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Effect of Smoking Duration on the Microbiological Quality of Cold-smoked Atlantic Cod, *Gadus morhua* (Linnaeus, 1758)

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ABSTRACT

Cold-smoked Atlantic cod, *Gadus morhua* that floods the smoked fish market of the South-Western region of Nigeria are not microbiologically shelf-stable; hence, the need for a study on varying smoking durations in order to produce smoked fish with longer shelf life; safe for consumption. This study evaluated the effect of varying smoking durations of 6, 6.5, 7 and 7.5 h on the microbiological quality and percentage moisture content of cold-smoked Atlantic Cod. The fish samples were cold-smoked using the traditional smoking drum. Four batches of the smoked fish were stored in metal baskets at ambient temperature (25-28°C) for 12 days. Each batch was assessed for; Total Plate Count (TPC), Total Coliform Count (TCC), Mould Count (MC), *Staphylococcus aureus* (SA) count and presence or absence of *Escherichia coli*. Analyses of the smoked fish samples were carried out at the initial stage (day 0) and subsequently every alternate days. The result obtained showed significant variations ($p<0.05$) for all the microbial counts of the four smoked fish samples. The best microbiologically stable cold-smoked samples were that smoked at the longest duration of 7.5 h which recorded the least mean TPC range of 1.50×10^{3}, 0.0 MPN g^{-1} (TCC), 1.32×10^{3} (MC) and 3.0×10^{3} CFU g^{-1} (SA). The moisture content ranged from 15.2 to 25.3%. All the samples tested negative to *Escherichia coli* and met the specified microbiological limit. It can be concluded that the cold-smoking *Gadus morhua* at 7.5 h will preserve the fish more and make it safe for consumers.

Key words: Cold-smoking, *Gadus morhua*, smoking durations, microbiological qualities, shelf life, moisture content

INTRODUCTION

In Nigeria, fish is cooked fresh and eaten, preserved or processed. Therefore, the need to improve the quality and shelf-life of fish products cannot be over-emphasised (Bolorunduro *et al*., 2005). Smoking is carried out in fishermen camps in chambers of traditional kilns made of clay, cement blocks, or drums. The traditionally smoked fish is typical of the Lake Chad region of Nigeria (Abobarin, 2000). According to Shimang (1990), in the 1970's, most of the fishes caught in the Lake Chad were smoked before the advent of drought. Similarly, out of the 100 million tonnes estimated world's fish production in 1989, fifteen percent were cured and one third of the cured fish were smoked (Da Silva, 2002). Technically, the duration of smoking determines the moisture content of fish product. Also, the moisture (or water activity) of a smoked fish has been found to determine the rate of microbial growth and invariably the shelf life or keeping qualities of the fish. Microbes e.g.,

L. monocytogenes has been found in crabmeat samples and smoked fish samples (Gram, 2001); they are ubiquitous in nature and able to grow at low temperatures and in high salt concentration up to 10% (Da Silva, 2002). Hence, preservation by drying is effected by lowering the water activity of the fish to a level which micro-organisms can no longer grow (Eyo, 2001). In cold smoking, the temperature of the smoke does not exceed 30°C (Eyo, 2001). Cold smoking is an uncommon preservation technique in Nigeria, due to the need for a supporting or alternative method of preservation (Waterman, 1976). Also, study has revealed that cold-smoked fish products do not last long (Moses, 1983). According to Oyelese (2006), cold-smoked samples had the highest moisture content in comparison with moisture content of hot-smoked and oven dried tilapia samples. This is because the cold-smoked fish is not fully dehydrated. However, Eyo (1981) compared cold-smoked and hot-smoked fish and observed that cold-smoked fish possess higher nutritive value than hot-smoked fish which became cooked in the process. Therefore, the objective of this study is to establish the most effective smoking duration on the microbiological quality of the cold-smoked Atlantic Cod, *Gadus morhua*.

MATERIALS AND METHODS

Sample site and study duration: This research was carried out around Bells University, Ota, Ogun State, Nigeria. The drum-type smoking kilns were used for the study. Furthermore, the study was carried out within 12 consecutive days in June, 2010.

Preparation of fish samples: In this study, 20 kg carton of Atlantic Cod (*Gadus morhua*) with each of the pieces ranging between 150-200 g was purchased from a reputable frozen foods dealer in Ota and taken to an artisanal fish smoking site located at Bells Junction, Ota, Ogun State.

Smoking procedure and storage: Following standard processing steps, 68 pieces of the frozen fish were sorted out for cold smoking at temperature around 30°C. They were thawed, sorted into four batches of 17 pieces per batch and rinsed in clean water. Each piece of fish in the batches were folded into a round shape and held in place with a sharp stick. The smoking was done on a drum-type smoking kiln with a single smoking rack which was fabricated from a 44 gallon drum. The batches of folded *Gadus morhua* were arranged on the smoking rack and were subsequently subjected to different smoking durations of 6, 6.5, 7 and 7.5 h at temperature ranging between 30 35°C. The temperature was controlled by regulating the burning of the fuelwood. Smoking temperature was monitored at intervals by checking the heat that gets to the fish at the rack and dipping a thermometer into the flesh of the fish. After smoking, the fish samples A-D were cooled and separately stored in four labelled metal baskets each containing 17 pieces of the cold-smoked fish. The samples of cold-smoked *Gadus morhua*, were coded based on the different smoking durations they were subjected to. The cold-smoked fish were then taken to the Microbiology Laboratory of Bells University of Technology, Ota for analyses. The samples of the smoked fish were stored in metal baskets on the shelf at an ambient temperature for 12 days.

Analyses of cold-smoked fish samples: Analyses of the smoked fish samples were carried out at the initial stage (day 0) and subsequently every alternate days (i.e., days 2, 4, 6, 8, 10 and 12); making up seven testing days. The methods of microbiological analysis described by Lyne (1976), were adopted for the analysis of samples of the cold-smoked *Gadus morhua*. The parameters determined were Total Plate Count (TPC), Total Coliform Count (TCC), Mould count,

Staphylococcus aureus count and *Escherichia coli*. Percentage moisture content was also determined by AOAC (1990) method.

Statistical analysis: Data obtained from the different smoking durations against the storage days were subjected to Analysis of Variance (ANOVA) at 5% level of significance ($p<0.05$), while Duncan's multiple range test was used to determine significant differences between the means.

RESULTS AND DISCUSSION

The microbial load (TPC, *Staph.*, mould and TCC) and moisture content of the frozen fish sample was higher than that of the smoked fish (Table 1). For example, TPC reduced from 4.3×10^4 in frozen cod to an average of $1.50\times10^3\pm0.05$ CFU g^{-1} in fish smoked at 7.5 h in day 0. Similar reduction patterns were observed for the values of *Staph. aureus*, mould, TCC and percentage moisture content of the smoked Atlantic cod. Also, the values of the microbial loads of the four batches of *Gadus morhua* cold-smoked at different durations and expressed in colony forming per gram (CFU g^{-1}) were significantly different ($p<0.05$). The microbial load and moisture content of the smoked fish increased with increased days of storage. In addition, for all the parameters assessed, the values reduced with increased smoking duration (Table 2). The initial 2 days of analyses recorded very low microbial loads in all the four smoked samples, A-D. Sample D which was smoked for the longest duration of 7.5 h had the lowest TPC of 1.50×10^3 CFU g^{-1} at day 0 which rose to 2.0×10^5 CFU g^{-1} at day 12. This shows the usefulness of TPC in measuring the effectiveness of time profile and heat treatment as processing procedures (FNB/NRC, 1985). In addition to this, the phenolic fraction of wood smoke has been said to possess the highest inhibiting ability on bacteria. The dual effects of heat treatment and phenolic fraction of wood smoke as displayed in sample D are made evident in the enumerated TPC values of the smoked fish samples. This result is in agreement with the findings of Ikeme and Gugnani (1988), who, in a comparative assessment of the effect on varying periods (i.e., 4, 5, 5.5 and 6.5 h) of smoking on the acceptability and storage stability of mackerel, reported that the samples of mackerel smoked for the longest period of 6.5 h (lowest moisture content), were the most stable. The TPC of the frozen sample of *Gadus morhua* was recorded to be 4.3×10^4 CFU g^{-1}.

Total coliform counts are particularly useful as indicators of contamination when they occur in small numbers. Their occurrence in large numbers indicates mishandling such as temperature abuse (Mossel, 1967; Silliker and Gabis, 1976). The differences in the values of TCC of the four cold-smoked samples were significant ($p<0.05$). At day 0, *Gadus morhua* cold-smoked at 6, 6.5, 7 and 7.5 h had TCC values of 3.0, 1.0, 1.0 and 0.0 MPN g^{-1}, respectively. Subsequently, by the 12th day, these have increased to 17.0, 17.0, 14.0 and 12.0 MPN g^{-1}. All the samples recorded fairly consistent TCC values on initial 2 days of analyses. The occurrence of these microorganisms in the four study samples almost throughout the storage days can then be attributed to contamination

Table 1: Microbial load of the frozen sample of *Gadus morhua* before smoking

Frozen fish sample	Total plate count (CFU g^{-1})	*Staph. aureus* (CFU g^{-1})	Mould (CFU g^{-1})	Moisture content (%)	Total coliform count (MPN g^{-1})
1st reading	5.0×10^4	4.5×10^4	1×10^3	77.73	9
2nd reading	3.8×10^4	3.5×10^4	1×10^3	78.88	7
3rd reading	4.2×10^4	3.1×10^4	0.9×10^3	76.92	6
Average	4.3×10^4	3.7×10^5	1×10^3	77.84	7.3

Table 2: Microbial and moisture content (%) of cold-smoked *Gadus morhua* stored for 12 days

Microbial parameters	Storage days	Smoking durations 6 h (A)	6.5 h (B)	7 h (C)	7.5 h (D)
Total plate count (CFU g^{-1})	0	$3.05\times10^{4}\pm0.05$	$2.55\times10^{4}\pm0.45$	$1.25\times10^{4}\pm0.25$	$1.50\times10^{3}\pm0.05$
	2	$6.15\times10^{4}\pm0.85$	$3.35\times10^{4}\pm0.35$	$3.20\times10^{4}\pm0.20$	$2.15\times10^{3}\pm0.35$
	4	$1.35\times10^{6}\pm1.50$	$2.95\times10^{5}\pm1.25$	$4.40\times10^{4}\pm0.40$	$4.40\times10^{5}\pm0.40$
	6	$1.65\times10^{6}\pm0.75$	$1.55\times10^{6}\pm1.50$	$6.80\times10^{5}\pm0.20$	$3.90\times10^{5}\pm0.10$
	8	$2.20\times10^{6}\pm1.00$	$1.95\times10^{6}\pm0.50$	$7.90\times10^{5}\pm0.40$	$4.05\times10^{5}\pm0.95$
	10	$2.50\times10^{6}\pm0$	$2.35\times10^{6}\pm1.50$	$1.20\times10^{6}\pm1.00$	$1.90\times10^{5}\pm0.60$
	12	$2.65\times10^{6}\pm0.50$	$3.50\times10^{6}\pm1.50$	$1.70\times10^{6}\pm1.00$	$2.00\times10^{5}\pm0.40$
Total coliform count (MPN g^{-1})	0	3.0±0.5	1.0±1.0	1.0±1.0	0.0±0
	2	5.0±0.5	3.0±2.5	2.0±1.5	2.0±0.0
	4	5.0±1.5	4.0±1.0	3.0±2.5	2.0±2.0
	6	7.0±2.5	7.0±2.0	5.0±4.5	5.0±2.0
	8	14.0±4.5	10.0±2.5	6.0±1.0	5.0±2.0
	10	17.0±1.0	12.0±6.5	11.0±1.5	8.0±3.5
	12	17.0±1.0	17.0±1.0	14.0±4.5	12.0±55
Mould count (CFU g^{-1})	0	$1.65\times10^{4}\pm0.05$	$1.40\times10^{4}\pm0.10$	$1.05\times10^{4}\pm0.25$	$1.32\times10^{3}\pm0$
	2	$3.10\times10^{4}\pm0.20$	$3.15\times10^{4}\pm0.35$	$2.53\times10^{4}\pm0.20$	$2.15\times10^{4}\pm0.15$
	4	$3.00\times10^{5}\pm0$	$1.55\times10^{5}\pm0.35$	$1.00\times10^{5}\pm0$	$3.5\times10^{4}\pm0.15$
	6	$3.00\times10^{5}\pm0$	$3.50\times10^{5}\pm0.50$	$2.15\times10^{5}\pm0.45$	$1.45\times10^{5}\pm0.15$
	8	$4.40\times10^{5}\pm0.40$	$4.20\times10^{5}\pm0.20$	$2.25\times10^{5}\pm0.75$	$1.55\times10^{5}\pm0.25$
	10	$6.90\times10^{5}\pm0.20$	$5.80\times10^{5}\pm0.30$	$5.4\times10^{5}\pm0.47$	$1.65\times10^{5}\pm0.25$
	12	$9.5\times10^{5}\pm0.15$	$8.5\times10^{5}\pm0.25$	$5.60\times10^{5}\pm0.40$	$2.55\times10^{5}\pm0.25$
Staphylococcus aureus (CFU g^{-1})	0	$5.70\times10^{4}\pm3.70$	$3.35\times10^{4}\pm0.55$	$4.0\times10^{3}\pm0$	$3.0\times10^{3}\pm0$
	2	$8.45\times10^{4}\pm0.35$	$3.50\times10^{4}\pm0.20$	$1.30\times10^{4}\pm0.20$	$3.3\times10^{3}\pm0.05$
	4	$5.50\times10^{6}\pm0.50$	$5.30\times10^{5}\pm3.70$	$1.10\times10^{5}\pm1.90$	$7.0\times10^{4}\pm0.10$
	6	$5.50\times10^{6}\pm0.46$	$1.35\times10^{6}\pm0.15$	$1.20\times10^{5}\pm0.10$	$1.00\times10^{5}\pm0.20$
	8	$6.0\times10^{6}\pm0$	$5.0\times10^{6}\pm0$	$1.35\times10^{5}\pm1.34$	$1.10\times10^{5}\pm0$
	10	$6.0\times10^{6}\pm1.00$	$5.15\times10^{6}\pm0.85$	$3.95\times10^{5}\pm2.05$	$1.20\times10^{5}\pm0.10$
	12	$6.5\times10^{6}\pm0.45$	$5.50\times10^{6}\pm0.50$	$5.00\times10^{5}\pm4.00$	$1.35\times10^{5}\pm0.15$
E. coli (MPN g^{-1})	0	0	0	0	0
	2	0	0	0	0
	4	0	0	0	0
	6	0	0	0	0
	8	0	0	0	0
	10	0	0	0	0
	12	0	0	0	0
Moisture content (%)	0	43.9±0.20	41.1±0.60	38.10±0.70	25.30±0.25
	2	23.95±1.75	20.90±0.70	23.81±0.21	22.97±0.15
	4	20.42±0.32	20.19±0.32	21.05±0.15	22.13±0.07
	6	19.95±0.85	17.47±0.27	19.34±0.26	17.25±0.55
	8	19.61±1.29	17.25±0.35	18.15±0.05	16.21±0.19
	10	18.59±0.71	16.38±0.26	14.99±0.29	15.22±0.92
	12	16.0±0.70	15.09±0.95	14.30±0.70	15.20±0.20

Values are Mean±SD of triplicate experiments of each sample

from processing utensils, water, handlers and probably storage materials. The values of mould count showed that variations of all the four cold-smoked samples were significant ($p<0.05$). Mould

count of the frozen sample of *Gadus morhua* was 1×10^3 CFU g^{-1} which was lower than the values of the four cold-smoked samples. The four cold-smoked study samples A-D recorded low levels of mould growth in the first 2 days of analysis. Sample D recorded the lowest values of mould count throughout the storage days, compared to sample A that recorded the highest mould count. Hence, the effectiveness of heat treatment and time profile in processing procedure is again established. According to Olsen (1976), yeast and mould are more resistant to inhibitory influence of smoke even up to concentration of 60 mg kg^{-1}. Despite the relatively low percentage moisture content of sample D, these food borne moulds were able to grow on the low moisture fish samples, because of their relatively low moisture requirements (AOAC, 1990). The values of *Staphylococcus aureus* count showed that variations for the four cold-smoked *Gadus morhua* samples were significant ($p<0.05$). *Staphylococcus aureus* count of the frozen sample of *G. morhua* was 3.7×10^5 CFU g^{-1} which was higher than all the values obtained for the four cold-smoked samples at the initial stage (day 0) of analyses. Hence, on the subjection of the fish to heat treatment, the values of the *S. aureus* count of the raw material dropped. This establishes inhibitory effects of phenolic fraction of smoke on *Staphylococcus aureus*. The bactericidal effect of smoke as established by Olsen (1976) is associated with the smoke constituents especially the phenols as well as the combined heating and drying process during smoke curing. *Staphylococcus aureus* grows poorly in competition with large numbers of other microorganisms. Small numbers are to be expected in products handled by humans. Therefore, the presence of large numbers in any food material indicates possible faulty sanitary or production practice (ICMSF, 1986). According to Huss *et al.* (1995), any handling of fish, and the associated sanitary practices from the point of harvesting, however, has the potential to contribute to the microflora on the final product. All the four batches of cold-smoked *Gadus morhua* stored for 12 days tested negative to *Escherichia coli* throughout the storage days. The resistance of *E. coli* to adverse physical and chemical conditions is low which makes *E. coli* less useful as indicator organisms in examination of frozen or otherwise preserved fish products. There was significant difference ($p<0.05$) between the values of the percentage moisture contents of the four smoked fish samples. The highest percentage moisture content of 43.9 was recorded for sample A at day 0 which reduced to 16.0 by day 12; this can be attributed to the fact that this sample was exposed to the least duration of smoking (6 h). This agrees with the submission of Eyo (1981) that the moisture retention of cold-smoked fish product is usually high and may be in the order of 35-45%. Sample D that recorded the least percentage moisture content of 25.3 at day 0 was cold-smoked for the longest study duration of 7.5 h. Despite the consistent decrease in moisture content of the cold-smoked study samples, the susceptibility to infestation by microorganisms increased with storage period, such that, the microbial load picked up from day 4 and remained increasingly high till the last day. This also agrees with of Eyo (2001) that cold-smoked fish, being not well cooked, has shorter shelf life and is easily infested by microorganisms such as bacteria and moulds if not properly stored. The season in which the study was carried out could have contributed to the decrease in moisture content of the cold-smoked *Gadus morhua* stored for 12 days.

CONCLUSION

Findings from this study show that fish cold-smoked for 7.5 h with a range of moisture content level of 25.3 to 15.2% can be microbiologically stable and safe for consumption even by 12 days of storage. The other three smoking durations, though not as effective in processing fish products are recommended be supported with refrigeration storage in other to extend the shelf-stability of the product.

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