Pest and mould infestation of smoked shrimp and preventive measures for storage in cottage industry

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Samples of entire smoked shrimp (ESS) and smoked shrimp powder (SSP) were randomly collected from retail markets in South Benin to evaluate the quantitative and the qualitative infestation with moulds. Furthermore, polyethylene barrel has been tested for smoked shrimp storage and compared to palm branch basket used as traditional storage method. All the samples of both ESS and SSP examined were contaminated with moulds. Mould count were 2.4 and 2.5 Log10 (CFU g-1) for ESS and SSP, respectively. About 14 mould species from 6 genera were identified from stored smoked shrimp. Aspergillus genus was the predominant strain in the investigated samples, followed by Epicocum, Mucor, Penicillium, Scopularus and Fusarium. Storage in barrel preserves shrimp from pest and mould development, 6 weeks longer than storage in baskets.

Keywords: Smoked shrimp, Pest, Mould, Infestation, Storage, Polyethylene barrel.

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Sea foods are one of the cheapest animal protein sources used to correct protein deficiency in human diets in the tropics1. In this group, fish is an excellent source of protein, essentials amino acids, polyunsaturated fatty acids, minerals and vitamins. Fish consumption provides important nutriment to a large number of people worldwide and then makes a very significant contribution to nutrition2. Severable technics have been reported for their preservation including fermentation, drying and smoking3. Out of microbial and enzymatic change stopped by food processing, heavy losses occur during storage and marketing4,5. In Benin, cottage smoking is the main technique used for shrimp preservation for local markets and consumption. More than 67% of fresh shrimp produced each year is processed in cottage industry5. Smoked shrimp is stored for 2.5 - 6 months and sold later during shortage period, to maximize the profit6. Infestation by insects and moulds are the main spoilage problems associated to storage of smoked shrimp in cottage industry7,8. These factors affect consumer acceptability, commercial value and income of shrimps producers and sellers. Post-harvest losses of fish due to spoilage have been estimated to about 10 to 12 million tonnes per year8.

Many medicinal plants and spices such as leaves of Borcia senegalensis, Capsium frutescens L., Dennettia tripetala Baker f., Myrcianthes fragrans (Sw.) McVaugh, Eugenia aromatica O. Berg, Monodora myristica (Gaertn.) Dunal and Piper guineense Schumach. & Thonn. have been cited as pest control agent of smoked fish5,9,10,11,12. Furthermore, as reported by Khan & Khan4, many insecticides such as malathion, gamoxine and endrine have been reported to be used on dried fishes for preservation from infestation. Insecticides are potential sources of chemical hazards causing blurred vision, dizziness and vomiting, while plant and species are sources of quality loss factors such as moulds13. In a previous study on spoilage effects of insect infestation on shrimp during storage, Bello-Olusoji et al.7 recommended that shrimp storage must be in air tight container and the old stock of shrimps must not be mixed with new stock. This study aimed to investigate the mycoflora associated with smoked shrimp sold in Beninese markets and to propose solution to preserve smoked shrimp quality during storage.

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Material and methods

Quantitative and qualitative evaluation of mycoflora of stored smoked shrimp

Smoked shrimp collected from market for this study are caught from Nokoué and Ahémé lakes and lagone of Porto-novo in the South Benin. Fresh shrimps are traditionally smoked (or smoked and grounded), stored and commercialized in conditions described by Kpoclou et al.5. As reported by these authors, Cotonou, Comè and Porto-Novo are cities where fresh and processed shrimp from cottage industry are strongly commercialized.

Samples collection

A total of 24 samples comprising 12 samples of entire smoked shrimp (ESS) and 12 samples of smoked shrimp powder (SSP) were randomly purchased (January-February, 2012), from retail markets of Ganhi and Saint Michel (Cotonou), Comè (Comè center city) 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 own glass package. Samples were transported to the laboratory within 2 hrs for immediate microbiological and water activity analysis.

Storage experiments

Storage experiments were performed for 6 month (from June to November 2013). Wild fresh shrimps (Penaeus duorarum) of size of 75-85 pieces/kg were obtained from fishermen. Smoking was carried out in chorkor kiln with Acacia auriculiformis as firewood as described by Kpoclou et al.14. After cooling, smoked shrimps were separated into two batches, one (batch A) stored in palm branch basket as usual in producers’ practices and the other (batch B) stored in tightly closed (25 dcm3) retrained polyethylene barrel (Devarson industries PVT. LTD, Ahmedabad, India) half-filled up. Shrimp samples were taken out from each batch, at two weeks intervals during 16 weeks. Samples were examined immediately for microbiological and physico-chemical analysis.

Enumeration of smoked shrimp microflora

Twenty-five gram (25 gm) of each sample were 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 No BA 6021, Seward, London, UK). Serial decimal dilutions were prepared in buffer peptone water as described by ISO 6887-315, and inoculated on different media for enumeration of total viable cells and moulds counts, as described by Kpoclou et al.16.

Isolation and identification of moulds microflora

Moulds colonies obtained were purified by successive sub-culturing on Malt Extract Agar (MEA, LAB 37, United Kingdom) and then three points were inoculated on MEA and Czapeck Yeast Extract Agar (M1335, HiMedia, India), as described by Sessou et al.17. Microscopic characters were observed using the identification schema proposed by Samson et al.18 and Pitt & Hocking19.

Measurement of water activity

Water activity (a_w) was measured as described by Kpoclou et al.16, using a thermo-hygrometer recorder (Rotronic HygroLab 2, 8303 Bassersdorf).

Data analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 16). Data are presented as means with standard deviation. Significance was set at P < 0.05 and means were separated using Student, Newman and Keuls (SNK) range test.

Results and discussion

Mycoflora of smoked shrimps

The mould load of each kind of smoked shrimps and the related species identified are presented in Table 2 and Fig. 1, respectively. Data showed that all

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Table 1—Shrimp samples collected for analysis

<table>
<thead>
<tr>
<th>Retail markets</th>
<th>ESS</th>
<th>SSP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ganhi (Cotonou)</td>
<td>3*</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Saint Michel (Cotonou)</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Comè (Comè city center)</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Ouando (Porto-Novo)</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>12</td>
<td>24</td>
</tr>
</tbody>
</table>

ESS = Entire smoked shrimp; SSP = Smoked shrimp powder; *number of samples.

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Table 2—Mycoflora load and water activity in the two types of smoked shrimps collected from beninese markets

<table>
<thead>
<tr>
<th>Type of product</th>
<th>Mycoflora load (Log10 (CFU g−1))</th>
<th>Water activity (a_w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>Maximum</td>
<td>Median</td>
</tr>
<tr>
<td>ESS (n = 12)</td>
<td>2.1</td>
<td>3.1</td>
</tr>
<tr>
<td>SSP (n = 12)</td>
<td>1.8</td>
<td>3.9</td>
</tr>
</tbody>
</table>

ESS = Entire Smoked Shrimp; SSP = Smoked Shrimp Powder; *CFU: colony forming units; bStandard deviation; *data expressed as mean ± standard deviation; SD: standard deviation; n = number of samples.
ESS and SSP samples collected from markets are contaminated with moulds. The maximal loads detected: 3.1 Log$_{10}$ (CFU g$^{-1}$) for ESS and 3.9 Log$_{10}$ (CFU g$^{-1}$) for SSP are lower than acceptable limit of 4.0 Log$_{10}$ (CFU g$^{-1}$) recommended for dried heat processed foods$^{20}$ and food condiments like spices$^{21}$. Therefore, smoked shrimp may be safe from a mycological point of view. A total of 14 mould species belonging to the following genera: Aspergillus, Epicoccum, Fusarium, Mucor, Penicillium and Scopularus, were isolated from smoked shrimps (Fig. 1). A. carbonarius, A. niger, A. wentii, E. nigrum, and A. flavus were the dominant strains in increasing occurrence order. Moulds enumerated in ESS and SSP samples examined, would be introduced during post-smoking handling. Indeed, moulds are spore forming microorganisms and smoking process is assimilated to pasteurization$^{22}$. After processing, smoked shrimps are stored in palm branch basket, generally used for several lots of shrimps with sun-drying after each use. This practice could bring a share of contamination to the product. Furthermore, in retail markets, ESS are sold in bulk and SSP is obtained from ESS after sun drying and grinding$^{16}$. Also, when the smoked dried fishery products are not well ventilated, pest can easily gain access into the stores as a result of bad storage practices as reported by Bukola et al.$^{23}$. Furthermore, the environment in which enter smoked shrimps are exposed in the market is not always hygienic and this is another way for microbial contamination. Essien et al.$^{24}$ have attributed the presence of moulds in smoked fish from market, to continuous contamination of the item through frequent handling and to the general poor sanitary condition of many West African markets. Each microbial species (or group) has an optimum, maximum, and minimum $a_w$ level for growth. In general, the minimum $a_w$ value for growth of most moulds is 0.8, with 0.6 for xerophilic moulds$^{25}$. The $a_w$ needed for spore-forming bacteria to sporulate, for the spores to germinate, and for the toxin-producing microorganisms to produce toxins is generally higher than the minimum $a_w$ needed for their growth$^{25}$. The $a_w$ values measured for ESS and SSP (0.61 and 0.54, respectively) suppose that the risk for mycotoxin production in smoked shrimp would be low. However, because of the detection of probable aflatoxin producing moulds such as A. flavus in examined samples (50% ESS and 58% SSP), further studies are needed to investigate mycotoxin in smoked shrimp.

### Microbiological and physicochemical changes in smoked shrimps during storage in polyethylene barrel and baskets

Table 3 shows microbiological count of smoked shrimp during storage in barrels and baskets. Data showed that after 16 weeks, total viable count (TVC) increased significantly ($p < 0.05$), from 2.7 to 3.2 Log$_{10}$ (CFU g$^{-1}$) in barrel and from 2.70 to 5.5 Log$_{10}$ (CFU g$^{-1}$) in basket. *S. aureus* and *Enterobacteriaceae* were not detected both in basket and barrel throughout the storage (data not shown). Moulds were not detected in shrimp stored in barrel throughout the experiment period; however moulds were detected in shrimps stored in basket from the 2nd week until the end of the experiment period.

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Barrel</th>
<th>Basket</th>
<th>Barrel</th>
<th>Basket</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.7±0.0a</td>
<td>2.7±0.1a</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>2</td>
<td>2.7±0.1a</td>
<td>4.3±0.1b</td>
<td>&lt; 1</td>
<td>1.6±1</td>
</tr>
<tr>
<td>4</td>
<td>2.8±0.1a</td>
<td>4.4±0.1b</td>
<td>&lt; 1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>6</td>
<td>2.9±0.1a</td>
<td>4.5±0.2b</td>
<td>&lt; 1</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>8</td>
<td>3.0±0.0a</td>
<td>4.6±0.0b</td>
<td>&lt; 1</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>10</td>
<td>3.0±0.2a</td>
<td>4.7±0.1b</td>
<td>&lt; 1</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>12</td>
<td>3.2±0.1a</td>
<td>5.3±0.2b</td>
<td>&lt; 1</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>14</td>
<td>3.2±0.2a</td>
<td>5.3±0.2b</td>
<td>&lt; 1</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>16</td>
<td>3.2±0.3a</td>
<td>5.5±0.01b</td>
<td>&lt; 1</td>
<td>2.3±0.1</td>
</tr>
</tbody>
</table>

TVC = Total viable counts, *Data expressed as mean ± standard deviation; a, b: data follow by different letters on the same line for two consecutive columns, are significantly different ($p < 0.05$); n = number of samples.
16th week (from 1.60 to 2.3 Log_{10} (CFU g^{-1}) with the presence of pharaoh ants (Monomorium pharaonis). The increase of TVC count in barrel would be due to contamination from air circulation as minimal it could be, when barrel is closed or during opening for sampling. In basket, TVC increasing could be due not only to air circulation which could be higher due to the nature of the material, but also to pest introduction. The TVC and the mould counts attained at the 16th week both in plastic barrel and in basket did not exceed the acceptable limits specified for dried heat processed foods and food condiments like spices (7.0 Log_{10} (CFU/g) and 4.0 Log_{10} (CFU g^{-1}), respectively). Moulds detected in basket during storage, could have been introduced by air circulation and by pests. Invasion of shrimp stored by pest is a serious worry for smoked shrimp producers and sellers. To prevent this phenomenon, the processors have developed unhygienic practices consisting to packaging the smoked shrimps in baskets previously lined with cotton clothes and alternating shrimp layers with peppers (Capsicum annuum L.) or camphor. Furthermore, basket is made from palm branch which is not easy to maintain clean. Therefore, basket could bring a share of contamination to the product. Previous study on spoilage effects of insect infestation on shrimp during storage, recommended that shrimp storage must be in air tight container and the old stock of shrimps must not be mixed with new stock. This study showed that pest invasion could be prevented and post processing contamination would be reduced by storing in plastic barrel which is easier to maintain clean. Water activity (a_{w}) increased from 0.52 to 0.71 in barrel, and from 0.52 to 0.76 in basket after 16 weeks (Fig. 2). According to Ray & Bhunia, the minimal a_{w} value for xerophilic mould growth is 0.6.

During storage experiments, this minimal value was attained after 10 weeks for storage in barrel and 4 weeks for storage in basket. Data showed that storage of smoked shrimp in plastic barrel preserved shrimp from moulds growth and presumably mycotoxin production longer (10 weeks) than storage in basket (4 weeks) (Fig. 2). Therefore, preservation in plastic barrel is an effective preventive measure to avoid infestation by moulds and pest identified as the major constraints for smoked shrimp preservation in cottage industry.

**Conclusion**

This study revealed that smoked shrimp sold in retail markets in Benin, are contaminated with moulds. Fourteen moulds species belonging to 6 genera have been identified from which some have been reported to be toxinogenic. Smoked shrimp storage in plastic barrel has been proposed to prevent pest infestation and to reduce the increase of water activity, the main factor which influences moulds growth and toxin production during storage.

**Acknowledgement**

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