

Microbial and chemical quality of selected dried fish varieties available in Sri Lankan market

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Abstract Dried fish is one of the traditionally preserved foods in Sri Lanka. Since dried fish is often an important component of daily meal, this study was aimed to assess the quality of selected dried fish varieties in the local market. Dried fish samples of nine selected varieties were collected under three categories, viz. locally produced, imported dried fish before and after distribution to retail market. They were analyzed for microbiological and chemical parameters. Water activity of the samples was also measured. Samples were found to be negative for *Escherichia coli*, *Staphylococcus aureus* and halophilic bacteria. Aerobic Plate Count and yeast and mould count of the majority of the samples were within the acceptable limit. Histamine content exceeded 100 mg/kg level in 33% and 13% of imported and local samples respectively. Water activity of the samples was <0.75 except for imported prawn samples of retail outlets. There was no significant difference ($p>0.05$) between the analyzed parameters of all three categories.

Keywords: dried fish, histamine, microbiological quality, salt

INTRODUCTION

Seafood acts as a main component in the diet of most countries around the world and contributes as a main supply of animal protein. 17% of the animal protein intake of humans globally, is derived from seafood. In Sri Lanka 55% of the animal protein intake is from fish and fishery products (FAO 2012). Since fish is a highly perishable food, most fish tend to spoil within few hours after capture at tropical temperatures. Therefore, as a measure to prevent the spoilage, fish should be processed or preserve quickly. Cooking, lowering moisture content by salting, smoking and drying and lowering the pH are the methods which can be used to preserve fish (Fellows and Hampton 1992).

Drying is regarded as a traditional method of preservation of fish. In Sri Lanka dried fish is one of the oldest processed foods. In 2014, the total dried fish production of Sri Lanka was 71,810 tonnes while imported amounts of dried fish were 35,280

tonnes and 33,000 tonnes in 2014 and 2015 respectively (MOFAR 2016).

The quality of dried fish is affected by the presence of microorganisms. Determination of microbiological quality of dried fish and other processed fish becomes very important when the consumer's health and hygiene is considered (Lilabati et al. 1999). Fungi are one of the major groups of microorganisms which affect the quality of dried fish, especially along the distribution channel from manufacturer to the consumer's end (Chakrabarti and Varma 2003) causing off flavours, softening of the flesh and some producing potentially dangerous mycotoxins when conditions are favourable for fungal growth (FAO 1982). Common sources of contamination include dust in and around fish processing place, contaminated coastal water and soil and unhygienic handling (FAO 1982; Prabhakaran and Gupta 1990).

Histamine is a chemical compound found in spoiled scombroid fish and other marine fish contain high levels of histidine which is a free amino

acid in the fish muscle (Pan and James 1985). Formation of histamine, which can cause health problems, is a result of time and temperature abuse (USFDA 2011). High degree of bacterial and fungal contamination and elevated histamine levels in dried fish has been reported in a previous study done in Sri Lanka (Ranasinghe et al. 2014).

Since dried fish plays a major role in Sri Lankan diet as a rich source of protein and a popular food item among people, it is very important to evaluate its microbial and chemical quality. Therefore, this study was done to assess the microbiological and chemical quality of selected varieties of both locally manufactured and imported dried fish available in the local market.

MATERIALS AND METHODS

A total of 71 dried fish samples of selected varieties namely; tuna (*Katsuwonus pelamis*), sprats (*Stolephorus* sp.), catfish (*Arius* sp.), sailfish (*Istiophorus* sp.), katta (*Scomberoides lysan*), shark (*Carcharhinus* sp.), prawns (*Penaeus* sp.), herrings (*Amblygaster* sp.) and pannawa (*Johnius* sp.) were obtained from locally produced dried fish (n=52), imported dried fish before distributing to the local market (n=19) and imported dried fish being sold at the local market (n=27) during March - November 2014. Each sample was weighed approximately 500 g and transferred into polythene bags and transported to the laboratory. Samples were analyzed for microbiological (Aerobic Plate Count or APC, Total Coliforms, *E.coli*, *Staphylococcus aureus*, yeast and mould, halophilic count) and chemical (salt, histamine; for histamine forming varieties only) parameters. Water activity of the samples was measured according to ISO 21807:2004 (E) standard (ISO 2004) using Novasina AW sprint water activity meter.

Aerobic Plate Count (APC)

APC was carried out using SLS 516: Part 1:1991 (SLS 1991a). Ten grams of the sample was weighed aseptically into a sterile stomacher bag and 90 ml of diluents (maximum recovery diluents - Oxoid) was added and blended in a stomacher blender for 1-2 minutes to make up the 10^{-1} dilution. Four dilutions were used starting from the 10^{-2} dilution for plating. 1 ml of 10^{-2} dilution was transferred to each of the

two sterile petri plates using sterile pipettes. Same procedure was repeated with the other dilutions. About 15 ml of standard plate count agar (Oxoid) medium at $45\pm 0.5^{\circ}\text{C}$ was poured into each petri plate and mixed with the inoculums and allowed to solidify. Plates were incubated at 37°C for 48 hours. After the incubation period, bacterial count was taken. NaCl (0.5%) was added to the culture media. Media control and air control were carried out in parallel to the analysis as internal quality control.

Yeast and mould

SLS 516: Part 2: 1991 (SLS 1991b) method was used to enumerate yeast and mould. Sample preparation was done as mentioned in the APC method and spread plate method was used. One millilitre of the 10^{-2} dilution was transferred on to the surface of previously prepared Potato Dextrose Agar (PDA) plates. Four dilutions were tested. Then the plates were incubated at $25\pm 1^{\circ}\text{C}$ for 5 days. After the incubation colonies were counted using colony counter. Here too, media control and air control were carried out in parallel to the analysis as internal quality control.

MPN method for coliforms and *E. coli*

For enumeration of coliforms, and *E. coli* SLS 516: Part 3: 1982 method was used. Ten grams of the dried fish sample was weighed in a sterile stomacher bag and 90 ml of the diluent (maximum recovery diluent-Oxoid) was added and blended. Then 10 ml of the 10^{-1} dilution was inoculated into each three tubes containing 10 ml of double strength MacConkey broth purple (Oxoid). Then 1 ml of each 10^{-1} dilution was inoculated to each three tubes containing 10 ml of single strength MacConkey broth purple. The same procedure was repeated for 10^{-2} and 10^{-3} dilutions. Tubes were incubated at $36\pm 1^{\circ}\text{C}$ to 24 - 48 hours. Tubes were examined for acid and gas production. Tubes showing acid and gas production after incubation is considered as presumptive positive for coliforms. Positive tubes were sub cultured into two tube sets containing 10 ml of Brilliant Green Bile Broth and one set was incubated at $36\pm 1^{\circ}\text{C}$ for 48 hours for total coliforms and the other tube set was incubated at $44\pm 0.1^{\circ}\text{C}$, for 48 hours for faecal coliforms. The tubes with gas production were considered as positive results.

From the positive faecal coliform tubes loop full of cultures were streaked onto eosin methylene blue agar (Oxoid) and incubated at $36\pm 1^\circ\text{C}$ for 24 hours. Typical *E. coli* colonies were examined and colonies were inoculated into tubes containing peptone water and incubated at $44\pm 0.1^\circ\text{C}$ for 24-48 hours. Production of indole was tested by adding indole reagent. Cultures showing indole production were considered as *E. coli* positive cultures. *E. coli* pure culture as the positive control and *Staphylococcus aureus* as the negative control was inoculated into broth tubes parallel to the analysis.

Enumeration of *Staphylococcus aureus*

SLS 516: Part 6: 1992 (SLS 1992) method was used to enumerate *S. aureus*. Sample preparation was done as mentioned in the APC method. 1 ml of the 10^{-1} dilution was transferred on the surface of three small previously prepared Baird-Parker agar plates (90mm). Then the plates were incubated at $37\pm 1^\circ\text{C}$ for 18-24 hours. After the incubation typical and atypical colonies were selected and confirmation was done using the coagulase test. For the coagulase test inoculum was transferred to a tube containing brain heart infusion broth and incubated at $37\pm 1^\circ\text{C}$ for 20 - 24 hours. Then the 0.1 ml of each culture was added aseptically to 0.3 ml of the rabbit plasma in sterile tubes and incubated at $37\pm 1^\circ\text{C}$. Clotting of the plasma was examined after 4-6 hours. *S. aureus* pure culture was used as the positive control.

Halophilic bacterial count

Halophilic bacteria was enumerated using the method prescribed in SLS 643:2007. 50 g of the sample was blended with halophilic broth and 0.1 ml of each dilution was placed on halophilic agar plates with the spread plate technique. Plates were incubated at $33 - 35^\circ\text{C}$ for 5 - 12 days. Media control and air control were carried out parallel to the analysis as internal quality control.

Determination of salt content (SLS 643:2007; SLS 2007)

One gram of the dried sample was weighed to the nearest milligram, into a 250 ml Erlenmeyer flask. 50.0 ml of standard silver nitrate solution and 20 ml of dilute nitric acid were added and boiled on a hot plate for about 45 minutes until all soluble solids are dissolved. Then it was allowed to cool and 50 ml of distilled water and 5 ml of indicator solution was added. Then the titration was done against standard potassium thiocyanate solution until a permanent brown colour appeared. The salt content was then calculated as follows:

$$\text{Salt content (as \%NaCl)} = \frac{5.85 (V_1N_1 - V_2N_2)}{m}$$

where, V_1 = Volume in ml of the standard silver nitrate solution used; N_1 = concentration of the standard silver nitrate solution; V_2 = volume in milliliters of the standard thiocyanate solution used; N_2 = concentration of the standard thiocyanate solution; and m = mass, in grams of the dried sample taken.

Determination of histamine content

Histamine was determined by the method described in Jinadasa et al. (2015). High-performance liquid chromatography (HPLC) model Shimadzu, SIL 20A was used to analyze samples. Ten grams of the sample was blended in 20mL of 10% trichloroacetic acid solution and 20mL of distilled water for 30 seconds. Final volume was made up to 100mL with distilled water and filtered through a Whatman No. 1 filter paper. Ten millilitres of the filtered sample was passed through Amberlite CG-50 resin. Fluorophore standards and samples were prepared by mixing 5mL of column chromatography elute with 0.1% O-Phthaldialdehyde solution. For each injected batch, a set of five standards with a concentration of 0.5, 1.0, 1.5, 2.0 and 3mg/mL of histamine and a recovery sample were run at the beginning. Samples were done in duplicates. Concentration of histamine (mg/kg) was calculated by using the following equation.

$$\text{Histamine concentration (mg/kg)} = \frac{\text{Measured concentration of histamine in extract } \left(\frac{\text{mg}}{\text{L}}\right)}{\text{Sample weight (g)}} \times 100$$



RESULTS

According to microbiological results obtained (Table 1), samples of all three categories were negative for *E. coli*, *S. aureus* and halophilic bacteria. Total coliforms were absent in all locally produced samples. Of the 46 imported samples examined, three samples were detected with total

coliforms both before and after distribution to retail outlets but were less than 100 MPN/g in two samples. One sprat sample had >1100 MPN/g. APC of locally manufactured samples were in the range of $1.7 \times 10^4 - 2.0 \times 10^7$ cfu/g and imported samples both before and after distribution to the market had $9.0 \times 10^2 - 2.4 \times 10^8$ and $7.6 \times 10^4 - 9.7 \times 10^6$ cfu/g respectively.

Table 1 Microbiological parameters of dried fish samples analyzed. Sample types: I – Locally manufactured dried fish samples; II – Imported dried fish samples before distribution; III – Imported samples available at the retail market. ND – Not detected.

Sample type	APC (cfu/g)	Total coliform (MPN/g)	<i>E. coli</i> (MPN/g)	<i>S. aureus</i> (cfu/g)	Yeast and mould (cfu/g)	Halophilic bacterial count (cfu/g)
I	$1.7 \times 10^4 - 2.0 \times 10^7$	ND	ND	$< 1.0 \times 10^1$	$1.0 \times 10^1 - 6.0 \times 10^4$	ND
II	$9.0 \times 10^2 - 2.4 \times 10^8$	ND - >1100	ND	$< 1.0 \times 10^1$	$8.5 \times 10^1 - 3.0 \times 10^3$	ND
III	$7.6 \times 10^4 - 9.7 \times 10^6$	ND - 9	ND	$< 1.0 \times 10^1$	$2.0 \times 10^1 - 8.0 \times 10^3$	ND

In locally manufactured samples 58% of samples contained APC less than 1.0×10^5 which is acceptable. 33 % of the samples had APC more than 5.0×10^5 cfu/g which is unacceptable. 60 % of the imported samples obtained from the retail outlets were acceptable in terms of APC with counts $< 1.0 \times 10^5$ cfu/g. 22% were in the rejection level ($> 5.0 \times 10^5$ cfu/g). APC of imported samples before distribution had 68% and 21% in the acceptable, and rejection limits respectively. According to SLSI limits yeast mould count of dried fish is acceptable if the count is < 1000 cfu/g and unacceptable if it is greater than 10,000 cfu/g. In locally manufactured samples 92% were good in quality and 4% were of bad quality. In imported samples obtained from retail outlets 82% were of good quality and 7% were in the rejection limit. 84% of the imported samples before distribution contained yeast and mould count in acceptable levels and 16% of the samples were in the rejection level.

According to Table 2, it can be seen that highest acceptable percentage (68%) of dried fish in terms of APC belongs to the imported samples before distribution. In yeast and mould count highest acceptable percentage (92%) is from locally manufactured samples. Thirty three percent of the locally manufactured dried fish were in the unacceptable APC levels. Imported samples before distribution had the highest unacceptable percentage (16%) of yeast and mould count. When

considering samples of all three categories 55.7% of locally manufactured, 52.6 % of imported before distribution and 55.5% imported after distribution, were in the acceptable range for all the microbiological specifications mentioned in SLS.

Table 2 Acceptability percentages of dried fish based on APC and yeast and mould counts. I – Locally manufactured dried fish samples; II – Imported dried fish samples before distribution; III – Imported samples available at the retail market.

Sample type	Acceptable %		Unacceptable %	
	APC	Yeast & mould	APC	Yeast & mould
I	58	92	33	4
II	68	84	21	16
III	60	82	22	7

Thirty three percent of imported dried fish samples and 13% of local samples exceeded 100 mg/kg level which is the maximum recommended limit for histamine content according to SLSI standard. According to Table 3, it can be seen that the average values of histamine content is high in the both categories of imported tuna samples. Nevertheless, in hurulla (*Amblygaster*) samples, locally manufactured samples showed a high average value. Sprat samples showed a less amount of histamine.

Table 3 Average histamine values (mg/kg) of selected dried fish species. I – Locally manufactured dried fish samples; II – Imported dried fish samples before distribution; III – Imported samples available at the retail market.

Sample type	Tuna	Hurulla	Sprat
I	57.0	94.0	5.6
II	134.0	28.0	7.6
III	131.0	Not available	7.8

Ninety one percent of the imported samples and 54% of local samples had salt content >12%. From Table 4, it can be seen that only prawn samples contained average salt content less than 12%. Compared to imported samples locally manufactured samples contained less amount of salt. Water activity of all the samples were less than the maximum level according to SLSI standards which is 0.75 except for tested prawn samples (0.76) available at retail market.

Table 4 Average salt content (%) in analyzed samples. I – Locally manufactured dried fish samples; II – Imported dried fish samples before distribution; III – Imported samples available at the retail market.

Sample	I	II	III
Tuna	11.8	17.5	15.0
Sprat	14.0	14.6	15.0
Catfish	12.5	24.0	16.0
Sailfish	11.3	18.8	14.0
Katta	15.6	14.2	17.0
Shark	18.2	9.0	19.0
Prawn	12.0	8.1	10.0
Hurulla	10.4	16.4	-

When considering all the parameters tested 15% of locally manufactured, 4% of imported samples obtained from retail market and 20% of imported samples before distribution were complying with Sri Lankan Standards. There was no significant difference (t-test; $p > 0.05$) between the analyzed parameters of locally manufactured samples and imported samples and also between imported samples before and after distribution to retail outlets.

DISCUSSION

Although none of the samples contained *S. aureus* in this study several studies conducted around the world have detected *S. aureus* in dried fish (Yam et al. 2015; Singh and Kulshrestha 1993; Suleiman and Mustafa 2012). *S. aureus* can survive in low water activity (aw) and can grow in media containing up to 18% salt (Sanjeev and Surendran 1996). Another study conducted in India has proved that if salt curing and sun drying procedures are followed accurately for fish, it is possible to get fish products free from enterotoxigenic staphylococci and its enterotoxins even from the raw material containing enterotoxigenic staphylococci (Sanjeev and Surendran 1996). High APC and yeast and mould counts were also recorded in a similar study conducted in India (Saritha et al. 2012).

In this study all the samples were negative for *E. coli* which is an indicator organism. Similar study conducted in Sudan has also reported that indicator organisms like coliforms and pathogens like *Staphylococcus aureus* were absent in tested dried fish samples after processing (Suleiman and Mustafa 2012). Hamjeer et al. (2006) have found that high concentrations of NaCl has an effect on morphology of *E. coli* and *S. aureus* cells and also *E. coli* cells were damaged extensively. This may be a reason for absence of *S. aureus* and *E. coli* in the tested samples of this study.

In a previous study conducted in Sri Lanka has found to contain histamine in dried-Skipjack tuna and dried sprats in 60.0% and 64.0 % respectively (Gunaratne and Samarajeewa 1994). Also a significant amount of histamine has been found in dried mackerel and dried shrimp in India (Vijayan et al. 2003). Another study conducted in Sydney, Australia has found that dried anchovies contained an average value of 35 mg/kg histamine and reported values ranging from non-detected level to 483 mg/kg (NSW Food Authority 2009). According to a study conducted in Taiwan, of the dried fish samples analyzed, 30.4% contained histamine greater than 5 mg/100 g, which was within the allowable limit of the US Food and Drug Administration (FDA) for scombroid fish and fishery products (Huang et al. 2010). According to FDA Guidelines (2011) for tuna, common dolphinfish (*Coryphaena hippurus*) and related fish,



the toxicity level is 50 mg/100 g and 5 mg/100 g is the defect action level. In this study, average histamine values of all three categories of tuna (locally produced dried fish, imported dried fish before and after distribution to retail market) and locally manufactured dried fish of herring fall into defect action level of FDA limits. Reasons for elevated histamine levels can be raw material quality, handling and storage conditions.

Salt has the ability to reduce the water activity and preserve the food. In addition microbial cells loose water from cells as a result of osmotic shock due to salt. Therefore the microbial cells die or growth becomes slow (Potter and Hotchkiss 1995). Addition of salt to dried fish therefore is a benefit but the quality of salt should also be considered. Generally, when water activity is lower than 0.85 there is no microbial growth or toxin formation in food occur (Jonsson et al. 2007).

CONCLUSION

According to obtained results it can be seen that unacceptable percentages of both imported and locally manufactured dried fish in terms of APC, yeast and mould and histamine content are less than 50 %. Although the majority of the samples fall into acceptable category by percentage care must be taken to further reduce the unacceptable amount of dried fish by adopting better handling practices while preparing and storing as storage conditions may also play a key role in quality deterioration. 91% of the imported dried fish contained salt content >12% and 54% of the local samples contained >12% salt. Although high salt content maybe injurious to health, salt is a must to control spoilage. The only way to avoid this dilemma would be thorough washing prior to cooking, a practice done in Sri Lankan households anyway. Measures should be taken to produce dried fish in hygienic manner with good quality raw materials and routine checks should be done to imported dried fish to verify their quality and it is very important to check the microbial and chemical quality before they release to the market.

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