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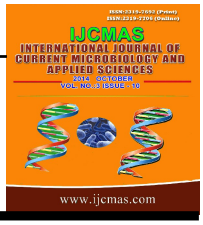

Original Research Article

Effect of packaging on the microbiological quality of chicken and fish viscera flour

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A B S T R A C T

Study aims on the one hand to know the microbial quality of chicken viscera, fish viscera flour and to select the packaging with the best suitable preservation condition.The flours were divided in three packages (plastic, jute bag and can) and three environments (refrigerated, ventilated, non-ventilated) for microbiological monitoring. A total of 36 samples of each flour were monitored and analyzed for two months, according to ISO and AFNOR standard methods for research of the following germs : *Escherichia coli*, yeasts, moulds, total and thermotolerant coliforms. The results obtained show that plastic bag and can packaging in a refrigerated or non-ventilated environments are favourable for the preservation of both flours for two months The results obtained on the production day of flours highlight the lack of germ spoilage indicator (*E. coli*, *Staphylococcus aureus*, sulphite-reducing anaerobes and coliforms). The microbial load was 2.95 and 2.84 $\log_{10}$ cuf/g respectively for chicken viscera and fish viscera flour.

Keywords

Chicken viscera flour, fish viscera flour, hygienic quality, packaging environment

Introduction

Current environmental policy encourages manufacturers to take into account the waste generated by all methods of fish processing. This is why, after the operation of noble parts of the fish, researchers are interested in the co-products. Co-products are defined as recoverable and unused parts during the traditional production operations.

100 million tons (FAO 2006). The increasing demand for protein on a global scale turns the focus on under- utilized protein sources (Liaset *et al*., 2000), and novel processing methods are needed to meet the demand for both human and animal feed use. During processing, solid wastes including heads and viscera are generated

and can contain 30 % of the original material. These wastes, which represent an environmental problem to the fishing industry, constitute an important source of protein. Consequently, large amounts of protein-rich by-products from the seafood industries are discarded or processed into fish meal (Ovissipour *et al*., 2009). Traditionally, this material has been converted to powdered fish flour as animal feed. New methods of processing are required to convert the underutilized wastes and by-products into more marketable and acceptable form. Fish viscera, one of the most important byproducts from any commercial fisheries, is a rich source of protein and polyunsaturated lipids but with low storage stability if not frozen or preserved (Raa *et al*. 1983). Annually, approximately 330 tons of sturgeon is caught off the south coast of the Caspian Sea (IFO 2006). Almost 20-25% of the weight of the sturgeon is viscera, which is produced as byproduct of sturgeon caviar and meat processing industries. The sturgeon wastes are discarded, except for swim bladder and notochord which are used for glue production and as soup ingredient, respectively. Sturgeon viscera could be a valuable protein source for animal feed and human food (Ovissipour *et al*. 2009). Rendering also helps minimize the release of animal tissues into the environment as potential biological hazards. Rendered meat and bone meal are valuable sources of protein in pig and poultry feed, pet foods, and even fertilizer (Wamalwa et Mohamed 2013).

In recent years, slaughterhouses have been expanding all over the world. This industry is among the most polluting industries because of the large amounts of waste it generates. Nonetheless, these slaughterhouse wastes are beneficial due to their potential as source of recoverable biomass and organic matter and fiber.

High production costs can be overcome by finding quality alternatives feed ingredients. Firstly, an alternative feed ingredients that have good potential as a substitute for fish meal is the waste of processed tuna industry, which is made of head, viscera, flesh and bone whose numbers may reach 10-25% of the total catch and harvest fish, estimated to about 500,000 tons per year, and can be utilized as a component of animal feed. Secondly, poultry by-products are eaten widely due to their low cost, their low content in fat and the short period of time for their preparation. However, the presence of pathogenic and spoilage microorganisms in poultry meat and in its by-products remains a significant concern for suppliers, consumers and public health o cials worldwide. Mesophiles, psychrotrophs, coliforms, *Escherichia coli* and *Staphylococcus aureus* are often followed in poultry products to assess microbiological safety, sanitation conditions during processing and to keep quality of product (Bean and Griffin, 1990; ICMSF, 1980; Nortje et *al*., 1990; Russell, 1997; Stolle, 1988; Tompkin, 1983). The majority of the microbiological studies on meat by-products had been carried out on beef and pork, but little attention has been paid to other meats. This study was designed to evaluate the bacteriological quality of retail chicken by-products (chicken parts and processed chicken products). The interest in poultry chicken production is due to the fact that live animals are hosts to a large number of different microorganisms residing on their skin, feathers or in the alimentary tract.

In Benin, fishing plays a relatively large role in the national socio-economic balance as it contributes to about 3% of gross domestic product (GDP) (Tossou, 2010). Fishing products are very popular with an average individual consumption of about 8.50 kg per year (FAO, 2000), with Tilapia fish

(*Oreochromis niloticus*) been one of the most consumed seafood in Benin. However, the waste produced by the latter is a cause of concern.

The aim of this study is to analyze the microbial quality of chicken and fish viscera flour with the goal of identifying the best packaging method for their preservation. To achieve this goal, we produced the different flours and keep them under different packaging and in different areas, for microbiological analyses.

Materials and Methods

Materials

Viscera chicken and fish were collected respectively from Agrisatch Society continental fishery port of Abomey- Calavi (Benin).

Methods

Production of flours

After chicken viscera of Cobb 500 receipt, they were emptied of their droppings. After dejection evacuation, they were washed, drained and cooked by fire. Viscera were drained again, spread on trays for drying an oven dried at 50°C. At the end, dried viscera were ground and packaged (Figure 1).

Immediately fish viscera of *Oreochromis niloticus* receipt, they were cooked, drained, spread on trays and dried in an oven at 50°C. Dried fish viscera were ground and packaged (Figure 2).

Chemical Analysis

Each flour was analysed for dry matter (DM), crude protein (CP) and total ash according to the method of AOAC (1990). Organic matter (OM) was calculated by subtracting the total ash from DM.

Assessment of microbiological quality

For microbiological analysis, each flour produced was packaged in three types of packaging: plastic bags, jute bags, cans. These packagings containing flours were then stored in three different environments: the refrigerator (4°C), a ventilated room and a room non-ventilated. Monitoring the quality over time was done by searching package and storage condition. The microbiological quality of flour was determined by the detection of yeasts, moulds, thermotolerant coliforms, *E. coli*, *Staphylococcus aureus*, sulphite-reducing anaerobes. A 10 g sample were diluted in sterile (90 ml) peptone water and then homogenized. Appropriate decimal dilutions were prepared for bacterial analysis according to ISO methods. Total Mesophilic Aerobic Bacteria were enumerated with Plate Count Agar (PCA) after incubation at 30˚C for 72 h. Yeasts and moulds were enumerated on Oxytetracycline Glucose Agar (OGA) after incubation at 25˚C for 5 days. Total and Thermotolerant Coliforms and *Escherichia coli* were counted in Brilliance *E.coli* Agar (Coliform Selective Medium) after incubation at 37°C for 48 h. *Staphylococcus aureus* was determined by the spread plate method using Baird-Parker agar with egg yolk tellurite emulsion (Oxoid Ltd.). The plates were incubated at 37°C for 48h (Lancette et Tatini, 1992). Sulfito-reducer Anaerobes were enumerated in Bacto Sulfite Agar (BSA) after incubation at 37˚C for 48 h.

Identifications were made during the two months of microbiological analysis using the method of Scotch (Samson *et al*., 1995) for the moulds investigation.

Results and Discussion

Manufacture of viscera

The most important unit operation of the technological diagram of the production of the chicken viscera flour is the evacuation dejection (figure1). It is essantially manual and tedious. This is a stage that determines the quality of flour. The result of the technological manufacturing of the fish viscera is shown in figure 2. According to Figure 2, pre-cook is the major operation of the manufacture of these fish viscera because of the fat.

Chemical analysis

Table 1 shows chemical parameters of the tested chicken and fish viscera.

The dry matters were similar and the levels of crude protein were 71.80% and 47.50% respectively in chicken and fish viscera. Total ash in fish viscera was higher than in chicken viscera.

Flour produced are of high quality nutrition for their protein content which are respectively 71.80 % and 47.50 % for the viscera of chicken and fish. Chemical analysis of the chicken viscera flour showed a dry matter content of 96.31 % ; 3.79 % for the total ash. These results are not consistent with those found by Giri *et al*. (2000) ; Hossein *et al*. (2007) ; Saroj *et al*. (2013). These differences are explained by a racially selected chicken, diet and technological diagram of the production. For Giri et *al* (2000), the chicken viscera contain 92.5 % organic matter, 32.5 % protein, 20% fat , 7.4 % ash and 40.1 % carbohydrate. Crude protein rate was higher than the result of Hussein *et al*. (2007) who showed a content of 60.5%. In another study of valorization chicken viscera, the content of protein is 57.90-63.44 % ; that of the lipids of 9.22-16.49 % and that of the ashes is between 4.97 and 12.90 % (Saroj, 2013). Proteins and total ash contents of the fish viscera, respectively 47.5% and 10.42% were not in conformity with the results of Giri et *al*.(2000) who found 38.8% to protein and 7.4% to ash. The nutritional value is estimated at 14,7% from protein and 33,6% of fat for catfish (Subramanian et *al.*, 2002). The viscera of *Epinephelus areolatus* and *Parupeneus indicus* respectively gave a content of 20,70% and 19,10% for protein (Nithin et *al.*, 2013).

Microbiological characteristics

From table 4, microbiological results obtained on the day of production show that the flour does not contain germs spoilage indicators but contain yeasts, moulds and total mesophilic flora whose quantities are very substandard. As shown in figures 3; 4; 5 and 6 the microbial load varied according to the two species in each environment.

Yeasts and moulds evolution in the chicken viscera flour

The observation in all environments (figure 3) was that the jute bag favored more yeast growth than other packages. After two months, the amount in the latter remained below the expected standard 4 $\log_{10}$cuf/g. The load of yeasts in chicken viscera flour preserved in all three environments after 15 days of conditioning, increased gradually over time and highest load of yeast 3.8 $\log_{10}$cuf/g was recorded in the ventilated environment (V). By comparing the curves (figure 4), in all environments, we noticed that the growth of mould in the jute bag was faster than in other packaging. The evolution was more important in jute bag than other packaging. After two months time storage the values remained below the standard and

highest load 3.9 log_{10}cuf/g was recorded in the ventilated environment.

No presence of coliforms or *E. coli* was detected during storage in all environments. The results obtained show that plastic bag and can packaging in a refrigerated (R) or non_ventilated (NV) environments are favourable for the preservation of chicken viscera flour for two months

Yeasts and moulds evolution in the fish viscera flour

The evolution of yeasts (figure 5) was more important in jute bag than other packaging in all environments. Comparing the environment, the ventilated room promotes more growth of yeast in fish guts flour. In refrigerated (R), non-ventilated (NV) and ventilated (V) environments (figure 6), the moulds are getting more numerous in jute bag than in other packaging. Comparing the environments, more ventilated environment promotes mould growth in the fish viscera flour. No presence of coliforms or *E. coli* was detected during storage in all environments.

The moulds of the genus *Aspergillus* and *Mucor* have been identified in the different flours. Flours are good and are in conformity to the European Regulation 178/2002/EC standards that set the threshold of contamination to 5 log_{10} cuf/g for the total flora.

The hygiene during production of flour rules is followed. This is what the results that do not show the presence of germs spoilage indicators. For Cartier (2007), the microflora of meat was mainly composed of saprophytic germs. Contamination by pathogens appears rarely. The occurrence of certain bacteria during storage may be due to packaging or storage environment. The presence of mould in the product's inherent quality of the raw material and safety related to the chain of processing and packaging (Oteng- gyank , 1984) of the latter. The evolution of their charge during storage reflects a trend in the age of the product (Leyral and Vierling, 1997). Their load, however, remain below the permissible standards.

The microbiological quality of cooled meals depends on the one hand the previous contamination brought by the hands of slaughterhouse staff and work tools for the slaughter and cutting operations and secondly the proliferation of flora contamination during cooling after their adaptation to conservation . The presence of moulds in products is inherent to the quality of raw materials (viscera chicken and fish), and safety related to the chain of processing and packaging of this latter (Bidossessi *et al*., 2013). Their presence in large numbers in the jute bag along two months is related to the material and the manner of making it. Indeed this bag is made of plastic permeable to oxygen. As stated Beaulieu (2007) plastic packaging are permeable to oxygen. Jute bags making parties plastic packaging having pores are permeable to oxygen. Moulds are usually aerobic germs and that hence their number. Their low growth in the refrigerated environment can be justified by the oxygen and temperature reduction. Observed in unventilated environment growth can be linked to the reduction of the flow of ambient air through the medium.

Contamination by fecal coliforms is indicative of poor hygiene and particularly indicative of faecal contamination and therefore defects or unhygienic behavior manipulators, since coliforms are saprophytic bacteria in the digestive tract of man (Basel *et al* . 1983).

Table.1 Chemical composition (% on DM basis)

Parameters	Chicken viscera	Fish viscera
Dry matter	96.31 ±0.5	96.92 ±0.1
Total ash	03.79 ± 2.1	10.42 ± 0.0
Organic matter	96.22 ± 0.1	89.58 ± 0.9
Crude protein	71.80 ± 8.2	47.50 ± 0.0

Table.2 Microbiological quality of chicken and fish viscera flours

Parameters $\log_{10}$ (cuf/g) \ Flours	Chicken viscera	Fish viscera	Safety standards
TMA	9.10^2	7.10^2	10^5
Yeasts	3.10^1	<1	10^4
Moulds	< 1	<1	10^4
Total coliform	<1	<1	10^3
Feacal coliform	<1	<1	10^3
E. coli	< 1	<1	10^2
S. aureus	< 1	<1	10^3
SRA	< 1	<1	10^5

SRA : Sulfito- Reducer Anaerobes
TMA : Total Mesophilic Aerobic

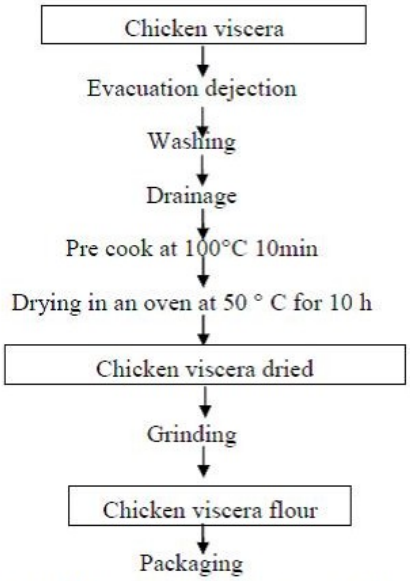

Figure 1: Technological diagram of production of chicken viscera flour

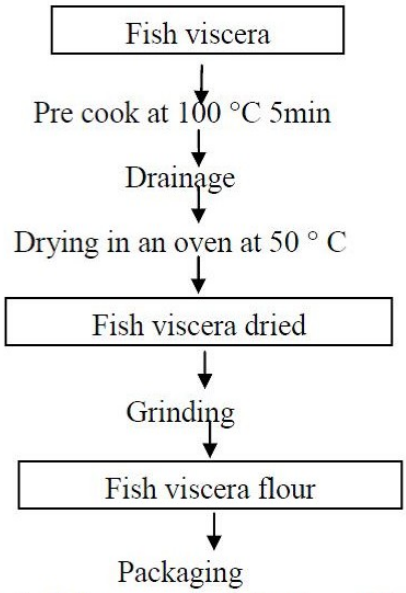

Figure2: Technological diagram of production of fish viscera flour

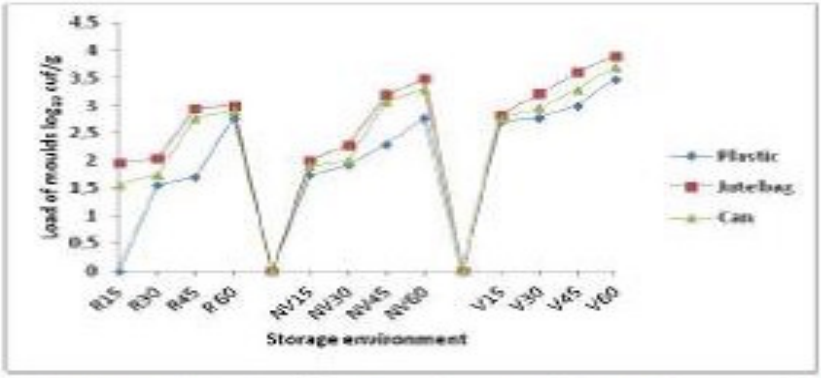

Figure 3 : Yeasts evolution in chicken viscera flour on refrigerated (R), non-ventilated (NV), and ventilated (V) environment at 15, 30, 45, 60 days

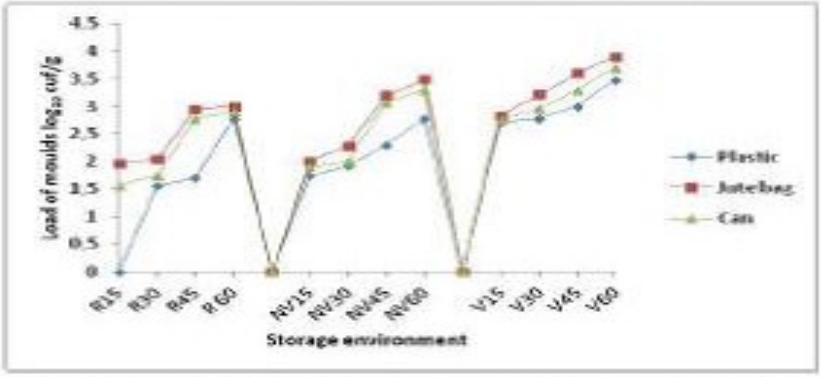

Figure 4 : Moulds evolution in chicken viscera flour on refrigerated (R), non-ventilated (NV), and ventilated (V) environment at 15, 30, 45, 60 days

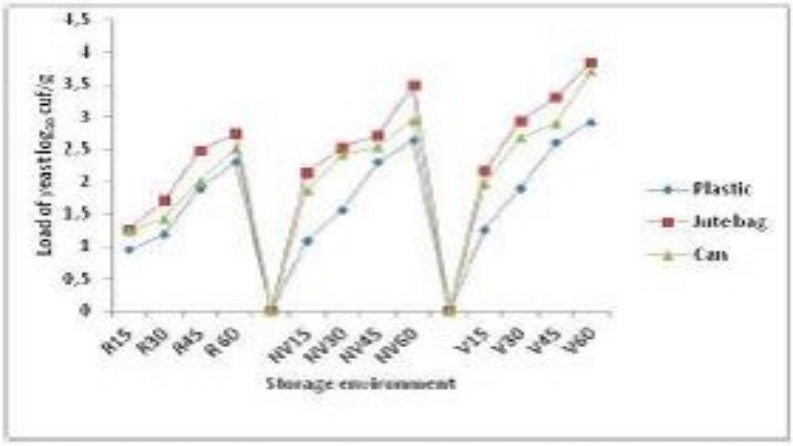

Figure 5 : Yeasts evolution in fish viscera flour in refrigerated (R), non-ventilated (NV), and ventilated (V) environment at 15, 30, 45, 60 days

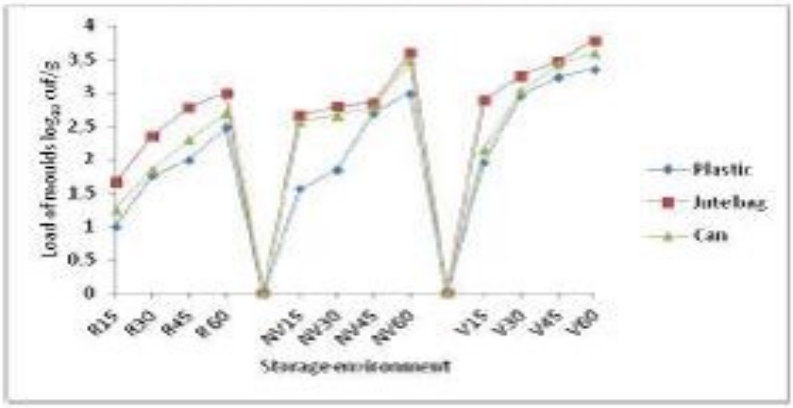

Figure 6 : Moulds evolution in fish viscera flour in refrigerated (R), non-ventilated (NV), and ventilated (V) environment at 15, 30, 45, 60 days

The origin of the contamination of flour could be fecal. According to Bonnefoy *et al*, (2002). 80% of product contamination is from human origin.

Given the results, we can say that the three environments are favorable for the preservation of flour. Just play on the packaging. In a refrigerated environment flours may be stored in three containers for two months without microbial spoilage. For ventilated or non- ventilated environment, the plastic and the can are suitable for conservation.

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