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Microbiological and Physico-Chemical Quality of Smoked Shrimp, An Expanding Food Condiment in Beninese Local Markets

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Abstract Entire Smoked Shrimp (ESS) and Smoked Shrimp Powder (SSP) are two food condiments widely used in Beninese local cooking practices. Twelve samples of each product collected from local markets were evaluated for safety assessment using standard methods. Regarding the microbiological status of the samples, the *Enterobacteriaceae* were detected in 83% and 75% of ESS and SSP respectively, whereas 25% of samples of each product were found to contain *E. coli*. Pathogenic bacteria such as *S. aureus* and *Salmonella* were absent. Except 8% and 17% of SSP sample exceeding the maximal limit of 10^6 UFC/g for Aerobic Mesophilic Bacteria and 10^4 UFC/g *Enterobacteriaceae* respectively, all the other samples were within the acceptable limits. Water activity values were low, ranging between 0.54±0.01 for SSP and 0.61±0.01 for ESS, showing a potential microbial stability. Considering the chemical hazards, 15 EU priority polycyclic aromatic hydrocarbon (PAHs) were detected in the samples examined with median Benzo(a) pyrene and PAH4 contents (91 µg kg^{-1} and 490 µg kg^{-1}respectively) exceeding the European maximal limit (5.0 µg kg^{-1} and 30 µg kg^{-1}). This study showed that smoked shrimps may be generally safe from a microbiological point of view, but they constitute a large source of exposure to possible carcinogenic PAHs.

Keywords Shrimp, Smoking, Polycyclic Aromatic Hydrocarbon (PAH), Microbiological Quality, Market

1. Introduction

In many tropical countries, the fishing surpluses are processed to be used as food condiments[1-4].

In Benin, smoked shrimp is a food condiment widely used in local cooking practices[5, 6]. Post-harvest processing of shrimp is essentially assumed by women of fishing communities. Shrimps are processed by artisanal hot smoking method to obtain a dry product. Then, they are stored in a basket at ambient temperature (30-33°C). Furthermore, smoked shrimps after solar drying (facultative) are ground and packaged in bottles or plastic bags. In Beninese traditional hot smoking practice, shrimps are in direct contact with wood smoke[7]. During smoking process, polycyclic aromatic hydrocarbons (PAHs) can be formed from the organic matter such as firewood[8]. More than 300 congeners constitute the PAHs family, among which 15 have been recognized as genotoxic by the European Union (EU) [9,10]. The benzo(a)pyrene has been recognized as carcinogenic for humans[11] and 6 other PAHs (benzo [a] anthracene, chrysene, benzo[b]fluoranthene, benzo [k] fluoranthene, dibenzo[a,h]anthracene, and indeno[1,2,3-cd] pyrene) have been classified as probable human carcinogens [12]. Furthermore, the microbiological quality of foods often reflects the hygienic status of the region where they are produced and manufactured. It is evident that as many condiments such as fermented fish[13, 14] and spices[15-17], smoked shrimps are exposed to a wide range of microbiological and chemical contaminations during the catch, the processing, the storage and in the retail markets. Previous studies have reported the presence of toxigenic moulds and mycotoxins such as aflatoxins and ochratoxins in smoked dry fish collected in retail markets in tropical

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conditions[18, 19]. Smoking treatment could not destroy all kind of micro-organisms and wood smoke is a potential source of many toxic contaminants. Thus, smoked shrimps may be considered as a potential vehicle for transmission of food borne diseases. The present work aims to assess the safety of smoked shrimps as sold in Beninese retail markets.

2. Materials and Methods

2.1. Samples Collection

A total of 24 samples comprising 12 samples of ESS and 12 other samples of SSP were randomly purchased from retail markets of Ganhi and Saint Michel (Cotonou), Comè (Comè city center) and Ouando (Porto-Novo) (Table 1). The entire smoked shrimps usually sold in bulk were collected in sterile stomacher bags while samples of smoked shrimp powder were collected with their glass package. Samples were transported to the laboratory within 2 h for immediate microbiological, pH and water activity analysis, or stored à -20°C until other chemical analysis.

Table 1. Smoked shrimp samples collected for analysis

Retail markets	ESS	SSP	Total
Ganhi (Cotonou)	3	3	6
Saint Michel (Cotonou)	3	3	6
Comè (Comè city center)	3	3	6
Ouando (Porto-Novo)	3	3	6
Total	**12**	**12**	**24**

ESS = Entire smoked shrimp; SSP = Smoked shrimp powder

2.2. Microbiological Analysis

Twenty-five gram (25 g) of each sample was suspended in 225 ml of buffer peptone water (Oxoid CM0509B, Basingstoke, Hampshire, England), and homogenized for 2 min using a laboratory blender (Stomacher Lab-Blender 400, model N° BA 6021, Seward, London, UK). Serial decimal dilutions were prepared in buffer peptone water as described by ISO 6887-3[20], and inoculated in different media: (i) plate count agar (PCA, Oxoid CM0463B, Basingstoke, Hampshire, England) for total viable counts; PCA plates were incubated at 30°C for 3 days[21]; (ii) Baird-Parker Agar Base (BP, Oxoid CM0275B, Basingstoke, UK) for *Staphylococcus aureus*; BP plates were incubated at 37 °C for 1-2 days, followed by coagulase test[22]; (iii) Violet Red Bile Glucose Agar (VRBG, Oxoid CM0485B, Hampshire, UK) for *Enterobacteriaceae*; the plates were incubated at 37°C for 1 day, followed by the confirmation of characteristic colonies using oxidase and fermentation tests[23]; (iv) TBX medium (Oxoid CM0945B, Basingstoke, Hampshire, England) for *Escherichia coli*; TBX plates were incubated at 44 °C for 1 day[24]; (v) Chloramphenicol glucose agar (Biokar diagnostics-zac de ther-allone-F60000 Beauvais) for moulds; the plates were incubated at 25°C for 3-5 days[25]. Results were expressed as colony forming units per gram of sample (detection limit = 10 CFU/g). Qualitative detection of *Salmonella* was performed by pre - enrichment in Buffered peptone water (37°C; 1 day), and selective enrichment (37°C; 1 day) in Rappaport-Vassiliadis broth (Oxoid CM0669B., Basing-stoke, Hampshire, England) and Muller Koffman broth (Oxoid CM0343B, Basingstoke, Hampshire, England). Cultures were plating-out (37°C; 1 day) on X.L.D medium (Oxoid CM0469B, Basingstoke, UK) and *Salmonella*, *Shigella* Agar (Oxoid CM0099B, Basingstoke, Hampshire, England) followed by confirmation of characteristics colo-nies using appropriate biochemical and serological tests for *Salmonella*[26].

2.3. Physico-Chemical Analysis

2.3.1. Moisture Content, pH and Water Activity (Aw) Determination

The pH of the samples was determined as described by Goulas and Kontomina[27] using a digital pH-meter (Inolab pH 730 WTW 82362 Wellheim, germany). The dry matter content was determined by oven drying of 5 g of grinded shrimp at 105°C until a constant weight was reached[28]. Water activity (Aw) was measured according to the method described by Anihouvi *et al.*[13], using a thermo-hygrometer recorder (Rotronic HygroLab 2, 8303 Bassersdorf).

2.3.2. Polycyclic Aromatic Hydrocarbons Determination

Individual polycyclic aromatic hydrocarbon (PAHs) standard solutions in acetonitrile (ACN) (purity: 98.5–99.9%) were purchased from Cluzeau Info Labo (Putteaux la Défense, France). The deuterated DiP-D14 (in toluene, purity: 99.7%), used as internal standard, was purchased from LGC Promochem (France). Working standard solutions were prepared by dissolving the commercial solutions in acetonitrile and stored at 4°C in dark vials sealed with PTFE/silicone caps.

High performance liquid chromatography coupled to fluorescence detector (HPLC/ FLD) analysis was carried out using a Model 600 E solvent delivery system, equipped with a Model 717 automatic injector, a MistralTM oven and both 996 PDA and 2475 Fluorescence detectors (all from WATERS). A C18 Pursuit 3 PAH (100 x 4.6mm, 3µm) equipped with a ChromGuard (10 x 3mm) precolumn, both from VARIAN, were used to separate the PAHs. The PAHs were extracted as described by Veyrand *et al.*[29]. Briefly, one gram of freeze-dried shrimp sample was extracted with Hexane/acetone (50:50, v/v) using the Accelerated Solvent Extraction (ASE) technique. The solvent was evaporated until 1 ml and reconstituted with 5 ml of cyclohexane. The reconstituted extract was purified by column chromatography using Envi Chrom P column (Supelco) conditioned successively with 15 ml ethyl acetate and 10 ml cyclohexane. After loading the sample extract, the column was washed with 6 ml cyclohexane/ethanol (70:30, v/v) and PAHs were eluted using 12 ml of cyclohexane/ethyl acetate (40:60, v/v). The solvent was evaporated until dryness to change solvent to 90 µl acetonitrile. The final extract was then spiked with 10 µl of deutered DiP (internal standard).

Five µl of this final extract was injected on HPLC column as described by Brasseur *et al.*, and Danyi *et al.*[30, 31]. The limit of quantification of the method was 0.85 µg/kg fresh weight for Benzo(j)fluoranthene and Indeno[1.2.3-cd]pyrene and was 0.21µg/kg fresh weight for all the over PAHs.

2.4. Statistical Analysis

For data of microbiological analysis, Geometric mean, standard deviation and median was calculated replacing value lower than the detection limit (< 10 CFU/g), by 5 CFU/g. Analysis of data was performed by the test T of student using Minitab 14.1. Statistical significance was set at $p < 0.05$ and means were separated using SNK (Student, Newman and Keuls) range test.

3. Results and Discussion

3.1. Microbiological Characteristics of Investigated Samples

The geometric means of microbial loads and the description of the contamination level in each kind of product are shown in Tables 2 and 3. No statistical significant difference was noticed through the different evaluated criteria (Table 2). Aerobic Mesophilic Bacteria (AMB) count was up to 1.4×10^4 CFU/g and 3.2×10^4CFU/g in ESS and SSP respectively. Moulds were detected in all the samples examined with a mean value of 2.7×10^2 CFU/g and 3.4×10^2 CFU/g in ESS and SSP respectively. *Enterobacteriaceae* count was 1.8×10^1 and 2.6×10^2 CFU/g in ESS and SSP respectively. *E. coli* was detected in 3 samples (25%) of each kind of product up to 1.3×10^1 CFU/g in ESS and 9.5×10^0 CFU/g in SSP respectively (Tables 2 and 3). Neither *S. aureus* nor Salmonella were detected in evaluated samples of the two kinds of product.

Table 2. Geometric mean of microbial loads of smoked shrimp samples (CFU/g)

Tests	ESS	SSP	P value*
AMB	1.4×10^{4a}	3.2×10^{4a}	0.896
Moulds	2.7×10^{2a}	3.4×10^{2a}	0.954
Enterobacteriaceae	1.8×10^{1a}	2.6×10^{2a}	0.968
E. coli	1.3×10^{1a}	9.5×10^{0a}	0.945
S. aureus	$< 10^a$	$< 10^a$	1
Salmonella	Absent/25g	Absence/25g	-

ESS = Whole smoked shrimp; SSP = Smoked shrimp powder; AMB = Aerobic Mesophilic Bacteria; aValues in the same line followed by the same letter are not significantly different ($p < 0.05$); *T Student test

The AMB count enumerated in ESS and SSP samples examined could be due to the growth of microorganism which resisted to the smoking treatment. It could also probably be due to the contamination during the post-processing handling. Indeed, hot smoking process is a pasteurization method and couldn't eliminate all the microorganisms of the raw shrimp. Plahar *et al.*[32] have reported that the initial microbial types and viable numbers decrease during traditional hot smoking (60°C-80°C for 2-5 hours), but are not completely eliminated. Similar results were reported on smoked dry fish collected in retail markets in Nigeria[18, 19]. *Enterobacteriaceae* are indicators of hygiene and contamination after processing because they are destroyed by hot treatment[33]. In our study, the high percentages of *Enterobacteriaceae* positive samples indicate a lack of hygiene and the detection of *E. coli* even in few numbers of samples (3 out of 12) point out the possibility of human or animal fecal sources of contamination during post-processing handling often correlated with contamination by digestive pathogen. Indeed, Plahar *et al.* [32] have shown that the traditional hot smoking (60°C-80°C for 2-5 hours) eliminate Gram negative bacteria such as fecal coliforms and *E. coli*, but microbial loads, however, increased again under the traditional post-processing handling and storage conditions.

In proportion to the use of smoked shrimp as food condiment, the evaluated products were compared to the ready-to-eat spices, because microbiological standards were available neither for smoked dry shrimp nor for other foods condiments of animal origin. The International Commission on Microbiological Specifications for Foods[34] set up maximum limits of 10^6; 10^4; 10^4 and 10^3 CFU/g of spice for AMB, moulds, coliforms and *E. coli*, respectively. The public health laboratory service[33] also specified a maximum limit of 10^2 and 10^4 CFU/g for *S. aureus* and *Enterobacteriaceae* and *Salmonella* should be absent in 25 g of smoked ready to eat fish. Considering these specifications as a guide, our results indicate a low level of microorganisms in smoked shrimp (Table 4). Except for 8% and 17% of the SSP samples which exceeded the maximal limit for AMB (10^6 UFC/g) and *Enterobacteriaceae* (10^4 UFC/g) specified respectively by the standards, the sample tested were in accordance with the standards (Table 4). These data revealed a high level of microbiological quality of the investigated ESS and SSP.

3.2. Physico-Chemical Characteristics of the Investigated Samples

3.2.1. Moisture Content, pH and Water Activity (a_w)

The pH, moisture content and water activity of the two kinds of smoked shrimp are given in Table 5. The pH values of 7.59 and 7.69 were recorded for ESS and SSP respectively without significant difference ($p > 0.05$) between the two kinds of product. The moisture content is significantly lower in the SSP (10.67±2.17) than in the ESS (13.99±1.78). The water activity value was also significantly lower ($p < 0.05$) in the SSP (0.54±0.01) than in the ESS (0.61±0.01). SSP is obtained from the ESS after a complementary solar drying and grounding. The lower moisture content in SSP may be due to this additional drying step of the product. Indeed, Kumolu-Johnson and Ndimele[35] have shown a decrease in moisture content in fish through sun drying. Water activity influences the stability of foods during storage, as some deteriorative processes in foods are mediated by water.

According to Prescott *et al.*[36], bacterial growth would be impossible in food products with a water activity value lower than 0.7. Thus, the low water activity values recorded in the investigated samples during this study are sufficiently low to inhibit the growth of pathogenic bacteria in both smoked shrimps.

3.2.2. PAHs Contents of Smoked Shrimps Investigated

The results obtained from the PAHs analysis in entire smoked shrimp (ESS) are summarized in Table 6. All the 15 EU PAHs investigated have been detected with a median total PAH concentration of 772 µg kg^{-1}.

Table 3. Description of microorganism load (CFU/g) in the two kinds of smoked shrimp

Type of product	Tests	Positive Samples[a] (%)	Minimum	Maximum	Median	SD[c]
ESS (n = 12)	AMB	12 (100%)	8.5×10^2	2.1×10^5	1.7×10^4	5.6×10^4
	Moulds	12 (100%)	1.2×10^2	1.2×10^3	2.5×10^2	3.0×10^2
	Enterobacteriacea	10 (83%)	< 10	8.2×10^1	2.0×10^1	2.0×10^1
	E. coli	3 (25%)	< 10	2.6×10^2	< 10	1.1×10^2
	S. aureus	0 (0%)	< 10	< 10	< 10	< 10
	Salmonella[d]	0 (0%)	-	-	-	-
SSP (n = 12)	AMB	12 (100%)	6.0×10^2	1.3×10^6	4.5×10^4	3.6×10^5
	Moulds	12 (100%)	6.4×10^1	8.7×10^3	2.9×10^2	2.4×10^3
	Enterobacteriacea	9 (75%)	< 10	4.7×10^4	8.6×10^1	1.4×10^4
	E. coli	3 (25%)	< 10	1.6×10^2	< 10	4.6×10^1
	S. aureus	0 (0%)	< 10	< 10	< 10	< 10
	Salmonella	0 (0%)	-	-	-	-

ESS = Entire Smoked Shrimp; SSP = Smoked Shrimp Powder; AMB = Aerobic Mesophilic Bacteria; aPositive sample= sample in which the number of detected colonies is >10; bCFU: colony forming units; cStandard deviation; d Presence/absence test in 25 g of sample

Table 4. Microbiological status of smoked shrimp according to the ICMSF standard

Tests	Status	ESS	SSP
		N° + sample1 (%)	N° + sample (%)
AMB2	Compliant	12 (100)	11 (92)
	Non-compliant	0 (0)	1 (8)
Moulds	Compliant	12 (100)	12 (100)
	Non-compliant	0 (0)	0 (0)
Enterobacteriaceae	Compliant	12 (100)	10 (83)
	Non-compliant	0 (0)	2 (17)
E. coli	Compliant	12 (100)	12 (100)
	Non-compliant	0 (0)	0 (0)
S. aureus	Compliant	12 (100)	12 (100)
	Non-compliant	0 (0)	0 (0)
Salmonella	Compliant	12 (100)	12 (100)
	Non-compliant	0 (0)	0 (0)

ESS = Entire Smoked Shrimp; SSP = Smoked Shrimp Powder; [1]N° + sample = sample in which the number of detected colony is > 10; [2]AMB = Aerobic Mesophilic Bacteria

Table 5. pH, moisture content and water activity in smoked shrimp samples

Parameters	ESS	SSP
pH*	7.59±0.06a	7.69±0.13a
Moisture content*	13.99±1.78a	10.67±2.17b
Aw*	0.61±0.01a	0.54±0.01b

ESS = Entire smoked shrimp; SSP = Smoked shrimp powder; * Data expressed as means ± standard deviations; [a] Values in the same line followed by different letter are significantly different ($p < 0.05$).

Table 6. PAHs levels in entire smoked shrimp from retail local markets (µg kg^{-1}) (n = 12)

PAHs	Minimum	Maximum	Median
Benzo[b]fluoranthene (BbF)	50	125	75
Dibenzo[a,l]pyrene (DlP)	2	28	5
Dibenzo[a,h]anthracene (DhA)	9	88	22
Benzo[ghi]perylene (BgP)	21	80	40
Dibenzo[a,e]pyrene (DeP)	24	78	44
Benzo[j]fluoranthene (BjF)	30	78	49
Benzo[c]fluorene (BcL)	18	257	75
Benzo[a]anthracene (BaA)	26	202	159
Chrysene (CHR)	33	274	189
5-methylchrysene (5MC)	0	66	27
Benzo[k]fluoranthene (BkF)	7	62	27
Benzo[a]pyrene (BaP)	21	197	91
Indenol[1,2,3-cd]pyrene (IcP)	7	169	33
Dibenzo[a,i]pyrene (DiP)	0	4	2
Dibenzo[a,h]pyrene (DhP)	0	1	1
Sum (PAHs)	326	1415	772
Sum (PAH4)	152	708	490

PAH4 = benzo[*a*]pyrene, chrysene, benzo[*a*]anthracene and benzo[*b*]fluoranthene)

The Beninese ministerial ordinance[37] set the maximum acceptable concentration of benzo(a)pyrene to 5 µg kg^{-1} (wet weight) for smoked fish and smoked fishery products, excluding bivalve molluscs. On the other hand, the European Food Safety authority[38] recommended the sum PAH4 (benzo[*a*]pyrene, chrysene, benz[*a*]anthracene and benzo[*b*]fluoranthene) as the most suitable indicator of the occurrence and effect of carcinogenic PAHs in food. This is confirmed here as chrysene (CHR) and benzo(a)anthracene (BaA) are the most abundant PAHs followed by benzo(a)pyrene (BaP) and benzo(b)fluoranthene (BbF). All the tested samples exceeded these standards individually. The median concentration of benzo (a) pyrene found in smoked shrimp (91 µg kg^{-1}) was 18 times higher than the Beninese national specification, while the median sum PAH4 content (490 µg kg^{-1}) exceeded 15 times the European maximum acceptable concentration limit of 30 µg kg^{-1}[39]. The high values of PAHs revealed in smoked shrimp samples examined might be attributed to the smoking process. Indeed, Soclo *et al.*[40] reported that total PAHs content in fresh shrimp (*Penaeus duorarum*) catched in Nokoue lake in Benin, was 32.63 µg kg^{-1} with Benzo(a)pyrene content of 0.50 µg kg^{-1}. We did the same observation on a limited number of fresh shrimps analyzed in the framework of another study where we found concentrations below 1 µg/g fresh weigh for individual PAHs (data not shown). Degnon *et al.*[7] reported that in Benin, shrimps are smoked by traditional method with wood smoke in direct contact with product. Furthermore, many studies have showed the reality of traditional hot smoking implication in food PAHs contamination[41, 42].

4. Conclusions

In the present study, smoked shrimp in its different forms sold in local markets show a good stability (low moisture content, low water activity and low microorganism load). However some microorganism indicators of fecal contamination have been detected. Furthermore, PAH content exceeded the maximal allowed limit in all investigated samples. These parameters may be considered as an important warning signal for human consumption. Therefore, important measures need to be taken to train local populations in hygienic practices as well as in controlled use of smoking technics.

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REFERENCES

[1] Abbey, L.D., Hodari-Okae, M., Osei-Yaw, A., 1994, Studies on traditional processing and quality of fermented fish (momone). Ghana/Netherlands Artisanal Fish Processing and Applied Research Project Report. Food Research Institute, Accra, Ghana, 48p.

[2] Achinewhu, S.C., Oboh, C.A., 2002, Chemical, microbiological and sensory properties of fermented fish products from Sardinella sp. in Nigeria. J. Aquat. Food Prod. Technol., 11 (2), 53–59.

[3] Anihouvi V.B., Hounhouigan J.D., Ayernor S.G., 2005, Production and commercialisation of “lanhouin”, a fermented fish-based condiment from the Gulf of Benin. Cah. Agric., 14 (3): 323-330.

[4] Paludan-Muller C., Madsen M., Sophanodora P., Gram L., Lange M.P., 2002, Fermentation and microflora of plaa-som, a Thai fermented fish product prepared with different salt concentrations. Int. J. Food Microbiol., 73: 61– 70.

[5] Djevi H., Outtier A., 2004, Forum « Accès au marché des produits agricoles», résultats du groupe thématique « Produits Séchés », rapport d'étude. Cotonou, Bénin, 22 p.

[6] Elwert-Kretschmer K., 2001, L'aspiration au changement alimentaire à Cotonou, Bénin, Rapport d'enquête, CIRAD, 71 p. http://afm.cirad.fr/documents/5_Agro_industries/ALISA/FR/Aspiration.pdf.

[7] Degnon G.R., Dahouenon-Ahoussi E., Adjou S.E., Sohounhloue K.C.D., 2013, Transformation artisanale des crevettes (Penaeus spp) au sud du Bénin: évaluation des performances techniques des équipements et procédés de fumage. Nat. Technol., 8: 23-31.

[8] Visciano P., Perugini M., Conte F., Amorena M., 2008, Polycyclic aromatic hydrocarbons in farmed rainbow trout (Oncorhynchus mykiss) processed by traditional flue gas smoking and by liquid smoke flavourings. Food Chem. Toxicol. 46: 1409–1413.

[9] EC (European Commission), 2002, Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food, SCF /CS /CNTM /PAH/29 Final. (http:// europa. eu. int/ comm/ food /fs /sc/scf/out153_en.pdf).

[10] Food Safety Authority of Irland, 2009, Polycyclic aromatic hydrocarbons (PAHs) in Food. Toxicology factsheet series 1: 1-9.

[11] WHO/IARC (World Health Organization/ International Agency for Research on Cancer), 2012, IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 100 F. International Agency for Research on Cancer, Lyon.

[12] US Environmental Protection Agency (US EPA), 2002, Polycyclic organic matter. Environmental Protection Agency, Washington, DC, Available from: bhttp:// www. epa.gov /ttn/atw/hlthef/polycycl.html.

[13] Anihouvi V.B., Ayernor S.G., Hounhouigan D.J., Sakyi-Dawson E., 2006, Quality characteristics of Lanhouin: A traditional processed fermented fish product in the Republic of Benin. AJFAND 6 (1), 1-5.

[14] Essuman K.M., 1992, Fermented fish in Africa. A study on processing, marketing and consumption. FAO Fish. Techn. Paper, Rome, FAO, 80 p.

[15] De Boer E.W., Spiegelenberg M. and Jansen W.F., 1985, Microbiology of species and herbs. Antonie Van Leeuwenhoek 51: 435-438.

[16] Garcia S., Iracheta F., Golvan F., and Heredia N., 2001, Microbiological survey of retail herbs and species from Mexican markets. J. Food Prot., 64 (1): 99-103.

[17] Pafumi J., 1986, Assessement of the microbiological quality of species and herbs. J. Food Prot., 49: 958-963.

[18] Essien J.P., Ekpo M.A., Brooks A.A., 2005, Mycotoxigenic and Proteolytic Potential of Moulds Associated with Smoked Shark Fish (Chlamydoselachus anguincus). J. Appl. Sci. En-viron. Mgt. 9 (3) 53 – 57.

[19] Bukola C.A., Abiodun A.O., Ukpe G.P., 2008, Mycofloral of Smoke-Dried Fishes Sold in Uyo, Eastern Nigeria. World J. Agric. Sci., 4 (3): 346-350.

[20] ISO 6887-3: 2004, Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 3: Specific rules for the preparation of fish and fishery products.

[21] ISO 4833: 2003, Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C.

[22] ISO 6888-1: 1999, Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium.

[23] ISO 21528-2: 2004, Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony-count method.

[24] ISO 16649-2: 2001, Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of β-D-glucuronidase-poistive *Escherichia coli* – Part 2: Colony-count technique at 44°C using 5 – bromo – 4 - chloro-3-indolyl β-D-glucuronidase.

[25] ISO 7954: 1988, microbiology – General guidance for enumeration of yeasts and moulds – Colony count technique at 25°C.

[26] ISO 6579: 2002, Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella spp*.

[27] Goulas E.A., Kontomina G.M., 2005, Effet of salting and smoking-method on the keeping quality of chub mackerel (Scomber japonicus): biochemical and sensory attributes. Food Chem., 93, 511-520.

[28] AOAC, 1995, Official methods of analysis. Gaitthersburg, MD: Association of Official analytical Chemists. Official method 985.14.

[29] Veyrand B., Brosseaud A., Sarcher L., Varlet V., Monteau F., Marchand P., Andre F., Le Bizec B., 2007, Innovative method for determination of 19 polycyclic aromatic hydrocarbons in food and oil samples using gas chromatography coupled to tandem mass spectrometry based on an isotope dilution ap-proach. J. Chromatogr. A, 1149: 333-344

[30] Brasseur C., Brose F., Pirlot A., Douny C., Eppe G., Maghuin-Rogister G., Scippo M.L., 2007, Validation of the analytical procedure for the determination of polyaromatic hydrocarbons in smoke flavourings using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. Accred Qual. Assur., 12: 535-542.

[31] Danyi S., Brose F., Brasseur C., Schneider Y.J., Larondelle Y., Pussemier L., Robbens J., De Saeger S., Maghuin - Rogister G., Scippo M.L., 2009, Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. Anal. Chim. Acta, 63: 293–299.

[32] Plahar A. W., Nerquaye-Tetteh A.G., Annan T. N., 1999, Development of an integrated quality assurance system for the traditional Sardinella sp. and anchovy fish smoking industry in Ghana. Food Control, 10 : 15-25.

[33] Public Health Laboratory Service (PHLS), 2000, Guidelines for the microbiological quality of some ready-to-eat foods at the point of sale. Comm Dis Public Health 3 (3): 163-167. http://www.phls.co.uk/publications/CDPHvol3/No3/cdph030

0.pdf

[34] International Commission on Microbiological Specification for Foods, 1974, Microorganisms in foods. Vol. 2. University of Toronto, Toronto press.

[35] Kumolu-Johnson C.A., Ndimele P.E., 2001, Effect of salting, brining and sundrying on the shelf-life of Clarias gariepinus (LACEPEDE). J. Res. Rev. Sci., 2: 21-25.

[36] Prescott L., Harley J., et Klein D., 2003, Microbiologie. 2e éd. Bruxelles : De Boeck, 1139 p.

[37] Arrêté 2007 N°0362/MAEP/D-CAB/SGM/DRH/DP/SA du 30 Octobre 2007 portant fixation des teneurs maximales pour certains contaminants dans les denrées alimentaires en répu-blique du Bénin, publié au journal officiel n°9 du 01 Mai 2008.

[38] EFSA (European Food safety autority), 2008, Polycyclic aromatic hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain. The EFSA Journal 724: 1-114.

[39] European Commission, 2011, Commission regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. Official Journal of the European Union, L 215, 4-8.

[40] Soclo H.H., Budzinski H., Garrigue P., Matsuzawa S., 2008, Biota accumulation of polycyclic aromatic hydrocarbons in Benin coastal waters. Polycyc. Aromatic Compounds, 28: 112–127.

[41] Garcia-Falcon M.S., and Simal-Gandara J., 2005, Polycyclic aromatic hydrocarbons in smoke from different woods and their transfer during traditional smoking into chorizo sausages with collagen and tripe casings. Food Add and Cont., 22 (1): 1–8.

[42] Silva O.B., Adetunde T. O., Oluseyi O.T., Olayinka O.K., Alo I.B., 2011, Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. Afr. J. Food Sci., 5(7): 384-391.