly sun-dried in raised racks. At KMFRI, all the treated fish samples (and the controls) were then placed in well labeled clear sterilized plastic containers, 500g for each treatment (salted, chlorinated, fried or control), sealed, and then taken to Biomedical Science and Technology laboratory in Maseno University for immediate microbial (bacteria and fungi) analysis and the remaining fish sealed again and stored for 56 weeks before again testing for microbial loads.

B. Bacterial Analysis

In the Biomedical laboratory at Maseno University, ten grams (10-15 whole pieces of *Rastrineobola argentea*) of the control or treated (salted, chlorinated or oil fried) fish samples from various fish landing beaches were removed from the respective labeled plastic containers, [sealing again and leaving behind remaining samples in the containers for 56 weeks storage in the laboratory before analysis again], and separately macerated for 3 min in a blender (Sanyo^{TM}) to make slurry. The resultant slurry was respectively transferred into sterile labeled 250ml flasks in readiness for bacteriological analysis. Sterile normal saline was added into each of the 250ml flasks containing the respective fish slurry, for each treatment or control, up to 100ml mark, and mixed thoroughly.

Bacterial analysis for the fish samples experiments was done using most probable number (MPN) of microbe determination method [18], and also using the aerobic plate count method which employs plate count agar according to AOAC method 966.23 [19] with colony forming units (CFU) determination. For MPN analysis, 10mls of phenol red lactose broth (HiMedia Lab. Pvt. Mumbai, India) was added into each of 3 sets of 25ml tubes (with inverted Durham's tubes' inserts). Each set contained three tubes (i.e., there were 9 tubes in total). The loaded tubes were sterilized by autoclaving. The tubes were allowed to cool and then inoculated with a ten-fold difference in respective fish treatments or control slurry mixtures (from the 250ml flasks) i.e., 0.1ml, 1ml, and 10ml per tube and incubated at 37^0C (Gallenkemp, Germany) to determine the number of coliforms in the respective fish treatments or controls, with the end point determined from the most probable number McCardys MPN table [18].

For each treated or control fish slurry sample from each beach, total viable bacterial counts analysis and CFU determination was done in triplicate. Briefly, using a micropipette and sterile tips, aliquots of 1 ml (or 1000µl) of the treated or control fish slurry sample (from the 250 ml flasks), were aseptically inoculated into sterile test tubes containing 9mls of molten plate count agar at approximately 35-40^{0}C, vortexed, and then plated into sterile Petri dishes and allowed to set for about 15 minutes. The loaded Petri dishes were then covered, inverted and incubated at 37^0C (Gallenkamp, Germany) for 24 hrs. By using Quebec colony counter, the number of colonies per plate (i.e. colony forming units, CFUs) was counted, and the means from the triplicate experiments for each fish treatment and control samples determined and recorded. The recorded mean values were multiplied by 10 to take care of the dilution factor of 1:10 to express the final results as CFUs per gram of fish sample. Bacteriological analysis of the treatment and control samples was repeated after 56 weeks of storage to compare bacterial loads and thus shelf lives of the fish samples.

C. Isolation and Identification of Fungi

For fungal analysis, similarly ten grams (10-15 whole pieces of *Rastrineobola argentea*) of the control or treated (salted, chlorinated or fried) fish were also removed from the respective labeled plastic containers, [sealing again and leaving behind remaining samples in the containers for 56 weeks storage in the laboratory], and grinded aseptically in a porcelain mortar and mixed in 90 ml of sterile peptone water [20]. From this mixture, 1ml was spread on potato dextrose agar (PDA) supplemented with oxytetracycline to inhibit bacterial growth [21]-[22]-[23]. The plates were incubated at 28 ± 2^0C and examined daily for 7 days. All observed colonies were subcultured in PDA to obtain pure cultures which were subsequently isolated, counted and identified using morphological characteristics, spore formation and production of fruiting bodies according to methods of Banenet and Hunter [24]-[25]. Each different appearing fungal culture isolate was transferred with a sterile needle to a sterile slide, teased apart and stained with lactophenol cotton blue and examined macroscopically and microscopically [26]. Microscopic

examination was done using an iroscope LMOD MX-T. Fungal culture isolates were identified to species by assessment of the sporulating bodies and mycelial growth using the keys of Samson *et al* [27].

D. Moisture Content Determination of Fish Samples

The moisture content was determined by oven drying of 5g fish tissue according to AOAC method 950.46 [19]. Briefly, 5g of fish tissue (5-7 whole pieces of *Rastrineobola argentea*) was oven dried at 105°C until a constant weight was reached [28], and resultant weight measured following the methods outlined in Cockerell *et al* [29] which determines the weight difference between dry and wet material and the moisture content expressed as percentage of the wet tissue.

E. Data Analysis

Data entry and analysis was done using Windows Excel 2003. The microbiological data (MPN and CFU) for different treatments compared with control from the four fish landing beaches (Luanda Kotieno, Usenge, Ndeda and Oyamo) were statistically analyzed by single factor ANOVA, those of fungal isolate counts presented descriptively because low counts after treatments and storage, and the differences in mean percentage moisture contents before and after storage determined by paired two-tail t-Test. Significant differences were accepted at $p < 0.05$.

III. Results

Table I shows that before storage, oil fried dagaa recorded the lowest mean bacterial counts of log 0.15 MPN/g, followed by salt treated dagaa with a mean of log 1.76 MPN/g while chlorinated solution treated dagaa recorded the highest bacterial mean count of log 2.25 MPN/g. After storage, oil fried dagaa still recorded the lowest mean bacterial count of log 0.10 MPN/g followed by salt treated dagaa with log 0.43 MPN/g and the chlorinated solution treated dagaa with a mean of log 0.73 MPN/g. Single factor ANOVA showed statistical significances of $P < 0.006$ between the treatments and control before and after storage.

TABLE I
BACTERIAL COUNT USING MOST PROBABLE NUMBER (MPN) METHOD BEFORE AND AFTER STORAGE OF DIFFERENTIALLY TREATED *R. ARGENTEA* FISH

Before Storage					
Treatments	Luanda Kotieno	Ndeda	Oyamo	Usenge	Mean
Control	2.92	2.29	2.53	2.48	2.56
Brine salt	2.2	1.22	1.98	1.65	1.76
Chlorinated solution	2.4	2.2	2.24	2.17	2.25
Fried	0.2	0	0.2	0.2	0.15
After Storage					
Control	1.03	1.19	0.51	1.06	0.95
Brine salt	0.68	0.20	0.00	0.84	0.43
Chlorinated solution	0.92	0.72	0.40	0.90	0.73
Fried	0.20	0.20	0.00	0.00	0.10

Table II shows bacterial counts results for aerobic plate counts done for the differentially treated dagaa before and after storage. Similar to MPN results, before storage oil fried dagaa showed the lowest mean count of log 0.46 CFU/g, followed by brine salted dagaa with log 2.04 CFU/g, while the chlorinated solution treated dagaa had the highest count of log 2.17 CFU/g. After storage, no bacterial growth was observed in oil fried dagaa, by aerobic count method while salt treated dagaa recorded a mean of log 0.06 CFU/g and chlorinated solution treated dagaa showed highest count of log 0.16 CFU/g. Single factor ANOVA showed significant differences in bacterial loads between treatments and controls before and after storage at $p<0.0003$.

TABLE II
BACTERIAL COUNT USING AEROBIC PLATE COUNT METHOD BEFORE AND AFTER STORAGE OF DIFFERENTIALLY TREATED *R. ARGENTEA* FISH

Before Storage					
Treatments	Luanda Kotieno	Ndeda	Oyamo	Usenge	Mean
Control	2.24	2.17	2.18	2.23	2.21
Brine salt	2.14	1.93	1.02	2.07	2.04
Chlorinated solution	2.24	2.1	2.18	2.17	2.17
Fried	0.72	0.36	0.26	0.52	0.46
After Storage					
Control	0.60	1.21	0.46	0.60	0.72
Brine salt	0.19	0.00	0.00	0.10	0.06
Chlorinated solution	0.30	0.10	0.10	0.10	0.16
Fried	0.00	0.00	0.00	0.00	0.00

The Table III shows the results of the total fungi isolates from differentially treated dagaa (*R. argentea*) before and after storage. Before storage, oil fired dagaa recorded zero fungi species isolates, brine salted dagaa recorded a mean of 2 fungi species isolates, and chlorinated solution recorded a mean of 4 fungi species isolates compared to the control (untreated dagaa) which recorded a mean of 5 fungi species isolates. After storage, all the treated dagaa samples recorded zero fungi species isolates compared to 3 fungi species isolates for the untreated (control) samples.

TABLE III
FUNGI SPECIES ISOLATES BEFORE AND AFTER STORAGE OF DIFFERENTIALLY TREATED R. ARGENTEA FISH

Before Storage					
Treatments	Luanda Kotieno	Ndeda	Oyamo	Usenge	Mean
Control	7	4	4	4	5
Brine salt	3	2	2	2	2
Chlorinated solution	4	3	3	4	4
Fried	0	0	0	0	0
After Storage					
Control	1	0	3	0	3
Brine salt	0	0	0	0	0
Chlorinated solution	0	0	0	0	0
Fried	0	0	0	0	0

Table IV shows the percentage moisture contents before and after storage of differentially treated dagaa. Before storage, brine salted dagaa recorded the lowest mean percentage moisture content of 14.30% followed by chlorinated solution treated dagaa with mean percentage moisture content of 16.38% and oil fried dagaa with mean percentage moisture content of 19.50%. Similarly, compared to before storage mean percentage moisture content of control 21.59, after storage brine salted dagaa recorded the lowest percentage mean moisture content of 6.47%, followed by chlorinated solution and lastly oil fried dagaa with percentage mean moisture contents of 9.65% compared to percentage

mean moisture content of 11.15% of untreated control fish samples. There was significant difference in percentage mean moisture content before and after storage by paired t-test, P = 0.0005.

TABLE IV
PERCENTAGE MOISTURE CONTENT BEFORE AND AFTER STORAGE OF DIFFERENTIALLY TREATED R. ARGENTEA FISH

Before Storage					
Treatments	Luanda Kotieno	Ndeda	Oyamo	Usenge	Mean
Control	21.37	22.43	22.5	20.04	21.59
Brine salt	14.02	13.9	15.6	13.67	14.30
Chlorinated solution	15.66	16.26	19.14	14.47	16.38
Fried	18.32	21.03	21.1	17.55	19.50
After Storage					
Control	12.58	9.92	10.04	12.07	11.15
Brine salt	6.89	6.92	5.97	6.11	6.47
Chlorinated solution	9.13	7.7	6.5	6.11	7.37
Fried	10.89	9.8	7.76	10.14	9.65

IV. DISCUSSIONS

Before storage oil fried dagaa recorded the lowest mean bacterial counts followed by salt treated dagaa while chlorinated solution treated dagaa recorded the highest bacterial mean counts. After storage, oil fried dagaa still recorded the lowest mean bacterial counts followed by salt treated dagaa and the chlorinated solution treated dagaa had the highest bacterial counts. The results of the total fungi isolates from differentially treated dagaa (*R. argentea*) before and after storage also showed similar trends as for bacterial loads. Before storage oil fired dagaa recorded zero fungi species isolates followed by brine salted dagaa, chlorinated solution and highest in the control (untreated dagaa). After storage, all the treated dagaa samples recorded zero fungi species isolates compared to three fungi species isolates for the untreated (control) samples. These results show the effectiveness of oil frying as an effective method for preserving dagaa fish.

Oil causes an anaerobic condition which makes the bacterial and fungal cells not to grow or sporulate (22). This suggests that the fried then stored *R. argentea* were free of any microbiological organism. Frying differs from other methods of processing in that the cooking medium is hot oil due to heating. Fried dagaa have the characteristic crispy outer surface and low water activity which inhibited the growth of bacteria and fungi (22).

Brine salting also had a significant effect on microbial load of fish. This effect was also present after long time of storage for fifty six weeks in sterile closed plastic containers. Salt preserves food in different ways but mainly by inhibiting bacterial growth through dehydration, chloride ion effect, oxygen removal, and preolytic enzyme [30]. During salting or brining two processes take place simultaneously: water moves from the fish into the solution outside and salt moves from the solution outside into the flesh of the fish. At a concentration of ≥3% (w/v), NaCl generally inhibits the growth of *Salmonellae* [31]. The preservative effect of salt is mainly due to the decrease in water activity and thus prevention of growth of many spoilage micro-organisms along with formation of a more membranous surface which further inhibits the growth of micro-organisms [4]-[5]. The preservation period of fish product is linked to the amount of salt added [32]. It is more likely that the microbial growth occurs as a result of attacking proteinaceous and other soluble nitrogenous compounds that exists in the fish juice. As the fish become drier there is decrease in water activity and this together with the accumulated salt in the flesh, results in suppression of bacterial growth [33].

Chlorinated solution was the least effective in reducing microbial growth in fish, though it has been reported to be highly effective and inexpensive solution used in the control of food-borne diseases [14]. The elimination of microbes using chlorine is by disrupting microbial (especially bacterial) cell walls and membranes through oxidation of chemical group known as the thiol group [14]. The reason for low effectiveness of chlorinated solution could be that sodium hypochloride efficacy is reduced with time and this is due to oxidation, it's more efficient during the first few minutes of its use [34].

Before storage, brine salted dagaa recorded the lowest mean percentage moisture content followed by chlorinated solution treated dagaa and moisture content was higher in oil fried dagaa. Similarly, compared to before storage, mean percentage moisture content of control dagaa was high. After storage, brine salted dagaa recorded the lowest percentage mean moisture content, followed by chlorinated solution treated dagaa and lastly oil fried dagaa, compared to percentage mean moisture content of untreated control dagaa samples. Interestingly, oil fried dagaa showed higher moisture contents compared to salted or chlorinated fish probably because much of oil was absorbed by fish tissue while frying and dried out faster than water during moisture content analysis. The results from this study show that moisture contents of fish after storage is lower, is encouraging but surprisingly because the container was air tight. Further research is needed to find out whether the sealed plastic containers allowed moisture to get out or the opening of the containers to sample the already sun-dried and treated fish for before storage experiments allowed the moisture to be released. The results of this study are important because it shows that vegetable oil fried fish are naturally microbe free especially after storage for along time in air tight plastic containers. Thus oil frying should be adopted for processing and preserving *Rastrineobola argentea* which is the most abundant and affordable fish species from Lake Victoria and can help alleviate poverty and economically empower the local community.

ACKNOWLEDGMENT

The authors acknowledge Maseno University, School of Public Health and Community Development, Department of Biomedical Science and Technology, for allowing the research to be undertaken in their premises, and the School of Graduate Studies for administrative support. This research was supported by funds from Commission of Higher Education (CHE), Kenya, held by Prof. Ayub V. O. Ofulla.

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