United International Journal for Research & Technology

Volume 06, Issue 01, 2024 | Open Access | ISSN: 2582-6832

Microbiological and Chemical Analysis of Commercially Important Smoked Fish in Pasacao, Camarines Sur, Philippines

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***Abstract*—** The study was carried out to determine microbial count and the proximate composition of the commercially important smoked fish such as; Smoked Sardines (Sardinella) Round Scads (Decapterus macrosoma) from Different Smoked Fish Enterprise (SFE) Location in Pasacao, Camarines Sur, Philippines. The samples were collected from the identified three (3) SFE and were packed in PPE pouches and brought to Department of Science and Technology Region V (DOST V} Laboratory for microbial analysis. The method used for analysis was the Association of Official Analytical Chemists method (AOAC 2021 Ed). Coliform count and E. coli was detected below the minimum microbial limit for both species of fish and to the three (3) SFE (<10). However, APC was detected above the maximum microbial limit in SFE 2-3 (9.3 x 107 - 6.4 x 107). On other hand, the overall nutritional composition of smoked fish showed that it is highly nutritive with crude protein having the largest quantity of the dry matter in the fish samples. Hence, smoked fish products can provide nutrients needed to maintain health and to provide reasonable levels of is recommended that the production of smoked fish products demands smoked products that are clean, wholesome and fit for human consumption. Therefore, it is the operator's obligation and local government to take all precautionary measures. The smoked Fish enterprise ensures that only wholesome fish products should be produced.

***Keywords*—** Chemical, Commercially Important, Microbiological, Smoked, Fish.

INTRODUCTION

Pasacao is a well-known fish landing area in Camarines Sur. Presently, there are 16 registered fish smoking processors using traditional processing methods and old types of smokehouse. Further the finished products are not properly packed where smoked can easily be contaminated with dust and other foreign matters. For safety purposes there is a need to examine the nutritional and microbiological status of the smoked products, hence, this study.

Over 80% of world fish production is used for consumption. The demand for fish is estimated to grow on all continents and reach even 17.9 kg per capita by 2020 (Bainy E.M., Bertan L.C., Corazza M.L., Lenzi M.K, 2015)[1]. The increasing consumer awareness of nutritional issues and appropriate composition of meals contributes to the growing interest in health-enhancing food. Fish are perceived as a significant component of a balanced and healthy diet, primarily due to the low fat content compared to animal meat and the content of fatty acids exerting a positive effect on the human organism, e.g., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) European Food Safety Authority (EFSA), 2014 and USDA Dietary Guidelines, 2015) [2].

Fish and fishery products are an important food component for a large part of the world's population, with an average consumption level of 20.1 kg per capita (FAO, 2016) [3]. In developing countries, fish is a relatively cheap and accessible protein source, suitable for complementing high carbohydrate-based diets. Among muscle food, fish is the most perishable and loses freshness after death due to autolytic and microbial spoilage (Dehghani, Hosseini, & Regenstein, 2018; Matak, Tahergorabi, & Jaczynski, 2015) [4-5]. In tropical regions, conservation of fresh fish remains a problem because of the lack of adequate infrastructures, and environmental and climatic conditions that contribute to its spoilage within few hours (Anihouvi, Kindossi, & Hounhouigan, 2012) [6]. Fish contains most of the important essential amino acids, particularly, lysine, methionine and tryptophan that are lacking in plant proteins. It is also an important source of vitamins and minerals which are important for good living (Abolagba, O. J., and O. O., Melle, 2008) [7].

Food processing methods, in particular high temperature, have a substantial effect on the maintenance or modification of food structure as well as loss of nutrients. Heat effects resulting in reduced fish

water activity allow better preservation causing microbial sterilization and thus minimize spoilage and increase the shelf life of fish products. They contribute to changes in the chemical composition and, hence, the nutritional value of processed food (García-Arias M.T., et.al., 2003) [8].

During the smoking process, fats and water drip from the fish, resulting in the physical loss of lipids, protein, and micronutrients. Smoking at high temperatures can also reduce the functionality of essential amino acids. Smoke particles can react with nutrients in fish meat and may lead to loss of important nutrients and antioxidants (Abraha B., 2018) [9]

To prevent fish spoilage and reduce postcapture losses, various preservation methods including frying, fermentation, drying, salting, and smoking are used (Adeyeye et al., 2015; Ikutegbe & Sikoki, 2014) [10-11]. Smoking consists in submitting fish to direct or indirect action of smoke during the incomplete combustion of sewdust as fuel. Smoking of foodstuffs improves food organoleptic characteristics, induces water loss, and reduces the microbial load, and the presence of aromatic and bactericidal substances (Chakroborty & Chakraborty, 2017; Yusuf et al., 2015) [12-13]. Smoked fish is highly desirable because of its enhanced flavour and texture in fish in addition to the protection offered by smoking against microbiological, enzymatic and chemical deteriorative alterations (Sowumi, A. A., 2007) [14].

Traditional smoking, one of the main methods used for fish preservation in the country (Dégnon et al., 2013) [15], generates two types of end products, smoked fish (SF) and smoked–dried fish (SDF), used for local consumption or exported to neighboring countries.

Fish and fish products are involved in 10%–20% of foodborne diseases (Pilet & Leroi, 2011) [16], and the presence of pathogenic bacteria such as Staphylococcus aureus, Salmonella spp., pathotypes of Escherichia coli, and Listeria monocytogenes has been reported in SF (Adeyeye et al., 2015; Ayeloja, George, Jimoh, Shittu, & Abdulsalami, 2018; Ineyougha, Orutugu, & Izah, 2015; Likongwe, Kasapila, Katundu, & Mpeketula, 2018; Nunoo & Kombat, 2013; Udochukwu, Inetianbor, Akaba, & Omorotionmwan, 2016) [17,18, 19, 20, 22,21,22]. Another concern is the contamination by fungi.

In this respect, various studies have reported the occurrence of aflatoxigenic fungi in SDF (Ayeloja et al., 2018; Babalola, Odebode, Ojomo, Ogungbemile, & Jonathan, 2018; Job, Agina, & Dapiya, 2016; Wogu & Iyayi, 2011), which under certain conditions can produce mycotoxins (Wogu & Iyayi, 2011). Bigueja, (2020) [23, 24, 25, 26, 27] reported that the extent of Adoption of Good Manufacturing Practices in of Small and Medium Smoked Fish Enterprise (SMSFE) in Bicol Region, Philippines is fair due to lack of capital, no available cold storage in the area, lack of proper training for workers, work load and time constraint. It is therefore necessary to take action by improving the microbiological quality of SF and SDF. Hence, the present study aims to investigate the microbiological quality of three commercially important smoked fish in Pasacao.

MATERIALS AND METHODS

Collection of the Samples

The samples of commercially smoked fish were obtained from the three Fish Smoking Enterprises (SFE) in Pasacao, Camarines Sur.

The newly processed smoked fish was packed in sterilized PPE and immediately brought to the DOST V for proximate and microbiological analysis. The proximate chemical and microbial assessment was determined.

Microbial Load/Count

The analysis of coliform count, E. coli count, Rapid yeast and molds count and Total plate count was determined using the Official Analytical Chemists method (AOAC 2021 Ed.). The Analysis was carried out in the DOST V Laboratory. This microbial parameter is the FDA requirements.

Proximate Analysis

The analysis of water content, protein, fat, and ash was determined using the Association of Official Analytical Chemists method (AOAC 2021 Ed). Analysis was carried out in the DOST V Laboratory.

Statistical Analysis

The results are expressed as means and standard deviation and analyzed using two ways analysis of variance (using SPSS version 17.0 where significant difference.

Results and Findings

Microbiological Analysis of Commercially Important Smoked Fish Product taken from Different Fish Smoking Establish in Pasacao

Commercially Smoked Fish samples were taken from three (3) fish smoking Enterprises where fish are hot smoked without gutting and drying, soak concentrated brine solution and used uncleansed equipment. Generally, the production of smoked fish was done in an environment that is not sanitary and water used does not undergo microbiological analysis.

Coliform Count and E. coli Count

Table 1 shows the microbial load of Smoked Sardines (Sardinella) from Different SFE Locations in Pasacao.

Table 1. *Microbial Count Smoked Sardines aand Round Scads from Different Enterprise Location in Pasacao*

Enterprise Location (EL)	Coliform Count (CFU*/g)	E. coli Count (CFU*/g)	Rapid Yeast & Mold Count (CFU*/g)	Aerobic Plate Count (CFU*/g)
Sardines				
SFE 1	<10	<10	<10	<300
SFE 2	<10	<10	$1.8X10^2$	$2.3 X 10^3$
SFE 3	<10	<10	<150	$1.8 X 10^3$
SFE 1	<10	<10	<10	$6.2 x 10^3$
SFE 2	<10	<10	$1.8 X 10^3$	9.3 x 107
SFE 3	<10	<10	<10	6.4 x 107

The results showed that Coliform count and E. coli count was recorded less than ten (<10 CFU*/g) for both smoked sardines and round scad and to the three SFEs. The result is within recommended microbiological limits for seafood (ICMSF, 1986) [28]. However, the detection of E. coli does not assure the absence of enteric pathogens (Mossel 1967, Silliker and Gabis 1976) [29, 30]. Coliform presence in food products raises the question of pathogen contamination occurring through a similar process. Many coliforms, including Escherichia coli (E. coli), a subgroup of coliform, can be found in the human digestive tract. While some strains of E. coli are harmless, other strains can cause serious illness. Similar to coliforms, if E. coli contamination is detected it indicates that conditions exist in which pathogens may be present (Washington State Department of Health, Questions & Answers, 2018) [31]. According to Adelaja, O.A., et.al., (2013) [32] testified that E. coli caused diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections while Salmonella caused gastroenteritis and typhoid fever. According to Ababouch (2008) [33], contamination of food indicates non-compliance with the rules of good manufacturing practice and breach of the hygiene rules. During collection the contamination of fecal coliforms may be associated by poor hygiene and unsanitary handling of fish. The Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP) and Standard Sanitation Operating Procedures (SSOP) were fairly or rarely adopted by the smoked fish operators in smoking the fish (Bigueja, M.C., 2020) [34].

Aerobic Plate Count (APC)

The Aerobic Plate Count (APC) is also known as standard plate count, aerobic mesophilic count, total plate count or aerobic colony count. The APC is used to estimate the bacterial population in a food sample. It provides an estimate of the numbers of microorganisms that can grow aerobically at mesophilic temperatures. The APC may be used to judge sanitary quality, sensory acceptability, and conformance with good manufacturing practices (GMPs). Results of the APC can provide a food processor with information on the quality or handling history of raw materials, food processing and storage conditions, and handling of the finished product. Detectable changes in food quality characteristics due to microbial growth and enzyme production generally occur when the APC increases reaches about 106–107 per g or ml (Mendonca, Aubrey and Gordon, André, 2020) [35].

The results for Aerobic Plate Count (APC) (Table 1), Smoked sardines from SFE 1APC has the Lowest APC (<300 CFU*/g) while smoked sardines from the 2nd and 3rd SFE the APC results is between the minimum and maximum limit (ICMSF, 1986), $2.3 X 10^3$ CFU*/g and $1.8 X 10^3$, respectively. On the other hand, The APC of round scad from SFE 2 has the highest APC (9.3 x 107 CFU*/g) followed by SFE 3(6.4 x 107 CFU*/g) Which exceed to the maximum limit (ICMSF, 1986), while

APC from SFE 1 between the minimum and maximum limit (6.2 x 10^3 CFU*/g).

The microbial flora associated with fish could be from the environment in which the fish are harvested and not specific to a particular species (Shewan, J. M. (2000) and Teugels, G. G., and Audenerde, D. F. E. (2003) [36,37]. In this study, the fish samples which were hot smoked using saw dust which are arranged in bamboo trays placed on dirty floors or untidy tables. Basti, A. A., A., Misaghi, T. Z., Salehi and A., Kamkar, (2006) [38] reported that processed fish are easily contaminated with microorganisms in nature, through handling, during processing and if the post-processing handling is not properly done under hygienic conditions. The quality of smoked products is dependent on several factors, including, the quality of the fish at the time of smoking, the preparation of the raw material, the nature of wood and the type of the smoking procedure employed (da Silva, L. V. A. (2002) [39]. Hence, with the result of this recent result, fish processors should be properly educated and be trained for food safety and GMP.

Rapid Yeast & Mold Count

The Yeast and Mold count were found higher for SFE 2 for both sardines and round scad , 1.8X10^2CFU*/g and 1.8 X 10^3 CFU*/g, respectively. This may be caused due to the improper processing and partial dehydration of fish during smoking. Due to the maintenance of inappropriate temperature and other parameters during smoking, smoked fish samples may have a relatively low water activity level which is a prerequisite for fungal growth (Dutta, Moon, et.al., 2018) [40]. In this study it was shown that the moisture content of the smoked fish from different SFE is relatively high which is favorable to the growth fungus. He presence of Aspergillus flavus and Aspergillus fumigatus in the fish samples can cause serious health concern because of their mycotoxigenic potentials (Adelaja OA, et.al., 2013) 41]. Essien et al. reported that Aspergillus flavus and Aspergillus fumigatus produced aflatoxins, which destroyed the liver and kidney in man resulting in death.

Chemical Composition of Commercially Important Smoked Fish Product taken from Different Fish Smoking Establish in Pasacao

Table 2. *Chemical composition Smoked Sardines (Sardinella) Round Scads (Decapterus macrosoma) from Different SFE Location in Pasacao*

Parameters	Smoked Sardines				Smoked Round Scad				Overall Mean + SD
	SFE1	SFE 2	SFE 3	Mean + SD	SFE 1	SFE 2	SFE 3	Mean + SD	
Moisture	42.72	49.16	51.32	47.73±3.65	62.21	53.38	60.29	58.62±3.79	53.18±6.59
Protein	39.72	32.74	34.65	35.70±2.94	29.19	32.85	30.34	30.79±1.58	33.24±3.39
Fat	2.86	3.03	3.49	3.12±.26	2.12	1.18	1.27	1.52±.2	2.3±.87
Salt	1.1	1.1	0.65	0.95±.21	0.46	0.99	0.54	0.66±.23	0.80±.26
Ash	13.86	13.06	8.65	11.85±2.29	6.08	11.72	6.97	8.25±2.47	10.05±2.98

Proximate analyses include moisture, proteins, fat, ash, and salt available per 100 grams of sample. The method use for moisture determination is TM-Ch-001 with reference to AOAC 952.08A 21st Ed., while protein, Fat, Salt and Ash is TM-Ch-004 with reference to AOAC 940.25 21st Ed., TM-Ch-003 with reference to AOAC 948.15 21st Ed., TM-Ch-019 with reference to R. Lees., and TM-Ch-005 with reference to AOAC 938.08 21st Ed., respectively. Table 2 presents the result of the Chemical Composition smoked Smoked Sardines (Sardinella) Round Scads (Decapterus macrosoma) from Different SFE Location in Pasacao. However, these values cannot be compared with the standard since no Philippine National Standard (PNS) for proximate analysis for smoked fish has been established so far. Thus, available literature was used for comparison and interpretation of results (Malaluan, Ivane N. et.al, 2018) [42].

Moisture composition of smoked fish

Table 2 shows the lowest moisture content is recorded to the smoked sardines in SFE 1 and the highest is from smoked round scad in SFE 1 too. This result indicates that SFE 1 is using the same processing method in any type of the fish. Moisture content of food is important to food manufacturers because it is a determining factor in food quality, preservation, and resistance to deterioration. Moisture content of 12% is the level

beyond which fish products begin to grow molds after a few days (Daramola et al., 2007)[43]. Analysis revealed that smoked fish has a mean moisture content of 47.73% for smoked sardines and for smoked round scad is 58.62% and the overall mean of 53.18%. This result suggests that immediate mold growth can occur in smoked fish samples upon storage apart from temperature effect at the point of storage and other storage conditions.

Protein

A safe protein intake level for adults is defined as the lowest level of dietary protein intake that will balance the losses of nitrogen from the body in persons maintaining energy balance at modest levels of physical activity (FAO/WHO/UNU, 2007) [44]. For all age groups (children and adults, male and female) ranging from 10 to >70 years old, %RNI of proteins are all above 20%.

In this study the mean proximate composition, the crude protein for smoked sardines and round scad is 35.70% and 30.79%, respectively. This results formed the largest quantity of the dry matter in the smoked fish samples. This provides the basis that smoked fish is a high source of protein. This result further coincides with the findings of Besharati in 2004 [45]. The percentage of protein increases during drying and smoking; this corresponds with the decreasing percentage of water, which can result in concentration of nutrients. This result suggests that smoked fish can be regarded as a rich source of protein, which implies that smoked fish can provide sufficient amounts of protein to meet their daily needs to build and repair body tissues for growth and maintenance (Malaluan, I. N. et.al, 2018) [46]

Fats

The Acceptable Macronutrient Distribution Ranges (AMDR) of fat for Filipinos is 15–30% for children and adults. The upper limit is the maximum intake level recommended by most dietary guidelines as a preventive measure against the risk of cardiovascular and other degenerative diseases.

As shown in Table 2 smoked sardines and round scad have only 3.12% and 1.52% respectively. Hence, fish product with low amount of fats indicates having low amount of fat and fatty acid content. Low fat content may be attributed to possible loss of fat due to the high temperature in the smoking process (Ahmed et al., 2011) [47]. This can be explained by oxidation and break down of crude fat into other components due to oxidation of poly-unsaturated fatty acids (PUFA) contained in the fish tissue (Daramola et al., 2007) [48].

Salts

Table 3. *ANOVA results for chemical composition smoked fish product*

Source of Variation	SS	df	MS	F	P-value	F crit	
Rows	12336.37	4	3084.091	158.739	7.54E-15	2.866081	
Columns	0.383547	5	0.076709	0.003948	0.999997	2.71089	
Error	388.574	20	19.4287				
Total	12725.32	29					

In the preparation of smoked fish products, salt also plays an important role, since it decreases aw and gives particular sensory attributes. These products usually present salt levels between 2% and 5.5%; however, the salt content can be up to 8% in the water phase in order to prevent the risk associated to Clostridium botulinum (Gram, 2001a). This latter level is very often unacceptable to the consumer. In mild hot smoked fish, the NaCl content in the flesh is usually low, about 2.5%, which corresponds to about 3.3% in the water phase. The combination of salt and temperature used in smoked fish products is not sufficient to guarantee the absence of Listeria monocytogenes growth, the use of additional hurdles being necessary (Gram, 2001b). In smoked products the salt content is critical for safety, given that the concentration in the water phase has to be high enough to inhibit the growth of pathogenic microorganisms (Pittia, Paola and Antonello, Paparella, 2016) [49]. In this research study, the overall mean of fat in smoked fish is only .80%. Hence, the fats obtained in smoked fish products in this recent study is very minimal which is acceptable to consumers. However, growth of pathogens may occur, as shown in table 1. Coliform and E. coli are detected in the smoked products.

Ash

Higher ash values in smoked sardines is obtained (11.85%) than smoked round scad (8.25%). Lower mean value of ash content may be due to moisture loss due to

the heat during smoked processing. Increased ash content in processed products has been reported by many workers (Akintola et al., 2013) [50]. The highest ash content was observed when salting and sun drying methods were combined. Bille and Shamkai (2006)[51]found that smoking significantly increased ash in the dagga fish more than the sun-drying method. An increase in ash content by a combination of processing methods involving smoking can also be attributed to protein denaturation due to a reduction in moisture and consequently loss of water holding capacity of the protein in the samples.

Significant difference

Table 3 shows there was no significant difference in chemical composition smoke sardines and round scad since the p-value is greater than (.05 level of significance). These results indicate that the three (3) SFE applying the same manufacturing practices.

CONCLUSIONS

Food safety is the scientific discipline describing handling, preparation, and storage of food in ways that prevent foodborne illness to avoid potentially severe health hazards. Generally, the density of microflora in fish and fishery products are related to environmental factors such as water pollution, hygienic conditions of processing environment, processing in appropriate methods, handling, transportation, commercialization and storage conditions. Furthermore, improper handling could also affect the nutritional value of the smoked. The detection of coliform, E. coli and higher value APC and yeast and molds count signifies that processors did not observe the high standard of GMP. Though the SFE who produces commercially important smoked products in the locality used traditional methods of smoking, the smoked produced with a good nutritional composition.

RECOMMENDATION

To preserve food safety, GAP (Good Aquaculture Practice), SSOP (Sanitation Standard Operating Procedure), HACCP (Hazard analysis and critical control points) etc. should be maintained in every step from culturing through processing to marketing of products.

To prevent the incidence of food contamination, there is a need to educate the related people such as fish processors, handlers, retailers or vendors about the importance of sanitation, hygienic measures and good food handling practices.

Proper hygienic conditions should be maintained at every step of culturing, catching, landing and transportation, processing, storing and marketing following HACCP steps for producing good quality fish and fishery products.

Production of smoked fish products demands smoked products that are clean, wholesome and fit for human consumption. Therefore, it is the operator‟s obligation and local government to take all precautionary measures. The smoked Fish SFE

ACKNOWLEDGEMENT

The authors wish to thank the management and staff of Partido State University, Sagnay Campus for financial support, to the Department of Science and Technology, Region V (DOST V) for making available their Laboratory facilities and to the Municipality of Pasacao for allowing the authors to conduct this research to their existing smoked fish establishment. Without them this study will not be successfully completed.

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