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Fermentation and Effect on the Microbial Loads of Atlantic Cod, *Gadus morhua*

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ABSTRACT: The study was carried out to evaluate the effect of fermentation on the microbial loads of Atlantic cod (*Gadus morhua*). Eviscerated samples of Atlantic cod were fermented in three media: sterile distilled water, 14% saline medium and 14% salt with potash for 24 hours. The fermented Atlantic cod samples were oven-dried at 60^0C for one hour and thereafter, 100^0C for three hours, cooled and stored in plastic baskets at ambient temperature for four weeks. Analyses of the dried fermented Atlantic cod was carried out weekly for *Salmonella shigella*, total bacterial, Staphylococcal, total fungal and total coliform counts. Results from the mean values indicated variations in the effects of the additives and processing treatments over the 24 hours fermentation period which were significant ($p < 0.05$). The dressed fish sample fermented with both salt and potash (DRSFP), recorded the least mean microbial counts during the 4-week storage period, followed by the sample which was fermented with salt (DRSF) while the highest occurrence of microorganisms was recorded in the sample which was fermented with sterile distilled water (DRF). Hence, fermentation of fish with salt and potash is recommended. Meanwhile, in relation to the standard recommended microbial limits, the loads of microbes on the fermented Atlantic cod samples from the three treatments still fall within the safe and acceptable level.

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Fish is highly susceptible to deterioration without any preservative or processing measures (Okonta and Ekelemu, 2005). It is a major source of proteins of high digestibility and are rich source of lysine and sulphur containing amino acids. In Africa, over 17.5% of the animal protein comes from fish while in Nigeria, fish constitutes 40% of the animal protein intake of the people (Olatunde, 2002). Its harvesting, handling, processing and distribution provide livelihood for millions of people as well as foreign exchange earning to many countries (Al-Jufaili and Opara, 2006). Traditional fish processing remains the predominant and most important method of fish preservation in Africa. The principal methods are smoking, sun-drying, salting, fermentation, grilling and frying (Abolagba *et al*., 2000; Zakhia *et al*., 2007). These may either be used alone or combined in order to get a well preserved product. Invariably, the final product is distinguished by peculiar qualities such as aroma, flavour and colour according to the consumer`s preference. The choice of a particular processing method is greatly influenced by the area`s geographical location, socio-economic factors and the food habits of the local people (Ghazala, 2003). Fermentation is one of the methods of fish curing in which the development of a bold flavour in the final product is the principal objective. Therefore, this product is mainly used as a condiment in the preparation of traditional sauces (Amano, 2001; Ito and Sato, 2009). However, according to Adams (1986), microorganisms play little or no part in aroma production. It can therefore be concluded that the microbiology of any salted, dried or fermented fishery product is greatly influenced by the natural microflora of the fish, the salt used and the conditions under which processing takes place (Huss and Valdimarson, 2010). Different processing techniques have been employed in fish fermentation from one region to another in order to increase the shelf-life of different fish species. In most African countries, the traditional fermentation of fish is carried out in artisanal way and the processing methods seem to be the same from one country to another with however a slight variants. Three basic methods were identified for fish fermentation in Africa: fermentation with salting and drying, fermentation with drying without salting and fermentation with salting but without drying. (Essuman, 1992; Dirar, 1993; Anihouvi, *et. al.* 2005; Gram, 2003). The combination of salting, fermentation and drying processes is needed in tropical regions mainly because of climate and the extreme perishability of fresh fish. According to Davies *et al*. (2008), the processed fishery products are still stored using traditional storage technologies. This is so as the

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long distance of distribution necessitates some processing and storage since preservation through refrigeration is not readily available (Agbon *et al*. 2002). However, there has not been any significant reported research work on fermentation of Atlantic cod *(Gadus morhua*) that could be presented to the local market as a new means of preserving fishery product. This justify the need for this work. The principal objective of this study is to isolate and enumerate the microbes associated with samples of Atlantic cod fermented in three media: sterile distilled water, 14% saline medium and 14% salt with potash medium for 24 hours respectively.

MATERIALS AND METHODS

The Atlantic cod samples were purchased from Oju-Ore market, Ota. The dressing and microbiological assessment of the fish samples were carried out at the Microbiology section of Bells University of Technology, Ota Research Laboratory.

Sample preparation: pieces of Atlantic cod were eviscerated and subdivided into three batches:
Batch 1: DRF- Dressed, fermented in sterile distilled water, not salted;
Batch 2: DRSF- Dressed, fermented in 14% saline solution;
Batch 3: DRSFP- Dressed, fermented in 14% salt solution with potash

Fermentation procedure: Three batches of the Atlantic cod samples were dipped in the prepared media and left to ferment for 24 hours after which the samples were allowed to drain for about 30 minutes before drying.

Drying: Each batch of the fermented fish samples were dried in electric oven at 60ºC at first for 1 hour and then at 100 ºC for 3 hours and then allowed to cool down. They were then stored in three different plastic baskets and kept on the shelves at room temperature for 4 weeks. Microbiological analyses of the samples were carried out immediately after oven-drying.

Microbial analysis: The dried fermented samples were subjected to microbiological evaluation: *Salmonella shigella*, total bacterial, Staphylococcal, total fungal and total coliform counts for four weeks.
Other laboratory procedures carried out include fish sample and media preparation, sterilisation, cultivation and enumeration of microbes, Isolation, characterisation and identification of microorganisms in the fish samples, gram staining and biochemical tests.

RESULTS AND DISCUSSION

The weekly results of the microbiological analyses of the oven-dried fermented Atlantic cod (*Gadus morhua*) stored at ambient temperature for four weeks are presented in Tables 1 to 3. Mean values of the analysed microbiological parameters were measured in colony forming unit per gram (cfu/g). Also, the Biochemical tests result and probable microorganisms is presented in Table 4. Fish sample fermented simply with sterile water (DRF) for 24 hours recorded the highest values of micro-organisms compared to those fermented with salt only or salt with potash (DRSF and DRSFP), in a progressive pattern. Irrespective of the treatments, in Tables 1, 2 and 3, there was no (nil) record of microbes- *Salmonella shigella*, total bacterial, Staphylococcal, total fungal and total coliform counts in week 0. Similarly, in Tables 1, 2 and 3, there was no record of Total Coliforms Count (TCC) in the fermented fish samples throughout the 4-week period of storage. This is corroborated by Tawari and Abowei (2011) who reported that successful preservation of fish by biological fermentation method is depended on the production of lactic acid. Bacteria ferment the sugars present to organic acid resulting in the lowering of pH which inhibits growth of pathogen organisms and putrefactive organisms. *Salmonella shigella*, total bacterial, Staphylococcal, total fungal and total coliform counts were too numerous to count (TNC) in fish fermented ordinarily with water by the 4th week (Table 1).

In Table 3, the mean values of microbes in fish fermented with 14% salt and potash were the lowest: *Salmonella* (0.20), Bacteria (8.10), *Staphylococcus* (2.60) and Fungi at 2.20 $\times 10^1$cfu/g respectively. However, in Table 4, after the biochemical tests, the probable micro-organisms were basically *Salmonella* and *Staphylococcus* species.

Table 1: Dressed, fermented fish samples with sterile distilled water (DRF)

	Mean values in cfu/g X 10^2				
Microbes	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Salmonella shigella	Nil	1.08	1.32	TNC	TNC
Total bacterial count	Nil	1.10	1.42	TNC	TNC
Staphylococcus count	Nil	TNC	TNC	TNC	TNC
Total fungal count	Nil	1.20	1.44	1.80	TNC
Total coliforms count	Nil	Nil	Nil	Nil	Nil

TNC = Too Numerous to Count

Table 2: Dressed, Fermented with 14% saline solution (DRSF)

Microbes	Mean values in cfu/g X 10^2				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Salmonella shigella	Nil	Nil	Nil	Nil	0.50
Total bacterial Count	Nil	Nil	Nil	8.41	9.77
Staphylococcus count	Nil	Nil	0.90	4.70	7.90
Total fungal count	Nil	0.70	1.21	3.02	5.50
Total coliforms count	Nil	Nil	Nil	Nil	Nil

Table 3: Dressed, Fermented with 14% salt and potash solution (DRSFP)

Microbes	Mean values in cfu/g X 10^1				
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Salmonella shigella	Nil	Nil	Nil	Nil	0.20
Total bacterial Count	Nil	Nil	Nil	4.40	8.10
Staphylococcus count	Nil	Nil	0.70	1.50	2.60
Total fungal count	Nil	0.50	0.80	0.80	2.20
Total coliforms count	Nil	Nil	Nil	Nil	Nil

Table 4: Biochemical Tests Result and Probable Microorganisms

R Color of colonies	Code	Coagulase	Catalase	Gram staining	Citrare	H_2S	Gas	Mortility	Indole	Slant K	Butt A	Probable organisms
Light yellow	12	+	+	+Cocci	+	+	+	+	-	+	+	*Staphylococcus aureus*
C Cream	13	+	+	-Rod	+	+	+	+	-	+	+	*Salmonella* sp.
Yellow dark	14	+	+	-Rod	+	+	-	+	-	+	+	*Citrobacter freundii*
Cream	15	+	+	-Rod	+	+	+	+	-	+	+	*Salmonella* sp.
Posha pink	03	-	+	+Cocci	+	+	-	+	-	+	+	*Staphylococcus epidermidis*
Cream	04	-	+	+Cocci	+	+	+	+	-	+	+	*Staphylococcus epidermidis*
Posha cream	05	-	+	+Cocci	+	+	-	+	-	+	+	*Staphylococcus epidermidis*
Orange	02	+	+	+Cocci	+	+	-	+	-	+	+	*Staphylococcus aureus*
Creamy pink	01	+	+	-Rod	+	+	-	+	-	+	+	*Salmonella* sp.

Note: 12, 13, 14 and 15 were obtained from MSA plates, 03, 04 and 05 from NA plates and 02 and 01 obtained from SSA plates.

Generally, it should be noted that a major goal for the food processing industry is to provide safe, wholesome and acceptable food to the consumer (Pal *et al*, 2015). For generations, Faroe Islanders have prepared Ræstur fiskur, a home-made air-dried and fermented fish dish made of Atlantic cod-Gadus morhua (Ingvar, 2015). Many factors govern the end product of the fermentation process. These include the initial preparation of the fish, whether the fish was gutted or whole, the fat content of the fish, the amount of salt added and at what stage salt was added and the temperature at which the fish is allowed to ferment (Olokor, 1997). In this study, irrespective of the treatment-water, salt or salt with potash, there was increase in the total microbial loads (*Salmonella shigella*, *Staphylococcus* Total bacterial and total fungal count) of the dried fermented fish samples during storage; except the Total Coliforms count-which had no value (nil). The increase in the microbial loads of the fermented oven-dried *Gadus morhua* samples are factors which indicates the proliferation of spoilage organisms in the fish during storage and also affect the nutritional quality of the fish. Meanwhile, the fish samples of *Gadus morhua* were subjected to fermentation for 24 hours, the effect of the media-water, salt and salt with potash on the microbial loads of oven-dried fish was significantly different ($p < 0.05$). This affirmed the importance of salt and potash as a good preservative useful in fish fermentation. Salt is believed to play an important role in fish fermentation as it induces moisture loss and has direct inhibitory effects on micro-organisms responsible for fish spoilage (Bello, Unpublished). Through the result of this research, salt and potash mixture have shown to be a means of reducing microbial load on raw fish samples in order to have fermented products that will not readily spoil; thereby prolonging the shelf and keeping quality.

Conclusion: Fermentation of Atlantic cod subjected to 14% salt and potash concentrations for 24 hours is recommended as it had the greatest preservative effect by inhibiting the growth of spoilage organisms, caused moisture loss and increased the shelf-life stability of the product. This processing method of fish-fermentation and drying is highly available to local fish traders in tropical countries. Sun-drying of fermented fish products, as being done in Northern part of Nigeria can be adapted where there is no facility for oven-drying.

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