

CURRENT GOOD MANUFACTURING PRACTICES COMPLIANCE AND MICROBIAL EVALUATION OF SMOKED FISH FROM SELECTED ENTERPRISES IN NAGA CITY AND PASACAO, CAMARINES SUR, PHILIPPINES

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Abstract — Compliance to Good Manufacturing Practices (GMP) requirements and microbial quality of smoked fish from selected enterprises in Naga City and Pasacao, Camarines Sur, Philippines was evaluated. Seven enterprises were identified and were profiled as to processing techniques which were shown to be different among enterprises. Assessment to GMP compliance revealed that enterprises have very low conformity to organization, personnel, equipment and utensils, and hygiene and sanitation requirements. Three enterprises with more than 20 years existence, annual production of 10,000 kg to 30,000 kg and wider scope of distribution were chosen as source of smoked fish to be focused of microbial evaluation. Total plate count of the product revealed that only one of the enterprises produces smoked fish that is not beyond the standard limit 5×10^5 CFU/g. Detection for coliform revealed that values were low having values ranging from <50 CFU/g to <100 CFU/g however, smoked fish was found to be positive for contamination and presence of *Escherichia coli*, results showed counts ranging from <10 CFU/g to <20 CFU/g which is beyond the limits set by Philippine National Standards.

Keywords — Smoked fish, enterprise, good manufacturing practices compliance, microbial quality

INTRODUCTION

Smoked fish is an important diet as a protein source for developing countries due to its relevant cheapness and longer shelf life than other protein source. Smoked fish and other traditional products of fish such as salted, dried or fermented products produced by small scale family establishments are common in the Philippines (FAO, 1990). According to Bureau of Fisheries and Aquatic Resources smoking as a means to preserve fish is not as prevalent as drying but is seen as an important industry in Camarines Sur. In fact, the Department of Trade and Industry of the province provides assistance and trainings that aims to improve quality of produced smoked fish.

Smoking method lessen chances of possible spoilage by microorganisms and enzymatic reaction (Kobajashi et al. 2012) but smoked fish can still be contaminated with pathogenic microorganisms, due to the following factors namely; improper smoking conditions, distribution and improper storage (Agu et al., 2013, Akinwumi & Adegbehingbe, 2015) resulting to the products' limited shelf life. In fact, several studies set in Africa reported by Olaleye & Abegunde in 2015, Gbolagade et al. in 2012, and Akinwumi & Adegbehingbe in 2005 showed instances of detection in alarming levels of pathogenic microorganisms such as coliforms, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* in smoked fish products.

In the Philippines however, only few studies about evaluation of its quality especially in terms of microbiology are published. Information is scarce especially of smoked fish in Camarines Sur.

This study is in consonance to the increasing public interest and concern over food safety, as well as commercial pressure to improve food quality and shelf-life (Bell et al., 2005). It is aimed to evaluate the degree of compliance of smoked fish enterprises

to the different requirements of good manufacturing practices. More importantly, this may address scarcity of information about microbial quality of smoked fish in Camarines Sur, Philippines.

MATERIALS AND METHODS

This study included interview of smoked fish producers as to profile practices, techniques and knowledge in handling smoked fish. Smoked fish samples were obtained from identified smoked fish enterprises in the Province of Camarines Sur. The study was carried out in two phases: survey and experimental.

Conduct of survey

Modified survey questionnaire designed by Kyangwa & Odongkara (2005) which gathered information on the processors' knowledge and handling practices of smoked fish and survey questionnaire utilized by Canini et al. (2013) to investigate on the characteristics of site and personal hygiene of identified respondents were used. Likewise GMP checklist (AO no. 153 s. 2004) intended to assess compliance of facilities to lay standards was also used. Focus of the interview was referred facilities by Bureau of Fisheries and Aquatic Resources and Department of Trade and Industry which were located in Brgy. Abella and Brgy. Sangay in Naga City and Brgy. San Cirillo in Pasacao, Camarines Sur.

After interview and initial assessment of identified enterprises, top three enterprises were selected based on the length in the fish smoking industry, average amount of processed smoked fish per year and the scope of distribution.

Sample collection

Sampling was done from February to March 2017, with a frequency of once a week during the same day of smoked fish production. Collection of samples was done every production schedule of the enterprises which were Tuesdays or Wednesdays. Five

samples of 250 grams each were obtained from each enterprise. Gathered samples were packed on original packaging used in the enterprises where smoked fish are wrapped on newspaper before packed on plastic bags. Evaluation of sample was done on the same day samples were acquired; prior to evaluation, samples were stored in freezer with temperature ranging from -4 to -10°C.

Microbial Analyses

Methods for microbial analyses of smoked fish sample used are procedures described in Bacteriological Analytical Manual (BAM), Association of Analytical Chemists (AOAC, 15th and 17th edition) and Food Microbiology and Laboratory Practices (Bell et al., 2005). Smoked fish samples were subjected to total plate count, coliform and *Escherichia coli* enumeration using Hygiena-MicroSnap rapid detection device.

Aerobic Plate Count

Twenty five grams of smoked fish sample was added with 225 mL distilled water and was blended for 2 minutes, resulting to 10-1 dilution. Prepared solution was regarded as the homogenate.

Using sterile micro pipettor, dilutions was prepared out of the homogenate. One mL of the homogenate was added to 9 mL of diluent to make dilutions; 10-3, 10-4 and 10-5 were plated out.

Detection and measurement of RLU (Relative Lights Unit)

Before transfer of enriched sample, detection device was allowed to equilibrate to room temperature before used. To bring the extractant liquid to the bottom of the tube, tube was flicked downward forcefully once. Then, aseptically, enriched sample from the enrichment device was transferred to the detection device by inserting swab to transfer sample up to the filling level indicated on the detection device. It was noted that filling was up to the fill line only

as over filling may result to inconsistent and increased reading. The same as the enrichment device, the snap valve of the detection device must be bent and broken for activation. After activation, tube was shaken to mix liquids and right after was inserted to luminometer to initiate reading; displayed results were recorded as RLU.

Coliform and *E. coli* Homogenization of sample

Sample was homogenized through the same process described for APC.

Enrichment of sample

One (1) mL of aliquot from dilutions 10-1 was transferred to the enrichment device for coliform and *E. coli*. The same procedures and techniques described for MicroSnap Total Detection Device were employed.

Detection and Measurement of RLU

After transfer of enriched sample and activation of detection device, incubation at 37°C for 10 minutes followed. After incubation, detection device was inserted to the luminometer to initiate reading; displayed results were recorded as RLU and were transformed to equivalent CFU/g using the table provided by the manufacturer.

RESULTS AND DISCUSSION

Selection of enterprises

Based on the information given by Bureau of Fisheries and Aquatic Resources and Department of Trade and Industry, a total of seven enterprises were assessed and visited for this study; four of which located in Pasacao and three from Naga City. Based on the given criteria (existence in the industry, scope of distribution, average production per year); two facilities in Pasacao and one in Naga City were chosen as respondents for this study.

Based on the data presented on Table 1, it showed that Enterprise 1, 4 located in Pasacao and Enterprises 5, situated in Naga City are the top three facilities to meet

Table 1. Summary of information gathered from the enterprises visited.

Enterprise	Existence (years)	Scope of Distribution	Average annual production kg/yr.
Pasacao			
Enterprise 1	+25	Partido, Pili, Ocampo (there are direct buyers)	36,000
Enterprise 2	1	Pili, Tinambac, Calabana	10, 080
Enterprise 3	15	Pasacao, Calabanga	36,000
Enterprise 4	+20	Pili, Naga, Calabanga	36,000
Naga City			
Enterprise 5	+55	Naga, Iriga, Buhi, Nabua, Naga (supplies some restaurants such as Graceland)	13,440
Enterprise 6	10	Francia (Naga)	6,720
Enterprise 7	17	Naga	13,440

the criteria given and thereby were chosen as respondents for the research where samples of smoked fish was acquired.

Compliance to GMP requirements

In order to evaluate the present condition and state of visited enterprises, GMP checklist developed by Zamudio et al. (2013) was adopted. Results gathered were correlated to the results obtained during microbial analyses. Enterprises were assessed based on the sections stated in the GMP requirements which include; organization, premises, equipment and utensils, sanitation and hygiene, and warehousing and distribution.

Organization

All of the enterprise has adequate number of personnel that compensate labor requirement probably because these are family establishments that involves all family members to the operations. However, no organizational structure with determined responsibilities is available; most of the time head of the family is the one who functions as quality assurance or production manager. Because of this, roles or functions of personnel are not specified

thereby responsibilities are performed less effectively. All enterprises do not have available written documents containing standard procedures for processing, production and cleaning. These documents are needed to check and validate if procedures employed in every production are standard for operations. Production records are of importance in implementing process controls and traceability of error in case of production rejects. Lack of these documents results to variation in day to day operations which consequently leads to inconsistent product quality and specifications.

Premises

All enterprises failed to conform on the sections specified for requirements in premises. Plant design and size is one of key factors in attaining sanitary operations. Walls, ceilings, and floors must be made of concrete with no impervious finish so as to allow easy cleaning and limit harborage of undesirable microorganisms. Lighting and ventilation must be adequate enough for comfort of workers and personnel, and likewise to avoid contamination of food (FDA, 2004).

During evaluation, problems in conformity to these requirements were evident on occasions where products were directly laid on floor because of insufficient space that may possibly result to cross contamination and deterioration of smoked fish quality. Anihouvi et al. (2019) related high microbial load of smoked fish to exposure in unhygienic environment.

There are facilities that smoke fish in open air although majority have enclosed or protected smoking facility (71.42%) that is more ideal than the open air smoking facility which is more susceptible to contamination. Likewise, it was observed that all enterprises are near residential houses since these facilities are family establishments with close proximity to the owner's house and still others the owner's house agreeing to the report made by FAO (1990). Moreover, a number of enterprises were located near highly contaminated areas such as toilet, drainage, dust bin and pig pen which poses contamination of food products as these areas are harbored with physical, chemical and microbial contaminants (Canini et al., 2013).

Equipment and Utensils

Smoked fish enterprises usually use baskets during the boiling procedure and weaved baskets made of bamboo slits called kaing during the smoking process. Buildup of grime, food debris and dirt were observed on the equipment and utensils which were evident during visit in the enterprises. Bamboo or wood has uneven surface which is difficult to clean hence become an ideal growing ground for microorganisms which in turn can be introduced to the smoked fish. Equipment and utensils have direct contact to food products, making them a primary source of contamination (Olaleye & Abegunde, 2015). Thus, a requirement that utensils and equipment should be made of stainless steels as these are corrosion resistant and so as to permit easy cleaning and sanitation. Likewise, correct washing and sanitation is also important to remove

food debris, filth and minimize microbial contamination. Non conformity to these articles results to smoked fish that is likely to be contaminated with microorganisms cross contaminated from the equipment.

Personnel Hygiene

Personnel working in direct contact with food and those involved in processing shall be free from illness or communicable diseases such as cough, lesions, and boils which are source of microbial contamination. It was found that personnel in the enterprises do not have current medical records because these are small scale fish processors where majority of workers are family members. Although, personnel are free from obvious communicable disease that shows the awareness on the importance of having healthy personnel to work in the plant.

Likewise, it is also important that personnel conform to hygienic practices to protect food from contamination. Majority of enterprises have personnel conforming to hygienic practices though only few have formal training regarding sanitation and hygiene. According to FDA (2004) personnel must have knowledge about the importance of sanitation and hygiene to establish production of safe and clean food.

Personnel were found to be aware on the basic hygienic practices to be applied when working, all wash hands at least three times a day, some were using clean clothes and had finger nails cut. Jay et al. in 2008 reported that bacterial pathogens may be transmitted from humans to food and that poor personal hygiene may facilitate transmission. Hence, hand washing is one of the most critical hygienic practices as enteric pathogens can survive on the hands for three hours or longer. However, no enterprise practices the use of hairnets in the site which can add more points to food safety and some females involved in processing has nail polish.

Sanitation

Sanitary facilities to support hygienic operations must also be present. Hand washing and toilet facilities are readily accessible for use. However, there are enterprises which have toilet facilities near the plant which poses the risk of food to be contaminated with fecal matter or other human excretions. Akinwumi & Adegbehingbe (2015), Udchukwo et al. (2016) reported that the quality of smoked fish is dependent on factors including site and personnel hygiene.

Production and Process control

Written handling and manufacturing procedures employed by the enterprises from receiving of raw materials, through processing and during the storage of finished products are not present. According to FDA, it should be ensured that production processes do not contribute to the contamination of food products making process control important. It should be noted however, that all enterprises are small scale food processors that do not have quality control officers to inspect and function as process and production control. Operations are carried out from knowledge passed on from family generations thus conformity to standards is not evaluated.

Warehousing and Distribution

All enterprises are practicing the first in-first out (FIFO) system. However, storage facilities of finished products are not secured from rodents, insects or other pests. Finished products are handled on the same area for processing thus products are exposed to spillage, dirt and garbage. The exposure of finished products may result to physical, microbial and chemical contamination that may pose health risks to consumer.

In general, all enterprises visited were found to have little conformance to requirements of Good Manufacturing Practices. This result is found to be in consonance to the report that small scale

fish processors or entrepreneurs in the Philippines are often non-compliant to set standards especially that of concerning hygiene and sanitation (FAO, 1990). It was also reported that only fish processing facilities that are for export conforms to the standard set by authorities (FAO, 1990) but it should be noted that smoked fish produced by these small scale processors are those accessible for majority of buyers thus its quality and microbial safety must be checked.

Characteristics of Operation and Site Hygiene

Site hygiene

Akinwumi & Adegbehingbe (2015), Udchukwo et al. (2016) reported that the quality of smoked fish is dependent on factors including site and personnel hygiene; the quality of raw materials used; the preparation employed for the raw materials; and the handling techniques used before, during and after processing. Thus, hygiene and sanitation in addition to handling and processing techniques have direct implications on the quality of smoked fish.

Processing Techniques

Based on the hot smoking procedure stated on the PNS/FDA26 (2010), questions to assess processing techniques carried out by the enterprises were developed. Washing of raw materials before processing significantly lowers the microbial load of materials, 42.85% of enterprises evaluated were found to wash raw fish 2-3 times before salting or brining. However, 2 out of 7 facilities do not wash and directly proceed to processing. With these numbers, four uses tap water while one uses well pump water.

In addition, ratio of fish to salt during soaking in brine solution and duration varies from enterprise. All enterprises do not monitor and has no standard time for soaking. Two enterprises soak for 30 minutes while the rest soaks until raw fish

becomes firm. Brine soaking does not just add to the salty taste of the smoked fish this also results osmotic pressure that draws out water from the fish and act as an additional preservation method (Oğuzhan & Angiş, 2012).

The same observation were made for the boiling of smoked fish in brine solution; in all the enterprises boiling temperature and duration is not monitored though some enterprise set indices and parameters to know if boiling is enough. Likewise, smoking temperature and duration is not monitored; during interview it was revealed that the smoking duration depends on the size of fish and amount of smoke. Others use the appearance of fish to indicate if smoked fish is already cooked. Only one of the assessed enterprises does not use artificial colorant but 85.71% apply artificial colorant at levels that are not regulated.

As reported by Adeyeye (2018), Andhikawati & Pratiwi (2021) and Hagos (2021), there are varying methods to produce smoked fish and that these methods has effects on the sensory and microbial quality of the finished product.

Results of Microbial Evaluation

Aerobic Plate Count

Figure 1 shows the microbial load in \log_{10} cfu/g of smoked fish for every week of sampling. Enterprise 1 was consistent into producing smoked fish that is of conformity to standard limit. Microbial load of Enterprise 4 was higher and was revealed that out of 5 sampling week, 4 had values that exceeded limit. Similarly, Enterprise 5, found to have the highest count was consistent in producing smoked fish beyond the standard limit.

The varying counts among the enterprises can be attributed to the differences in smoking techniques, variation in sanitation and hygienic practice (Gbolagade et al., 2012, Olaleye & Abegunde, 2013, Agu et al., 2013) that has

direct impact on the quality of fish.

Similarly, the difference in the microbial load among the smoked fish in each enterprise can be possibly attributed to absence of standard process control and process monitoring. Although different fish species were subjected to microbial analyses; Akinwumi & Adegbehingbe (2015) claimed that microbial flora associated with fish could be from the environment from which it is harvested and not specific to a particular species making initial microbial load of raw fish also a factor.

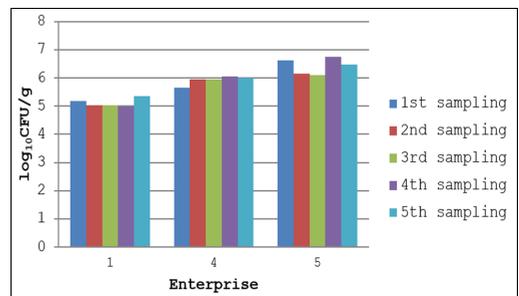


Fig. 1. Total plate count of smoked fish for every week of microbial evaluation.

In addition, Ibrahim et al. (2014) reported that microorganisms detected in smoked fish may be introduced from water used for washing, utensils and wrapping materials and the exposure of products or raw materials to high temperature and unhygienic condition.

Coliform and *Escherichia coli*

Coliforms are enumerated and detected in food products in microbial analyses as this microorganism is a general sanitary indicator of a food processing environment and it is also considered as a significant standard of hygiene and sanitation (FDA, 2013). Dutta et al. (2018), Akinwumi & Adegbehingbe (2015) and Olaleye & Abegunde. (2015) detected the presence of coliform in smoked fish and reported that the presence of this microorganism is an important public health concern. Coliforms

are easily killed by heat, thus are ideal when testing for post-processing contamination of cooked fish and fishery products (Jay et al., 2008).

Smoked fish samples from different facilities were evaluated as to the presence of coliform and it was revealed that all were detected to have presence of coliform. Table 2 shows the coliform RLU count of the smoked fish samples.

Results showed that the coliform count from the enterprises range from 4-12 Relative Light Units or <20-<100 CFU/g; on average, smoked fish from Enterprise 1 was found to have the lowest coliform RLU that is 5 RLU or <50 CFU/g while Enterprise 5 was found to have the highest count having mean of 9 RLU or less than 100 CFU/g. The difference in the level of detected microorganisms between enterprises can be attributed to varying factors such as differences of pre and post-smoking processes among enterprises (Agu et al., 2013), smoking and processing techniques and the hygiene and sanitation conditions among others (Akinwumi & Adegbehingbe, 2015). However, if these results are correlated to the information gathered about the enterprises. Enterprise 1 and 4 has lower coliform count possibly due to shorter holding time for the fresh fish as it is near the fish source in Pasacao Fish Port compared to that of Enterprise 5 in Naga City which utilized frozen or fresh fish. Although, it was expected that Enterprise 5 will have the lowest count because it has

conformity to GMP requirement regarding the use of stainless steel equipment and utensils.

On average, coliform counts of the samples indicates lower microbial load compared to the previous studies conducted about the microbiology of smoked fish (See Table 2). This may be attributed to the difference of sampling time, related studies focused on the microbial analyses at markets while this study focused on newly processed product from the enterprises. This coincides to the report of Agu et al. in 2013 that post processing contamination significantly affect the coliform count of smoked fish. Olaleye & Abegunde (2015) claimed that low counts for coliform may be attributed to conformance of food handlers to basic sanitary rules.

Escherichia coli

Escherichia coli is an enteric bacteria that is used as an index for fecal contamination and unsanitary processing of food products (FDA, 2013). Detection of this microorganism is important as its presence pose health risk and causes gastro intestinal illness. It is shown in Table 3 that smoked fish samples were detected to be positive with *E. coli*. Values were ranging from 0 RLU to 7 RLU equivalent to <10 to <50 CFU/g having values that were beyond the limit set by PNS/FDA 26 which was 11. Among the facilities, Enterprise 1 was the lowest; followed by Enterprise 4 and lastly Enterprise 5 that has the highest count.

Table 2. Coliform in estimated CFU/g of the smoked fish.

Enterprise	Sampling schedule and microbial load (Estimated cfu/g)				
	1	2	3	4	5
1	<50	<50	<50	<50	<50
4	<20	<20	<50	<50	<100
5	<50	<50	<100	<100	<100

Table 3. *E.coli* count estimated CFU/g of smoked fish.

Enterprise	Sampling schedule and microbial load (Estimated cfu/g)				
	1	2	3	4	5
Enterprise 1	<10	<10	<10	<10	<10
Enterprise 4	<10	<10	<20	<10	<10
Enterprise 5	<20	<20	<20	<20	<20

It can be seen that smoked fish sample from Enterprise 1 was consistent to having less than 10 CFU/g of *E. coli* and thus conforms to the standard value set by PNS/FDA 26 (2010) for smoked fish. All throughout the sampling collection, this enterprise produced smoked tamban (sardine species) a relatively small variety of fish thus it is concluded that *E. coli* count is directly proportional and affected by the fish size. In addition, there are limited sources of contamination in this enterprise; though it is near the owner's house, processing facility is physically separated from it.

Enterprise 4 produces the same variety of smoked fish but has higher *E. coli* count with 1 occasion out of 5 sampling instance exceeding limits, during the information gathering it was found out that a pig pen is located at the back of the facility that is of proximate distance from the processing area. The same personnel working in the processing area may be the same personnel working in the pen that causes cross contamination. In addition, proximity of the area where contaminants be, can justify these results. The facility is not physically separated from the owners' house and is surrounded by residential houses.

However, it can also be that the water source from which the raw fish was acquired has positive number of *E. coli*. According to FDA in 2011, harvest areas for some shellfishes are examined for safety of *E. coli*, this method however is not done for fishes thus the possibility that even before processing the microorganism is already present. According to Alikunhi et al. (2017) all fish and sea food are susceptible to contamination originating from the marine environment. This is further aggravated by the practice of not de-gutting or eviscerating fish for smoking.

CONCLUSION

Based on the data given by Department of Trade and Industry and Bureau of

Fisheries and Aquatic Resources, there are seven established smoking enterprises in Naga City and Pasacao, Camarines Sur, Philippines.

Smoked fish enterprises in Naga City and Pasacao, Camarines Sur, Philippines have been long existent, distribution of produced smoked fish are mainly in the province. It was revealed that annual production ranges from 6,720 kg-36,000 kg. Facilities were found to be family establishments that belong to the small scale fish processors that have low conformity to the GMP requirements. Likewise different processing techniques were employed by each facility that has consequent effect to smoked fish quality.

Only smoked fish from one of the enterprises was found to conform to the standard for total plate count limit; although coliform count was found to be significantly lower than the values reported from recent related studies conducted. However, all smoked fish evaluated were found to be positive in *E. coli*. Samples from Enterprise 5 were revealed to exceed standard limit for *E. coli*.

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