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Microbial and Chemical Quality of Selected Dried Fish Available in a Retail Market as an Approach for Assessing Health Safety

N.F. Nashath[1], A.C. Thavaranjit[1], G. Rajkumar[1]* and R. Srikaran[2]

ABSTRACT

Sun drying is an old practice of preserving fish that are not immediately consumed or sold within the right time. Determining the microbiological quality of dried fish is very important because this method is more vulnerable to get microbial contamination as it is done in the open environment. Hence, this study was conducted to determine the microbiological and chemical parameters of dried fish to reveal their suitability based on the Sri Lankan standards, aiming at giving some insight into the quality controllers and policy makers relevant to the industry. Seven dried fish types namely; Catfish *(Clarias batrachus)*, Tuna (*Thunnus thynnus*), Anchovy (*Stolephorus commersonii*), Bigeye scad (*Selar crumenophthalmus*), Ponyfish (*Leognathus* sp), Indian mackerel (*Rastrelliger* sp), Goldstripe sardinella (*Sardinella gibbosa*) were collected from different shops in a town market and their microbial parameters and chemical parameters were analyzed. Results revealed that *Clarias batrachus*, *Leognathus* sp, *Thunnus* sp, *Selar crumenophthalmus* and *Sardinella gibbosa* contained acceptable aerobic plate counts of less than 100X10^3 CFU/g while *Rastrelliger* sp exceeded the acceptable limit (105X10^3 CFU/g). The yeast and mould counts were in the acceptable range in all tested fish types. *Thunnus* sp showed zero results for the presence of *S. aureus* while the *Rastrelliger* sp contained the highest number of *S. aureus* count. Total coliforms and fecal coliforms were absent in all seven dried fish types. All samples contained acceptable salt and moisture contents. Out of seven samples, *Thunnus* sp was the only sample that was safe microbiologically for consumption. Hence, it could be noted that monitoring actions need to be undertaken to control the quality of dried fish by relevant authorities to assure the health-safety of dried fish.

Keywords: Chemical parameters, Dried fish, Microbial safety, Microbiological parameters

[1]*Department of Botany, Faculty of Science, University of Jaffna, Sri Lanka.*
[2]*Department of Chemistry, Faculty of Science, University of Jaffna, Sri Lanka.*

**gowrir@univ.jfn.ac.lk*
https://orcid.org/0000-0002-0783-6738

INTRODUCTION

A majority of people throughout the world use seafood as their primary

source of animal protein (17 %) (FAO, 2016). Fish and fisheries products account for 55 % of animal protein consumption in Sri Lanka (FAO, 2012). Drying is a common method in fish industry because it preserves the quality for an extended time and offers numerous benefits inclusive of insignificant alterations and minimum deterioration within the product. Dried fish provides high-quality protein at a low cost. Dried fish is one of Sri Lanka's traditional processed food. Sri Lanka produced 71,810 tons of dried fish in 2014 but imported 35,280 tons and 33,000 tons of dry fish in 2014 and 2015 respectively (MOFAR, 2016). Trincomalee, Mannar, Kalpitiya, Matara, and Jaffna are the main districts that produce marine dried fish. The dried fish industry is mainly carried out as a cottage-level industry. Traditional home scale techniques are mainly practiced for dried fish production by women as an extra source of income. The inland dried fish industry also has been developed in Anuradhapura, Polonnaruwa and Monaragala districts.

The moisture content of the material seems to be an index of the susceptibility of a product to undergo microbial impurity. Water content is the main factor, which determines the microbial, chemical and enzymatic stability of food (Amit *et al*., 2017). Other factors that affect drying are the addition of heat, removal of water, addition of bacteriostatic compounds and humectants, and pH changes. The phase of drying can last from minutes to hours, depending on the types of fish (lean or fatty), its preparation (high salted, semi salted, or not salted), and the severity of the external drying conditions (Doe, 1998).

Sun (solar) drying, heat pump drying, cold drying and osmotic dehydration are the common approaches of seafood drying (Nguyen *et al*., 2014). Sun-drying refers to the use of sunlight to dry the product. This is the cheapest approach and still in use in numerous countries. The dependence on rainfall, impurity from animals due to the open-drying method and exposure to dust are the biggest issues in this process. In traditional drying, the drying yards are situated near the seaside. The racks made of coconut leaves, mats made of coconut and palmyrah leaves are used for drying purposes. Here the fish is washed and salted and then it is exposed to sunlight for 24 hours for drying. Some fish are cut and opened while some are fully dried. In the traditional method, the

hygienic conditions are not satisfactory. Drying fish is carried out on sandy seashore in most of the places. So, fish will be contaminated by sand, blowflies and microorganisms. Incremental weather contamination, predation by animals and birds, dust and possible exposure to harmful microorganisms are the biggest issues in conventional sun drying in open spaces.

The main groups of microorganisms that affect the quality of dried fish are fungi and bacteria. When the consumer's health and hygiene are taken into account, determining the microbiological quality of dried fish becomes extremely important. Microorganisms are known to have a negative impact on the quality of cured fish and fungus growth generates off-flavours, softens the flesh, and some can produce potentially toxic mycotoxins in some circumstances (FAO, 1982). Yeasts and moulds are liable for the food spoilage and produce mycotoxin (Buchanan and Doyle, 1997). This is the reason for substantial decrease in the consumption of dried fish. Many bacteria capable of causing disease are considered saprophytic in nature, but they become pathogenic when fish become physiologically unstable, nutritionally deficient, or as a result of various stressors such as poor water quality or overstocking, allowing opportunistic bacterial infections to humans (Akinjogunla *et al*., 2011).

Many programs are being run by the government and non-governmental groups in Sri Lanka to improve the quality and health safety of dried fish. Many standards are established in the country, in order to make sure quality and safety. Protecting the consumers from unsafe conditions and reducing the postharvest losses of fish are the main objectives for establishing standards. The quality of dried fish items sold in the country must meet at least the requirements outlined in Sri Lanka Standards (1991a, 1991b and 1991c). And these standards vary from country to county as the environmental factors (temperature, humidity, pH, sunlight, and pollutants) determine the acceptable limits of microbial parameters.

Sri Lanka also, traditional drying technique is used to prepare the dried fish. Therefore, the objective of this research was to assess the microbiological and chemical parameters of selected dried fish species available in the retail market, which might help authorized

regulatory bodies to get decisions on controlling the health-safeness of dried fish.

MATERIALS AND METHODS

Collection of Different Types of Dried Fish

Seven dried fish types namely; Catfish (*Clarias batrachus*), Tuna (*Thunnus thynnus*), Anchovy (*Stolephorus commersonii*), Bigeye scad (*Selar crumenophthalmus*), Ponyfish (*Leognathus* sp), Indian mackerel (*Rastrelliger* sp), Goldstripe sardinella (*Saradinella gibbosa*) were collected during late 2020 and early 2021 from the retail market, using sterile polythene bags separately and brought to the laboratory for further studies (Figure 1). These fish types are commonly available and harvested during the particular period (October – June) and fishermen use these types for dry fish production at a great scale as they have the highest consumer preference and higher sales value.

Determination of Microbiological Parameters

The Aerobic plate count, yeast and mould count, enumeration of total coliform, fecal coliforms and *staphylococcus aureus* were determined according to recommended standard procedures.

Figure 1: Dried fish types used in this study.

Sample Preparation

The muscle tissue of a dried fish was aseptically cut out and 1 g was weighed by electronic balance. Then it was ground with the help of motor and pestle. The ground sample was transferred to a sterile Mac Cartney bottle. Then 9 ml of sterile saline water (0.85 %) was added to the sample and stirred well by using a mechanical stirrer for 1 minute.

Aerobic Plate Count

Plate Count Agar (PCA) medium was prepared according to the method reported in BAM (1998) under sterile conditions. About 12 – 15 ml of PCA medium at 45±0.5 ^{0}C was poured into each sterile petri dish and allowed to set. The sample was serially diluted up to 10^{-5} by using sterile saline water. A 0.1 ml of 10^{-1} dilution was taken with a sterile pipette and it was transferred to the center of the PCA plate. The inoculum was spread uniformly over the surface of the medium by using a sterile glass spreader. At the same time duplicates were also prepared. This procedure was repeated for each dilution and plates were incubated at 37 ^{0}C for 48 hours. After the incubation period, plates were observed for the development of colonies and the result was expressed in terms of Colony Forming Units (CFU) per gram of sample as reported in Sri Lanka Standards (1991a) (SLS 516: Part 1, 1991) The entire procedure was repeated for each sample three times .

Enumeration of Staphylococcus aureus

Mannitol Salt Agar (MSA) plates were prepared under sterile conditions. From the serially diluted samples, 0.1 ml of 10^{-1} dilution was taken with a sterile pipette and it was transferred to the center of the MSA plate. The inoculum was spread uniformly over the surface of the medium by using a sterile glass spreader. At the same time duplicates were also prepared. This procedure was repeated for each dilution and plates were incubated at 37 ^{0}C for 48 hours. After incubation period, plates were observed for the development of yellow colour colonies. The result was expressed in terms of Colony Forming Unit (CFU) per gram. The entire procedure was repeated for each sample three times (FDAUS, 1998 and Surendran *et al*., 2006).

Yeast and Mould Count

Yeast Mannitol Agar (YMA) plates were prepared under sterile conditions. From the serially diluted samples, 0.1 ml of 10^{-1} dilution was taken with a sterile pipette and it was transferred to the center of the YMA plate. The inoculum was spread uniformly over the surface of the medium by using a sterile glass spreader. At the same time duplicates were also prepared. This procedure was repeated for each dilution and plates were incubated at room temperature for 3 - 5 days. After incubation period, plates were observed for the development of colonies and the result was expressed in terms of colony-forming units (CFU) per gram of sample (Sri Lanka Standards, 1991b: SLS 516: Part 2). The entire procedure was repeated for each sample three times.

Enumeration of Coliform and Fecal Coliform Bacteria

Endo Agar (EA) plates were prepared under sterile conditions. From the serially diluted samples, 0.1 ml of 10^{-1} dilution was taken with a sterile pipette and it was transferred to the center of the EA plates. By using a sterile glass spreader samples were spread uniformly over the surface of the media and duplicates were also prepared. This procedure was repeated for each dilution and two sets of plates were prepared. One set was incubated at 37 ^{0}C (Enumeration of total coliform bacteria) and other set was incubated at 44.5 ^{0}C (Enumeration of feacal coliform bacteria) for 24 - 48 hours. After the incubation period, red/ pink colonies were counted and results were expressed in terms of colony-forming unit (CFU) per gram. Sri Lanka Standards, 1991c: SLS 516: Part 3). The entire procedure was repeated for each sample three times.

Determination of Chemical Parameters

Determination of Salt Content

A 1 g of ground dried fish was mixed with 10 ml distilled water and it was filtered with filter paper. The filtrate was taken and it was titrated with standard $AgNO_3$ solution using K_2CrO_4 as an indicator. In this method (Sri Lanka Standards, 2007: SLS 643:2007), a white precipitate of AgCl is deposited during the titration. Excess of Ag+ ion reacts with chromate ion showing an appearance of a red silver chromate precipitate which is the endpoint. The steps of the reactions are as follows.

$Ag^+ + Cl^- \longrightarrow AgCl$ (white precipitate)

$2Ag^+ + CrO_4^{2-} \longrightarrow Ag_2CrO_4$ (red precipitate)

The amount of NaCl was determined by the following formula,

$$\text{Amount of NaCl \%} = \frac{C1V1 \times \text{NaCl M}}{m} \times 100\%$$

where, C1= Concentration of $AgNO_3$, V1= Volume of $AgNO_3$ used at the end point, M= Molecular weight, m= mass of dried fish.

Determination of Moisture Content

Moisture content was calculated according to Helrich (1990). Small amount of ground dried fish sample was taken into a dried silica crucible with a lid. Then the sample was weighed and it was dried in an air oven at 100 ^{0}C - 105 ^{0}C for 3 hours. Then it was left to cool, to room temperature in a desiccator and weighed, repeated the heating, cooling and weighing at half an hour intervals until there was no further loss in mass. Moisture content was determined by the following equation.

$$\text{Moisture percentage by mass} = \frac{m_2 - m_3}{m_2 - m_1} \times 100\ \%$$

where, m_1= mass of crucible, m_2= mass of crucible and moist sample, m_3= mass of crucible and dried sample.

RESULTS AND DISCUSSION

Microbial Parameters

Aerobic Plate Count (APC)

Catfish, Ponyfish, Tuna, Bigeye scad and Goldstripe sardinella contained APC less than 100X103 CFU/g which is acceptable based on Sri Lankan standards (Figure 2). The Anchovy sample contained the APC with the margin of limit while Mackerel sample exceeded the acceptable limit having 105X103 CFU/g. Previous research conducted by Saritha *et al*. (2012) revealed that dried fish sample such as *Leiognathus dussumieri, Acetes* Sp, *Chirocentrus dorab, Pomadays maculates, Scomberomorus commersonnii, Stolephorus commersonnii* and *Paraupeneaus indicus* contained excessively high APC value (Above 1 X 106CFU/g). Post-harvest delay, unsuitable transportation, unsanitary handling during the salting and sun-drying process, contaminated working floor, and contaminated salt and water may be the main reasons for the bacterial contamination of sun-dried fish.

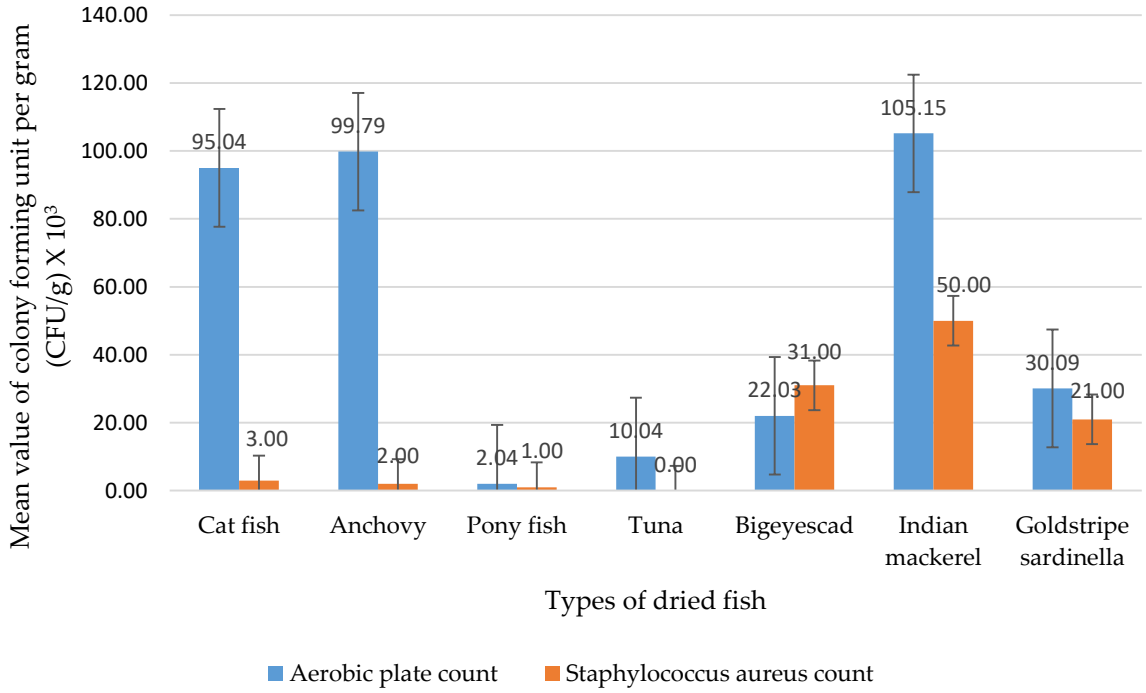

Figure 2: Mean value of aerobic plate counts and *Staphylococcus aureus* counts in different types of dried fish.

Staphylococcus aureus is a gram-positive bacterium and causative agent of several infectious diseases including skin infections, bacteremia, endocarditis, pneumonia and meal poisoning. It is very necessary to enumerate the *S. aureus* count in the food. Based on Sri Lankan standard, 2007 (SLS 643:2007), *S. aureus* count of dried fish should be less than 1×101 CFU/g. According to the above Figure 2, most of the samples contained unacceptable number of *S. aureus*. Tuna was the only sample that showed zero results for the presence of *S. aureus* while the Indian mackerel sample contained the maximum number of *S. aureus* count. *Staphylococcus aureus* was found in all other samples in this research. Many researchers have undertaken similar experimnets around the world and they claimed that none of the dried fish samples tested positive for *S. aureus* (Yam *et al*., 2015; Singh and Kulshrestha 1993, Suleiman and Mustafa, 2012).

Enterotoxigenic staphylococci do not form a part of the normal bacterial flora of fresh marine fish, however, they usually get contamination either from the handlers or from the surface with which they come in touch. The studies conducted by Simon and Sanjeev (2007)

have confirmed that 66.41 % of the *S. aureus* strains isolated from fish processing factory were enterotoxigenic and they produced enterotoxins A, B, C, D and E both singly or in combinations. A study in India showed that by correctly applying salt curing and sun-drying processes to fish, *S. aureus*-free fish products can be obtained (Sanjeev and Surendran 1996).

Yeast and Mould Count

Hundreds of species of microscopic foodborne yeasts and moulds (fungi) make up the enormous and diverse category of foodborne diseases. The capacity of those organisms to attack a wide range of foods is largely owing to their very adaptable environmental requirements. They have a wide range of acid/alkaline requirements for growth, ranging from pH 2 to pH 9, and they are osmotolerant. Their temperature range (10 - 35 ^{0}C) is also wide, with a few species capable of growing at temperatures below or above this range. Despite evidence that yeasts require a greater water activity, foodborne molds have low moisture requirements. Most species may thrive at a water activity (aw) of 0.85 or less. Both yeasts and moulds cause various degrees of deterioration and decomposition of foods. They can enter and grow on almost any sort of food, not only raw materials but even processed meals, at any moment. Because of their ability to produce poisonous compounds known as mycotoxins, some foodborne moulds and perhaps yeasts may be harmful to human and animal health.

Based on Sri Lankan standard (2007) (SLS 643:2007), yeast and mould count of dried fish should be between 1×10^3 - 1×10^4 CFU/g. Figure 3 shows that, Catfish, Anchovy, Tuna, Gold stripe sardinella, Indian mackerel and Bigeye scad contained yeast and mould counts in the acceptable range and the Bigeye scad sample showed zero (Figure 3). The Pony fish sample showed the highest count, which was $1X10^4$ CFU/g. Saritha *et al*. 2012 have reported that yeast and mould had been found in abundance in dried fish samples such as *Acetes* sp, *Chirocentrusdorab*, *Pomadays maculates*, *Leiognathus dussumieri*, *Scomberomorus commersonnii*, *Stolephorus commersonnii*, and *Paraupeneaus indicus* (Above 1.3×10^4 CFU/g).

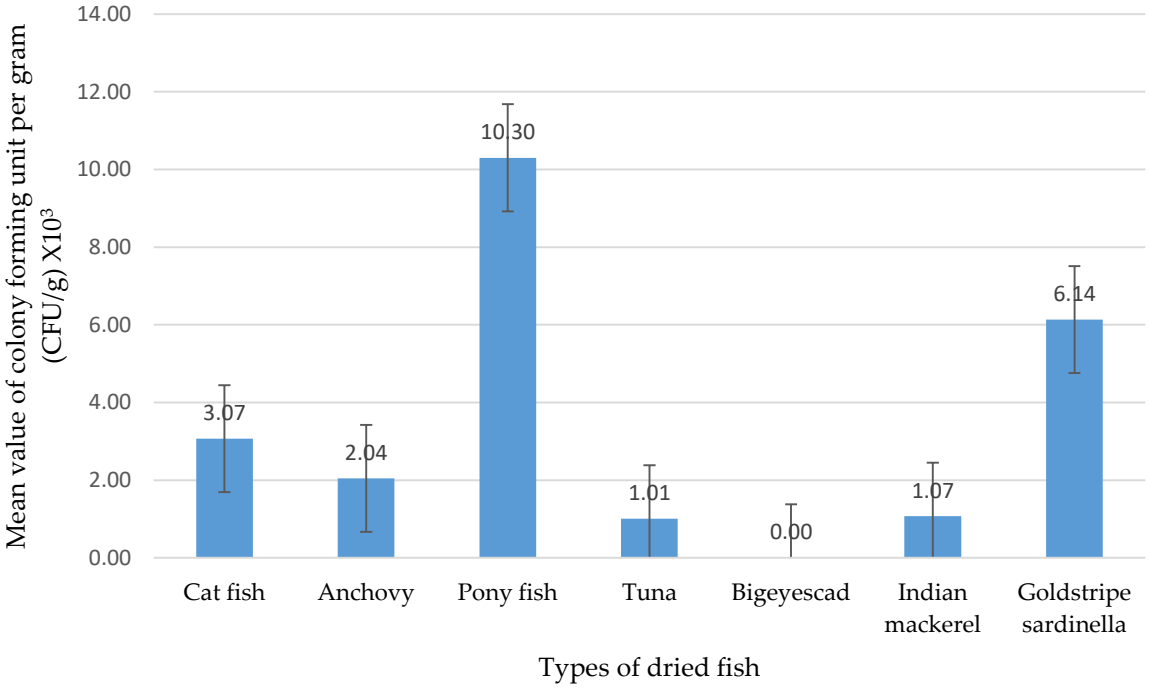

Figure 3: Mean value of yeast and mould counts in different types of dried fish.

Fungal species such as *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and *Fusarium* sp. are pathogenic to human beings (Köhler *et al*., 2014). According to Rawat (2015), food spoilage is caused by *Aspergillus* sp., *Mucor* sp., and *Penicillium* sp. As a result, the absence of these fungi is extremely important in terms of seafood safety and quality. Marine fungi may have invaded the dried fishes through the seawater used for washing or the salt used in brine preparation (Felicia and Jamila 2003). The occurrence of a high fungus count was reported by Logesh *et al*. (2012) and according to their results, fungal occurrence could be attributed to the brine used in the fishing curing yard in Cuddalore District. Because of the importance as one of the most worldwide trafficked foodstuffs and as a cheap protein supplement, fish and fishery products are at the forefront of food safety.

Coliform Bacterial Count

Total coliforms include microorganisms that are found in the soil and water that have been stimulated through surface water and human or animal waste. Fecal coliforms: are the group of the total coliforms that are considered to be present, especially in the gut and feces of warm-blooded animals.

Based on Sri Lankan Standards (2007) (SLS 643:2007), total coliform and feacal coliform count should be absent. The results of all the total and fecal coliform tests were satisfactory as all the dried fish were of good quality on the basis of total and feacal coliform count. Suleiman and Mustafa (2012) conducted a study in Sudan and reported that tested dried fish samples were free from indicator organisms like coliforms. According to Hajmeer *et al*. (2006), high concentrations of sodium chloride affect *E. coli* cell morphology and cause substantial damage to *E. coli* cells.

In accordance with a previous report (Nimrat *et al*., 2019), almost all gram-negative bacteria are generally inhibited at less than 0.93 water activity whereas gram-positive bacteria can thrive down at 0.85 water activity.

Determination of Chemical Parameters

Salt Content

Fresh fish consists of 75 – 80 % water, but in the case of very fatty fish, water percentage is 60 – 65 % (FAO, 1982) and this water can be removed partially by means of salt. A sufficient amount of common salt (NaCl) in fish can considerably minimize or prevent bacterial infection. When fish are immersed in a salt solution (brine) with a higher concentration of salt than the mixing with salt in the tissue, water will move from the tissue into the brine until the strength of the two solutions become equal, and salt will penetrate the tissue which is known as osmosis. Sodium plays a role in reducing the growth of microorganisms helping the removal of some water from the tissue. Before cooking salt from dried fish should be removed properly because excessive NaCl in the fish can lead to stroke, high blood pressure and heart disease. And also some of the calcium may be pulled from the bons causing cause calcium losses.

Based on Sri Lankan Standards (2007) (SLS 643:2007), the acceptable limit of salt should be between 12 - 35 %. Among the all samples Ponyfish showed the highest salt content, which was 23.98 %. And all the samples contained the acceptable salt limit (Figure 4).

High salt-containing foods can hamper the growth of organisms; only halophilic and halotolerant microorganisms prosper under extreme conditions. The tolerant ability of gram-

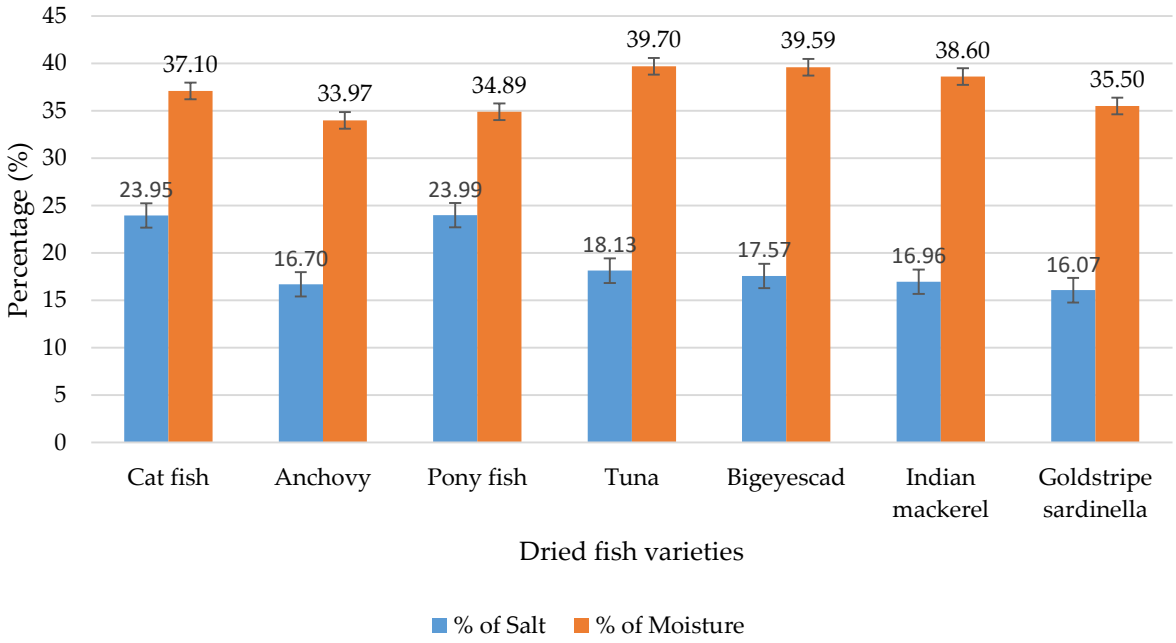

Figure 4: Salt and moisture content in different types of dried fish.

positive bacteria, especially cocci, results in survival under extremeconditions (Cotter and Hill, 2003). The incidence of halophilic and halotolerant bacteria can create health concerns and harm food safety. For example, there were several halotolerant bacterial species frequently being reported as active histamine-producing isolates e.g. *S. epidermidis, S. xylosus, Pantoaeagglomerans, Bur. cepacia* and *Bacillus*, isolated from various salted dried fish products (Aponte *et al*., 2010, Peconek *et al*., 1997).

Moisture Content

All the dried fish samples tested in this study were got from the retail market where the environment moisture content was more or less the same in all marketplaces. The moisture level of fish and dried fish has an important role in spoilage. The rate of drying of fish is controlled by a range of aspects, notably the type of the fish; (i) excessive fat content in the fish reduces the speed of drying; and (ii) the thickness of the fish; thicker fish takes a long time to reach the water in the middle layers to reach the surface. Based on the Sri Lankan standard (2007) (SLS 643:2007), the acceptable limit of moisture content

should be less than 40 %. Among all the samples highest moisture content was obtained from Tuna, which was 39.07 % and all the samples showed good quality based on moisture content (Figure 4). There is no microbial growth when there is less than 0.85 % of water content in food (Farkas *et al.*, 2007).

CONCLUSION

Out of all fish types, only the imported mackerel sample exceeded the acceptable APC limit having $105X10^3$ CFU/g while all other samples were in acceptable range. However, according to the Sri Lankan Standards (2007) (SLS 643:2007), all the dried fish contained unacceptable amount of *S. aureus* except Tuna compared to the acceptable limit which is less than $1X10^1$CFU/g. The maximum amount of *S. aureus* was found in imported mackerel.

According to the Sri Lankan standard, the acceptable limit of yeast and mould count should be between $1x10^3 – 1X10^4$ CFU/g and all tested types were in the acceptable range. Based on Sri Lankan Standards (2007) (SLS 643:2007) the total coliform and fecal coliform have to be absent and in the present study, both counts were zero in all of the samples.

In this study salt and moisture content of all the samples were under the acceptable limit showing 12 – 35 % and 40 %, respectively. Out of seven samples, Tuna was the only sample that was safe microbiologically for consumption. Therefore, proper measures have to be taken to produce dried fish hygienically and recurring checks have to be performed to confirm their quality, especially to check the imported dried fish as this study pointed out imported mackerel contained the highest number microorganisms. It is important to test the microbial and chemical quality of the dried fish before they are released to the market.

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