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Implication of Processing and Handling Methods to the Microbial Quality of Stored Fried Atlantic Cod

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Abstract
The microbiological keeping quality of Atlantic cod (*Gadus morhua*) fried and stored under ambient conditions was investigated. Raw Atlantic cods were subjected to two treatments in the laboratory of wheat flour-coated fried fish (LFS) and the other fried without wheat flour (LS) against the conventional flour-fried fish in the open market samples (MS). Microbial analyses were conducted on the three different fried fish samples at the initial stage and subsequently on a weekly basis. The result of the microbial analysis over the 4-week storage period revealed an increase in the total fungal count, bacterial count, *Salmonella* spp and *Staphylococcus aureus,* an enteric gram-negative bacterium. *Escherichia coli* was not isolated in any of the fried fish samples. The flour-fried fish samples obtained from the open market (MS) had no count in week 0, it recorded the highest total heterotrophic bacterial counts at 20.0×10^6 cfu/g in week 2; 22.2×10^6 cfu/g in week 3 and 24.0×10^6 cfu/g in week 4 respectively. Meanwhile, the fish samples which were fried without flour (LS) in the laboratory had the lowest count of total heterotrophic bacteria in week 2, week 3 and week 4 at 5.0×10^6 cfu/g, 8.2×10^6 cfu/g and 12.5×10^6 cfu/g respectively. Hence, based on the result of the microbial analyses of the fried Atlantic cod with or without flour, it is recommended that the fried fish under ambient conditions should be consumed within two weeks (14 days) of purchase or other preservation methods such as refrigeration or re-drying be applied.

Keywords: Atlantic cod, fry, flour, microbes, ambient, weekly

Introduction

Fish falls in the category of highly perishable food items in the world. Fish constitutes a rich source of essential nutrients in the human diet, which is necessary for their growth and healthy development at all stages (Abdullahi *et al*., 2001). The Atlantic cod (*Gadus morhua*) is a benthopelagic fish of the family Gadidae, normally consumed by humans. Predominantly, members of the genera *Pseudomonas, Alteromonas, Aeromonas*, *Flavobacterium, Salmonella* and *Escherichia,* were found to dominate the surface of the Atlantic cod. These pathogenic microorganisms in Atlantic cod pose a major hazard to human health as it is a commonly consumed fish product. Problems with pathogenic organisms can arise during the handling, storing and processing of these fish in conditions favorable for their growth (Ward and Beyens, 2015). According to Sapers *et al*. (2005), contamination of food is possible from handling to processing by infected handlers, with poor hygiene playing a major role in it. In most cases of food-borne illnesses or food poisoning in fish, processing approaches are relatively inadequate resulting in the spoilage of the processed fish. Smoking, drying, salting, frying, fermentation and or a combination of these traditional processing and preservation methods have been used to improve the shelf stability of fish (Ndife *et. al*., 2022). Meanwhile, frying is one of the major commercially adopted methods of processing fish such as Atlantic cod because it is a readily available means and fish get preserved. The inherent microbes in fish are killed by frying. Therefore, any contamination with microbes on the fried fish is considered to be from post-frying handling by the traders and from the selling location (Simiyu *et al*., 2021). However, there are hygienic measures necessary to be put into the processing of fried Atlantic cod, which could determine the safety of this ready-to-eat food to the consumer, as it is a major factor in the assessment of its quality (Teklemaria *et al*., 2015). Research has shown that fried fish products can be a source of public health hazards when they contain microorganisms that can cause foodborne illnesses such as *Listeria monocytogenes, Salmonella* spp. and *Clostridium botulinum* (Bintsis, 2017). Generally, the traditionally processed fried fish

products that dominate most local markets in developing countries are known to have poor shelf stability (Hansen *et al*., 2008; Teklemaria *et al*., 2015). This is due to poor quality of the food in terms of storage and handling (Oranusi *et al*., 2013) while Osatohanmwen *et al*., (2019) stated poor and excessive handling, the need for good personal hygiene and caution in the course of cooking, storing and packaging of cooked and fried ready-to-eat street foods, such as fish for sale. Therefore, there is a need to investigate the microbial succession and evaluate microbial spoilage patterns in fried *Gadus morhua*, by processing it in a controlled condition and exposing it to the same storage condition alongside commercially available ready-to-eat samples.

Materials and Methods

Sampling

The samples of fresh and fried Atlantic cod were purchased from Ota metropolis, and the binder, the wheat flour to prevent scattering and enhance firm product was procured from a local market (Iyana-Iyesi market) in Ado-Odo Ota, Local Government, Ogun State. The microbial analysis and the controlled exposure experiment were carried out in the Microbiology Research laboratory of the Department of Biological Sciences, Bells University of Technology, Ota Ogun State, Nigeria. A total number of 30 pieces of cod fish with weights ranging between 200g and 250g were used for the research. Ten (10) pieces were ready-to-eat fried fish purchased from a roadside fish seller; while twenty (20) pieces of the fresh fish samples were divided into two groups – a group of ten (10) was fried with wheat flour and the second group of ten (10) was fried without the addition of flour.

Processing of Fish Samples for Frying

The fish samples to be processed were eviscerated and washed thoroughly with sterile distilled water and properly drained. The first group of ten fish samples were dipped inside and coated with powdery wheat flour before frying with vegetable oil for about 40 minutes; they were turned at intervals to achieve proper frying. This procedure was repeated for the second group of ten cod fish samples, without the addition of flour prior to frying. The fried fish samples were stored in a perforated container for aeration; labelled as MS - Market sample fried with flour, LFS - Laboratory Flour-fried sample, LS - Laboratory sample fried without flour and stored for four (4) weeks.

Media preparation

The bacteriological and mycological agar media used for this study were prepared by following the manufacturers' instructions for preparation. Streptomycin Sulfate with a concentration of 30 mg/L was added to the Potato Dextrose Agar after preparation, before inoculation to prevent the growth of bacteria on this medium.

Microbial Analysis of Fish Samples

This was carried out following the method of Ajiboye *et al*. (2011). One gram of each fish sample was weighed and dispensed into a test tube containing 10 millilitres (10 ml) of sterile distilled water for serial dilution. A volume of one milliliter (1.0 ml) from this tube was transferred into the next test tube (containing 9 ml of sterile distilled water) upon proper mixing. The serial dilution was done in a one-tenth-fold stepwise order to the fifth dilution (10^{-5}). One milliliter (1.0 ml) from the 10^{-5} dilution tube was then inoculated by the pour-plate method in triplicates into Petri dishes containing freshly prepared Nutrient agar (microbes e.g. E. coli and S. aureus), Eosin Methylene Blue agar (gram-negative bacteria), Potato Dextrose agar (bacteria and fungi), Mannitol salt agar (Staphylococcus spp and *Escherichia coli*) and Salmonella-Shigella agar. For the bacterial culturing, the inoculated plates were inverted and incubated at 37°C for 24 hours after which the plates were examined for growth. The discrete colonies on the plates were counted and the mean counts were recorded as colony-forming units per gram of sample. For fungal culturing, the inoculated plates were inverted and incubated at 25°C initially for 48 hours after which the plates were further incubated for 72 hours in plates which showed no growth after the initial 48 hours. The discrete colonies on the plates were counted and the mean counts were recorded as colony-forming units per gram of sample (cfu/g).

Isolation and Characterization of Microorganisms in Fish Samples

Pure cultures of bacteria were obtained by sub-culturing. This was done by aseptically streaking representative colonies of different morphological types appearing on the culture plates, onto freshly prepared nutrient agar plates before incubation at 37°C for 24 hours. Subsequently, characterization was performed on the various bacterial and fungal isolates by following the methods of Olaniyi *et al*. (2018). Microbial analysis every week was conducted on all these samples to monitor the trend of microbial load in the samples over a period of four weeks.

Results and Discussion

Results

In Figure 1 and at the initial week (0), there was no growth in all cultured samples from the nutrient agar; that is, the Laboratory sample without flour (LS NA), Market sample (MS NA) and Laboratory flour-fried sample (LFS NA). For week 1, the market samples (MS NA) had the highest bacterial count among the groups of fish samples cultured at 7.4×10^{6} cfu/g while the sample with the lowest heterotrophic bacterial count was the laboratory flour-fried sample (LFS NA) at 4.2×10^{6} cfu/g. Microbial analysis carried out on the samples from week 2 to week 4 revealed the total heterotrophic bacterial counts for market samples (MS NA) was the highest: 20.0×10^{6} cfu/g in week 2; rose to 22.2×10^{6} cfu/g in week 3 and 24.0×10^{6} cfu/g in week 4 respectively. On the order hand, Laboratory samples without flour (LS NA) had the lowest values for the total heterotrophic bacterial count in week 2, week 3 and week 4 at 5.0×10^{6} cfu/g, 8.2×10^{6} cfu/g and 12.5×10^{6} cfu/g respectively.

Figure 2 revealed the total heterotrophic fungal count for each fish sample in this study. There was no growth in week 0 for the plated samples except the market samples (MS PDA) at 1.1×10^6 cfu/g. After the analysis in week 1, the laboratory sample without flour (LS PDA) had the highest heterotrophic fungal count at 12.6×10^6 cfu/g and the laboratory flour-fried sample (LFS PDA) had the lowest count at 7.2×10^6 cfu/g. From week 2 to week 4, the laboratory flour-fried sample (LFS PDA) had the highest fungal counts at 10.2×10^6 cfu/g, 13.0×10^6 cfu/g and 16.6×10^6 cfu/g. The Laboratory sample which was fried without flour (LS PDA) had the lowest total heterotrophic fungal count among all the samples cultured from week 2 to 4, with the following weekly values: 7.4×10^6 cfu/g, 8.2×10^6 cfu/g and 9.3×10^6 cfu/g respectively.

In Figure 3, the laboratory sample without flour (LS MSA) had 1.0×10^6 cfu/g for a staphylococcal count at week 0. By week 1, laboratory samples without flour (LS MSA) had the highest staphylococcal count at 5.2×10^6 cfu/g of cultured samples while the market sample (MS MSA) had the lowest at 4.4×10^6 cfu/g. The total staphylococcal counts recorded from week 2 to week 4 revealed that colonies of *Staphylococcus* were very high, in the range of 30×10^6 cfu/g for all cultured samples.

In Figure 4, no Salmonella growth was detected from all the fried fish samples in week 0. Meanwhile, for week 1, the market sample (MS SSA) was the only sample that had a total count of 3.0×10^6 cfu/g on the Salmonella/Shigella agar. From week 2 to week 4, laboratory flour-fried samples (LFS SSA) had the highest loads of 25.2×10^6 cfu/g, 26.0×10^6 cfu/g and 29.9×10^6 cfu/g respectively. However, the laboratory sample without flour (LS SSA) had the lowest count from the SSA plates among all the samples cultured with the following values 15.0×10^6 cfu/g, 16.2×10^6 cfu/g and 18.0×10^6 cfu/g respectively.

Throughout the 4 weeks of study, there was no growth of *Escherichia coli* on the Eosin Methylene Blue agar plates, in any of the stored fried fish samples as shown in Table 1.

Discussion

The proliferation of the target food-borne pathogens such as *Salmonella*, *Staphylococcus* spp., *E. coli* and other enteric organisms were monitored for the period of this study and for the total heterotrophic bacteria. Meanwhile, the occurrence of *E. coli*, *Salmonella* spp., *Staphylococcus* spp., and *Pseudomonas* spp in raw and sun-dried fishes has been documented (Nur *et al*., 2020). Also, Abebe *et al*. (2020) have reported *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, *S. aureus*, and *E. coli* as the most common of the thirty-one pathogens that may cause food-borne diseases globally. The flour-fried fish samples obtained from the open market (MS) had the highest microbial loads of all the samples cultured at the end of the research. This is probably due to the problem of handling, storage conditions and exposure. Also, is suggestive of the fact that the fried fish (MS) by the vendor, may not be adequately handled as the colonisation of the fish sample must have occurred after frying prior to sampling (Sapers *et al*., 2005; Peariso, 2005). Meanwhile, the fish samples which were fried without flour (LS) in the laboratory had the lowest count of heterotrophic bacteria. In the open markets in Ota metropolis, the desired effect of using wheat flour to coat before frying Atlantic cod is to help in keeping the tissue firm thereby preventing it easy scattering and improving the sensory/textural characteristics of the fried product. However, in this study, the use of flour in frying of the fish appeared not to hinder the growth of microbes as the dry outer crust created by coating with the wheat flour could still support the colonization of fungi prior to frying (Mohamed *et al*., 2019). The limited water encased in the flour-coated fried fish can still encourage bacterial growth as shown in this study. Total *Staphylococcal* count was recorded only from the laboratory fried without flour sample in week 0, this could be due to the colonization of the fried fish sample by staphylococcal species, as the surface might not be as crispy as the flour-coated samples from the open market or the laboratory flour-fried sample, hence the water activity would be high enough to support the growth of Staphylococcus present in the environment (Vaiyapuri *et al*., 2019). This suggests a relationship between the effect of the flour coating and staphylococcal species in the atmosphere or environment on the freshly fried Atlantic cod from the laboratory at the initial stage (week 0). Only the market samples were found with a microbial load of enteric gram-negative bacteria in week 1 of the study, as discovered on the Salmonella/Shigella agar. This could emanate from poor handling of the fried fish by the vendor, which is not limited to the handling but also, the utensils, the materials used for displaying the products and/or the storage material which must have introduced the contaminants (Hassanien *et al*., 2014). Generally, the relatively high microbial loads on flour-fried samples either from the open market or laboratory could be a result of the flour coating serving as an additional source of nutrients for the microbes to thrive (Ibrahim *et al*., 2015). The absence of *E. coli* in any of the samples throughout the period of study signifies that there was no form of faecal contamination since the fish samples were eviscerated prior to frying (Soliman and Shalby, 2001).

Conclusion

Generally, irrespective of the treatment employed, changes in the microbial parameters (bacterial, staphylococcus and fungal) were observed on the fried Atlantic cod gradually from week 1 (Day 7) and significantly from week 2 (Day 14) of storage under ambient conditions. This study revealed that storage of fried *Gadus morhua* at room temperature, beyond two (2) weeks might not be safe for human consumption based on the increase of microbes. The preparation of fried *Gadus morhua* under ambient conditions with proper hygiene could prevent it from being colonised by heterotrophic bacteria. The use of flour in frying Atlantic

cod could enhance the growth of fungi and most enteric microbial contaminants on the fried fish. Improper food handling practices could introduce microbial contaminants responsible for spoilage in *Gadus morhua* and food poisoning could emanate afterwards. Microorganisms that are indicators of faecal contamination such as E. coli should not be detected from fried Gadus morhua, under normal conditions of proper processing and disposal of intestines before adequate frying. Hygienic practices should therefore be encouraged and enforced among food handlers, especially the fishmongers and vendors who commercially fry *Gadus morhua* for human consumption. Other storage processes such as refrigeration, drying and salting could be employed to prolong the shelf life of fried *Gadus morhua*. Apart from the pleasant appearance that coating gives to fried *Gadus morhua*, it appears that there is no preservative function derived from it, rather undesirable proliferation of fungi and gram-negative enteric bacteria was observed. Hence, it is not necessary to fry *Gadus morhua* with flour coating. Therefore, based on the result of the microbial analyses and physical assessment in terms of firmness/texture and odour of the fried Atlantic cod with or without flour, it is recommended that the fish should be consumed within two weeks (14 days) of purchase or to apply other preservation methods such as refrigeration or re-drying.

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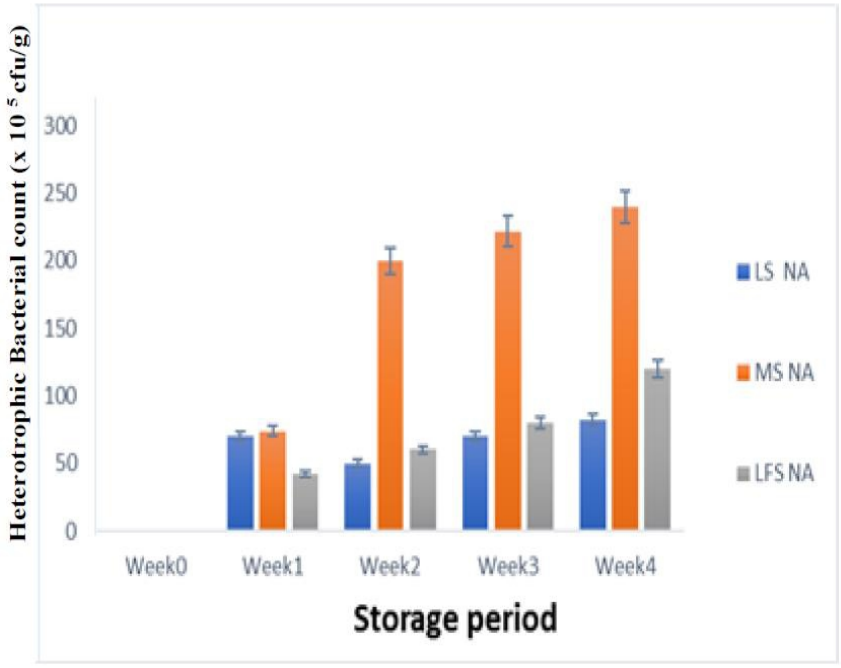

Figure 1:Total heterotrophic bacterial count from fried Gadus morhua samples stored under ambient condition
Key: LS NA – Laboratory sample without flour (on Nutrient Agar), MS NA - Market sample (on Nutrient Agar), LFS NA - Laboratory flour-fried fish sample (on Nutrient Agar)

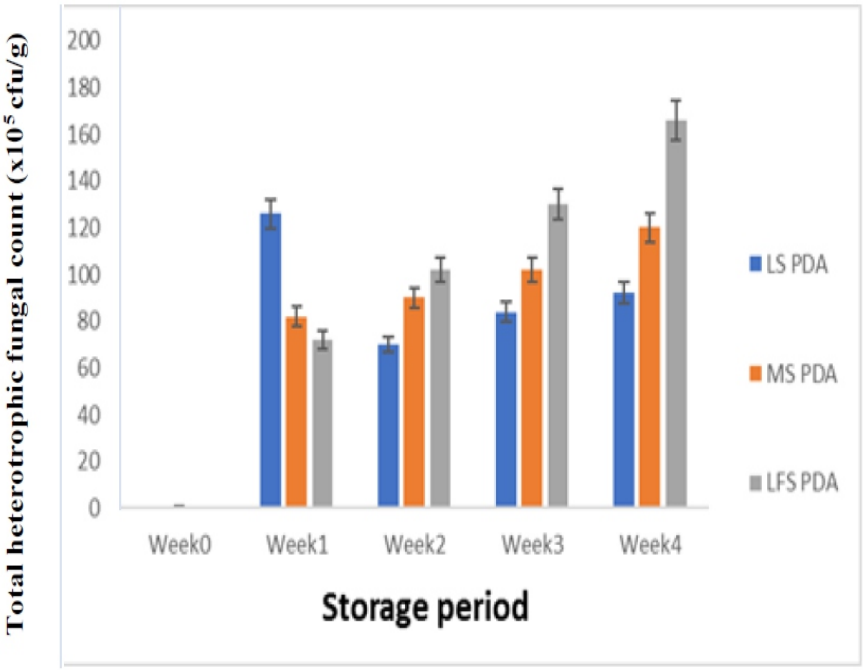

Figure 2: Total heterotrophic fungal count from fried Gadus morhua samples stored under ambient condition
Key: LS PDA - Laboratory sample without flour (on Potato Dextrose Agar), MS PDA - Market sample (on Potato Dextrose Agar), LFS PDA - Laboratory Flour-fried sample (on Potato Dextrose Agar)

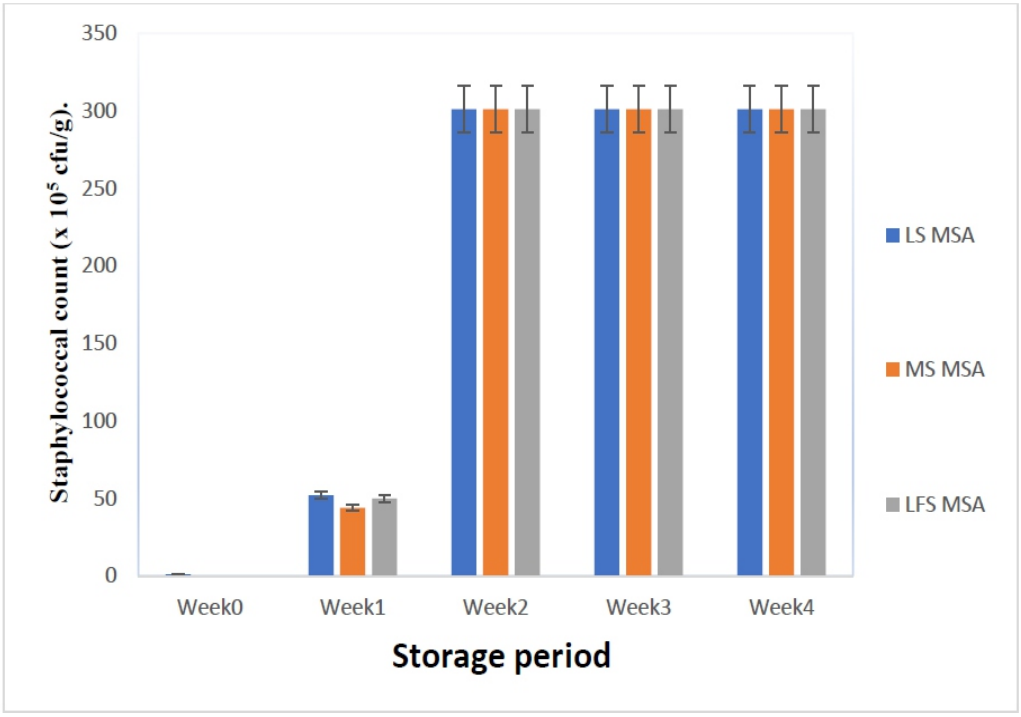

Figure 3: Total Staphylococcal count of fried Gadus morhua stored under ambient condition

Key: LS MSA - Laboratory sample without flour (on Mannitol Salt Agar), MS MSA - Market sample (on Mannitol Salt Agar), LFS MSA – Laboratory flour-fried sample (on Mannitol Salt Agar)

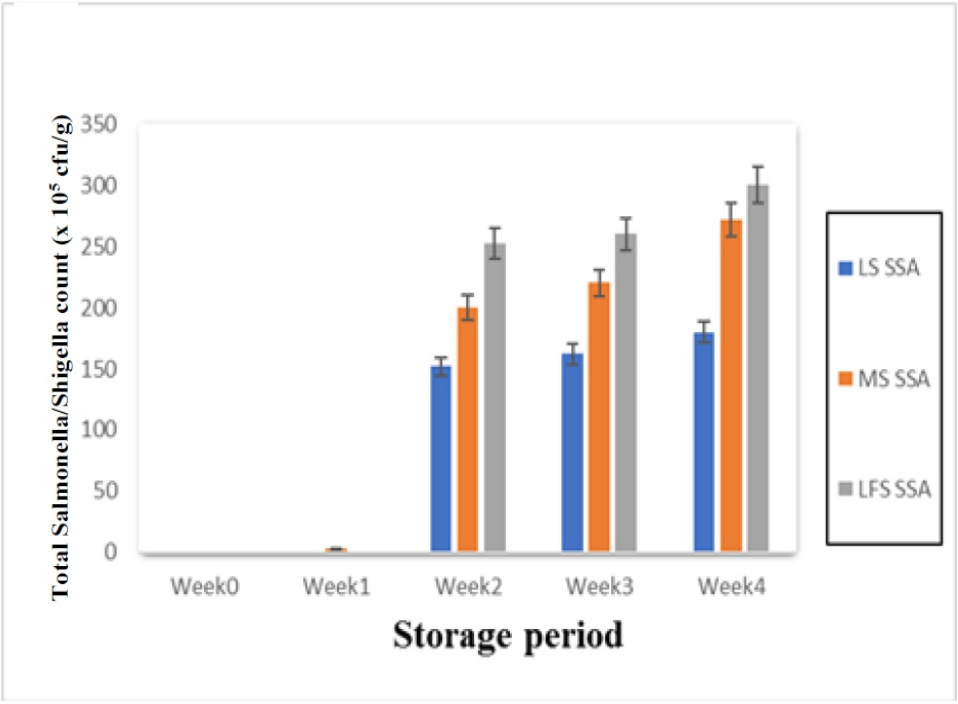

Figure 4: The occurrence of Salmonella in fried Gadus morhua stored under ambient condition

Key: LS SSA: Laboratory sample without flour (on Salmonella/Shigella agar), MS SSA: Market sample (on Salmonella/Shigella agar), LFS SSA: Laboratory flour-fried sample (on Salmonella/Shigella agar)

Table 1. Rapid Direct *E. coli* evaluation on EMB from fried *Gadus morhua* stored under ambient condition

	LS EMB	MS EMB	LFS EMB
Week 0	0	0	0
Week 1	0	0	0
Week 2	0	0	0
Week 3	0	0	0
Week 4	0	0	0

Key: LS EMB: Laboratory sample without flour (on Eosin methylene blue agar), MS EMB: Market sample (on Eosin methylene blue agar), LFS EMB: Laboratory flour-fried sample (on Eosin methylene blue agar)