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The Determination of Shelf Life and Some Nutritional Components of Catfish (*Clarias lazera*) After Hot Smoking

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تحديد فترة الصلاحية وبعض الخصائص الغذائية لسمك السلور (*Clarias lazera*) بعد عملية التدخين الساخن

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Abstract

Changes in the quality of hot smoked catfish *Clarias lazera* were determined just after smoking and during refrigerated storage (4°*C*±1). Chemical, sensory and microbiological analyses of the samples were carried out during the storage to test their shelf life and quality. Significant (p<0.05) differences were found in the chemical composition of fresh and smoked *Clarias lazera*. The process reduced moisture content and pH and increased crude protein, crude fat and ash. During storage at 4°*C*±1 the percentage of total protein, the ash contents and the pH increased significantly (p<0.05), whereas the moisture contents and the crude fat significantly (p<0.05) decreased. Microbiological analysis results demonstrated that the smoking techniques reduced the microbial content of the fish; total viable counts (TVC) of bacteria in fresh fish used as raw material had a total microbial load reaching 19.6×10^6 *cfu/g*. After smoking, TVC recorded 3.2×10^6 *cfu/g* and pathogenic micro-organisms *Escherichia coli* were counted at about 3.8×10^6 *cfu/g*, therefore *Salmonella* spp and *Staphylococcus aureus* were not detected in the fish samples. No yeast or mould was detected in the fresh and smoked samples. During storage at 4°*C* the weekly change in microbial loads of smoked *C. lazera* was increased, micro-organisms *E. coli* and *Salmonella* spp were observed, while *Staphylococcus aureus* was not detected. Sensory evaluation of smoked fish samples showed that the quality of the smoked fish might still be acceptable 28 days after smoking under refrigerator conditions.

Keywords: *Clarias lazera, Hot smoking, Shelf life, Nutritional component.*

الملخص

تم تحديد التغيرات في جودة سمك السلور(*Clarias lazera*) بعد عملية التدخين الساخن وخلال فترة تخزينه مبرداً عند (4°م±1). كما وأجريت التحاليل الكيميائية الحسية والميكروبيولوجية للعينات بعد عملية التجميد لاختبار مدى صلاحيته وجودته. لقد تم الحصول على فروقات معنوية (p<0.05) في التركيب الكيميائي لكلا من السمك الطازج والمدخن. حيث أن عملية التدخين العملية قللت كل من محتوى الرطوبة وقسمة الأسس الهيدروجيني بينما زادت كمية البروتين الخام، والدهون الخام، والرماد. خلال عملية التخزين عند درجة حرارة 4°م±1 فإن مستويات البروتين الكلية

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والرماد وقيمة الأس الهيدروجيني ازدادت بشكل معنوي ($p<0.05$)، بينما محتويات الرطوبة والدهون الخام الكلية تناقصت بشكل معنوي ($p<0.05$) . كما أن نتائج التحليل الميكروبيولوجي أشارت إلى أن تقنيات التدخين تقلل من محتوى الميكروبات بالأسماك، العدد الكلي للبكتيريا القابلة للنمو (TVC) بالأسماك الطازجة كمادة خام وصلت إلى 19.6×10^{6} *cfu/g*. بينما بعد عملية التدخين فإن قراءات TVC سُجلت عند 3.2×10^{6} *cfu/g* والعضويات الدقيقة الممرضة (*E. coli*) سُجل عددها عند 3.8×10^{6} *cfu/g*، بينما *Salmonella* spp و *Staphylococcus aureus* لم يُسجل لهما أي قراءات في عينات الأسماك. كما لم يتم تسجيل أي قراءة للخميرة في العينات الطازجة أو المدخنة. خلال عملية التخزين عند درجة حرارة 4°م فإن هناك زيادة ضئيلة في الحمل الميكروبي لأسماك السلور المدخنة، حيث وُجدت *E. coli* و *Salmonella* spp بكمية متزايدة بينما لا يوجد أي أثر لـ *Staphylococcus aureus*. الجدير بالذكر فإن التقييم الحسي لعينات الأسماك المدخنة أشارت إلى أن جودة أسماك السلور المدخنة سوف تبقى مقبولة حتى 28 يوم بعد تدخينها مادامت محفوظة تحت ظروف التجميد.

الكلمات الدلالية: *التدخين الساخن، مدى الصلاحية، مكون التغذية.*

1. Introduction

Fish is a very important food stuff, especially in developing countries, due to its high protein content and nutritional value of unsaturated fatty matter. However, fish is greatly perishable, quality losses might occur very rapidly after catch, especially in hot climates and tropical areas where cold preservation techniques are often missing. Various food preservation techniques have been utilized to improve the microbial safety and extend the shelf life of fish in general, including freezing, chemical preservation, salting, and smoking (Nickelson *et al*., 2001). With the over growing world population and need to store and transport food, fish preservation becomes necessary to supply the distant market, to produce a range of products with different flavors and textures and creation of conditions unfavorable to the growth or survival of spoilage organisms (Yohanna *et al*., 2011). Fish processing remains the predominant and most important method of fish preservation in Africa (Foline *et al*., 2011; and Kiin-Kabari *et al*, 2011). Preservation by salting, smoking, drying is called curing of fish. This term is defined as fish preserved without the need for refrigeration or freezing, but excluding sterilized products in air-tight containers. Drying, smoking, salting and fermentation processing remains the predominant and most important method of fish preservation in Africa (Foline *et al*., 2011; and Kiin- Kabari *et al*., 2011). These processes may either be used alone or combined in order to achieve the desired product (Turan *et al*., 2007). Up to 70% of the total fish catch in developing countries was preserved by smoking, a process through which volatiles from thermal combustion of wood penetrate fish flesh (Ward, 1995). Smoking is well known that it is one of the oldest food protection methods. Application of method is that with certain temperature and humidity, smoke sourced from plant material is applied to food. Smoking is not only increases resistance of food but changes appearance, taste and smell of foods. Food safety in such products depends upon combination of warm application and cold storage (Lidström *et al*., 2003). Besides, depending on countries, fish species, consumer demands, there could be differences in application style of fumigation. In literature, there is almost no research on *Clarias lazera* processing, especially on hot smoked (unsalted) *Clarias lazera* and its quality changes during storage. Therefore, the

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purpose of this study was to investigate the quality changes of hot smoked *Clarias lazera* at cold storage conditions. The objective of this work was to investigate the shelf life and changes in the chemical and microbiological parameters of *Clarias lazera* during smoking and storage period under cold conditions.

2. Materials and Methods

2.1. Raw Material

Clarias lazera samples were purchased from Jebl-Awlia reservoir, 45 *km* south of Khartoum. Totally eighty fish samples weighing 15.75 *kg* in total were packed in polyethylene bags equally with crushed ice and then transferred to the laboratory in fifty minutes. The average length of the whole fish was 37.78 *cm*±2.37 and average weight was 492.77 *g*±1.34.

2.2. Methods

Fish samples were gutted, washed and transferred to baskets to dry up while a thin cloth cover was placed in order to keep away insects. After draining the excess water for 60 *min*. All laboratory analyses were started 12 *hrs* after death. Hot smoking techniques was used. Using steel kiln whose fuel was composed of oak sawdust was used for smoking procedures. The kiln contained two parts one is smoke unit and the other for smoking and cooking unit. The processing time in the kiln was divided into three stages: (1) preliminary drying period (45 *min.*) at 30°*C*; (2) a smoking and partial cooking period (60 *min*) at 45°*C*; and (3) a cooking period (60 *min*) at 80°*C*. Total smoking process took 165 *min*. After cooking, fish samples were cooled at ambient temperature for 60 *min*. The samples were packed in aluminum foil since it was reported as a packaging material providing excellent protection from evaporation, loss of aroma and contamination (Anonymous, 1992), and then stored under refrigerator conditions (4°*C* ±1) during the analysis period.

2.3. Analysis

The determination of the crude protein, moisture contents, ash contents and crude fat of the fresh and smoked fish were carried out in triplicate in accordance to Association of Official Methods of Analysis. Moisture content was determined according to AOAC (1995) after the water in extract was removed, ash in extract was calculated. Crude protein content (NX6.25) was calculated using the Kjeldahl method while crude fat was determined to Soxhlet method described in AOAC (1995) pH was measured with a digital electronic pH meter with a glass electrode (WTW Mark 320). For microbiological analysis preparation of the samples was carried out according to (Harrigan, 1998). Total viable counts of bacteria (TVC) were determined using Plate Count Agar (PCA) (37^{o}*C*±1, 48 *hrs*). Presence of *Escherichia coli* was determined by applying IMVIC tests to the typical dark colonies from Violet Bile Agar. *Staphylococcus* spp was determined using Mannitol Salt Agar (MSA) (37^{o}*C*±1, 48 *hrs*), *Staphylococcus aureus* was determined by applying coagulase test on bright yellow halo colonies on (MSA). *Salmonella* spp was determined using Salmonella and Shigella Agar

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(SSA) (37^{o}C±1, 72 *hrs*). Potato dextrose agar (0130) was used for counting mold and yeast (22 ^{o}C±1, 5 *days*). All colonies were counted and the data was reported as colony forming units (CFU) per gram.

2.4. Sensory Evaluation

For examination purposes, end products were carried out by assessors composed of fifteen students from the school of fish sciences, section from faculty of agriculture and fish sciences. Questionnaires were used by panels and scoring was done on a weekly basis. Quality attributes investigations include texture, flavors, appearance and general taste. Panel members scored all factors on a 10-point scale (9 = excellent; 8-9 very good; 6.5-7.9 good; 5-6.4 fair; and <5 bad), using the score method as reported by (Afolbi *et al*., 1984).

2.5. Statistical Analysis

All measurements were performed in triplicate and the value expressed as the mean±standard deviation (SD). Statistical analyses were performed using SPSS 17.0 for windows. Analysis of variance (ANOVA) was used and statistical significance was set at $p< 0.05$.

3. Results and Discussion

3.1. Proximate Composition

The results of the proximate analysis carried out on the fresh and freshly smoked fish are presented in Table (1). The average values of moisture content, crude protein, crude fat and ash content of the fresh fish were, 76.56±5.22, 18.42±1.22, 2.22±0.78, and 1.27±0.51% respectively. These average values fall within the range given by various authors in earlier studies (Egbal *et al.*, 2010; and Kumolu *et al.*, 2010). After the smoking process, there was significant (p<0.05) reduction in moisture content when the fish was hot smoked, this is due to loss in water during drying and cooking as the result of smoking process. Industrial specifications for smoked finished products generally recommend less than 65% water content in fish flesh (Cardinal *et al*., 2001). This is in agreement with our values of 43.0±3.61 that shown in Table (1). The percentage of crude protein and crude fat in smoked *C. lazera* were significantly (p<0.05) higher than the values in the fresh samples. There was an inverse relationship between the protein, fat content and moisture in the smoked fish. Protein and fat content in smoked catfish *C. lazera* increased (as shown in Table.1) due to an increase in the dry matter content per unit of weight following sample dehydration during smoking. Similar results for chemical composition of smoked fish have been reported in previous studies (Bilgin *et al*., 2008; Egbal *et al.*, 2013; and Kumolu *et al.*, 2010). Differences in ash content between fresh and smoked samples were significant (p<0.05). A similar result was reported by Unlusayin *et al*. (2001) for hot smoked fish. No significant differences in pH values were observed between the fresh and smoked samples. Although there was dropped in pH value after smoking, this reduction accompanies the onset of rigor mortis where the fish muscle stiffens and muscle filaments shorten temporarily. The pH first decreases with the onset of

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rigor but then progressively increases from microbial activity. The pH in fish tissues drops due to smoking, generally (Doe, 1998).

Table 1. Chemical composition (*g*/100*g* %) of fresh and smoked *Clarias lazera* before storage

Parameter	Fresh fish	Smoked fish
Moisture	76.56±5.22[a]	43.0±3.61[b]
Ash	1.27± 0.51[a]	1.50±0.46[b]
Crude protein	18.42±1.22[a]	19.26±0.58[b]
Crude fat	2.22±0.77[a]	2.70±0.64[b]
pH	7.56±0.45[a]	7.13±0.12[b]

[a]Values represent pooled means and standard deviations of triplicate determinations of wet weight.

[b]Values with different superscript letters horizontally in rows are significantly different (p<0.05)

3.2. Proximate Composition of Hot Smoked Cured Products During Storage Period Under Refrigerator Condition

Table (2) demonstrates the quality changes of smoked *C. lazera* packed in aluminum foil and stored at 4°*C*±1. The samples were analyzed until the 28th day; therefore all parameters were evaluated for these days. Storage period at refrigerated temperature (4°*C*±1) had significant (p<0.05) effect on water content during storage periods. With increasing storage period, water content decreased. The highest water content was in zero day storage (43.0%±3.60), whereas the lowest one in 28th day (32.0%±3.0). Similar observation was also found for hot smoked Atlantic bonito by Duyar *et al*. (2008) and for smoked Atlantic mackerel by Bhuiyan *et al*. (1986); and Egbal *et al*. (2013) on cat fish *C. lazera*. Cardinal *et al*. (2001) suggested moisture content of smoked fish below 65%. Our result for moisture content was 43.0%±3.60. Significant differences in protein content (p<0.05) as shown in Table (2). The highest protein content was in 28th day storage (24.76%±1.53), whereas the lowest one in zero day (19.26%±0.1). Decrease in water content increased protein content as expected. This result agreed with Egbal *et. al*. (2013) who found that the protein content of *C. lazera* reached 29.0%±1.0 in the 28th day of storage. With storage period ash content increased and reached 2.28%±1.09 in the 28th storage day. Cardinal *et al*. (2001) stated that relative and significant increases occurred in ash content of smoked fish samples during storage period. Storage time had no significant effect on the fat content of smoked catfish, although it began to decrease in the 14th day. It was 2.70%±0.64 in zero day and the value reached 2.30%±0.06 in 28th storage day as present in Table (2). Different results were found by Ramazan *et al*. (2009) who found that the fat content of *Tinca tinca* reached 5.2% in the 28th day. This may be due to the effect of salting, packaging and storage condition. The pH changes of smoked catfish stored for four weeks were represented in Table (2). The pH value was not significantly affected by storage time (p >0.05), but it was increased slightly during storage period. This due to the microbial activity, the same results were found by Vasiliadou *et al*. (2005).

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Table 2. The effects of storage (4^{o}C±1) on chemical composition (*g*/100*g*%) of smoked *Clarias lazera*

Days	Parameter				
	Moisture	**Ash**	**Crude protein**	**Crude fat**	**pH**
0	43.00±3.60^{a}	1.5±0.46^{a}	19.26±1.00^{a}	2.70 ±0.64^{a}	7.13±0.115
7	41.00±1.00^{b}	1.63±0.55^{b}	20.00±2.00^{b}	2.80 ±0.70^{b}	7.20±0.265
14	37.67 ±1.53^{c}	1.75 ±.50^{c}	22.00 ±2.00^{c}	2.40 ±0.72^{c}	7.47±0.473
21	35.67 ±1.52^{d}	1.93± 0.55^{d}	24.00 ±100^{d}	2.37 ± 0.55^{d}	7.57±0.513
28	32.00 ±3.00^{e}	2.28 ±1.09^{e}	224.68 ±1.53^{e}	2.30± 0.06^{e}	7.67±0.569

*Values represent pooled means and standard deviations of triplicate determinations of wet weight.

**Values with different superscript letters vertically in columns are significantly different (p<0.05)

Table (3) shows a comparison between the microbial loads of fresh samples of *C. lazera* and freshly smoked samples. The results revealed that the flesh of the *Clarias lazera* had Total Viable Counts (TVC) reaching 19.6×10^{6} *cfu/g*. The total viable bacteria counts were found with highest concentration of fresh sample because fish is a very good culture media. Fish is low acid food and therefore very susceptible to the growth of food poisoning bacteria (Yohnna *et al*., 2011). After smoking TVC was decreased when compared to the fresh samples, that recorded 3.2×10^{6} *cfu/g*. Similar observation was also found for smoking of *Clarias gariepinus* by (Kumolu *et al*., 2010). No pathogenic micro-organisms like *Salmonella* spp and *Staphylococcus aureus* were found except *E. coli* which recorded 3.8×10^{6} *cfu/g*. No yeast or mould was detected in our fresh and smoked samples. In the present study, during storage at 4°*C*±1 a microbial loads of smoked *C. lazera* were presented in Table (4). Generally, there is an increase in the microbial load of the smoked samples in the 7th day until the end of storage time (28th days) due to growth and multiplication of the microbes (Bilgin *et al*., 2008), in addition to smoking procedure (unsalted smoked). This view was supported by (Clucas *et al*., 1981). In addition, smoking also reduced *E. coli* and *Salmonella* spp, when *Staphylococci* were no detected Table (4). *Salmonella* population was reduced to 2×10^{6} *cfu/g* after smoking and stayed stable until the end of storage time. The TVCs of the samples were all below 4.5×10^{6} *cfu/g*, which is below the maximum total plate count for the processed food to be consumed safely was 10^{7}-10^{8} which stated by Connel (1985). Low levels of *E. coli* were detected and *Salmonella* counts were below 2×10^{6} *cfu/g*. The pathogens *Staphylococcus aureus* were not isolated from any of the smoked samples. The range of tests and specified microbiological limits recommended by the ICMSF (1986) for smoked fish reflected the potential hazard of the 48 product. The microbial results were, however, within the ICMSF limits, so the samples were of acceptable microbial quality up to 28 days of storage.

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Table 3. Types and population densities of bacteria isolated from the fresh and freshly smoked samples

Microbial populations (*cfu/g*)	Fresh samples	Freshly smoked samples
Total Viable Counts (TVC)	19.6×10^6	3.2×10^6
Staphylococcus aureus	*+ve*	*-ve*
Escherichia coli	7.88×10^6	3.8×10^6
Salmonella spp	*+ve*	*-ve*
Yeast-Moulds	*-ve*	*-ve*

-ve = Not detected
+ve =Detected

The organoleptic properties of the smoked samples (*C. lazera*) that the products were acceptable according to the panel's evaluation, though statistically there was significant difference ($p<0.05$) in the sensory evaluation during storage period based on the panel's score Table (5). In the present experiment, scores are the average of 15 panel taste sheets. It could be noticed that smoked samples at zero and 7th days had received higher scores, followed by 14th and 21th samples had received lowest scores at 28th. In all the sensory qualities examined, the smoked *C. lazera* scored above average, which indicates that they might still be acceptable 28 days after smoking. These results show that in The Sudan this type of product could be accepted by consumers. In conclusion, hot smoking process can be used only without added of salt for preservation catfish *C. lazera* in Sudan. The hot smoking technology that was used led to the production of a high-quality delicatessen food item, which could be an alternative to cooked fresh fish. Based on the presented data hot smoked *C. lazera* packed in aluminums foil had an extended shelf life to 28 *days* at 4°*C*±1 temperatures according to microbial, sensory and chemical analysis results.

Table 4. Types and Population Densities of Bacteria isolated from the smoked sample (*Clarias lazera*) during storage at 4 ^oC±1 for 28 *days*

Microbial populations	Days				
	0	**7**	**14**	**21**	**28**
Total Viable Counts (TVC)	3.2×10^6	3.9×10^6	3.5×10^6	3.7×10^6	3.8×10^6
Staphylococcus aureus	*-ve*	*-ve*	*-ve*	*-ve*	2×10^6
Escherichia coli	3.8×10^6	4.0×10^6	3.0×10^6	3.0×10	2.0×10^6
Salmonella spp	-ve	2.0×10^6	2.0×10^6	1.0×10^6	3.2×10^6
Yeast-Moulds	*-ve*	*-ve*	*-ve*	*-ve*	*-ve*

-ve = Not detected
+ve =Detected

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Table 5. Sensory evaluation of smoked *Clarias lazera* during storage time (4°C±1)

Parameters	Days				
	0	7	14	21	28
Flavor	8.23±0.1[a]	7.84±.06[a]	6.97±0.55[b]	6.18±0.27[c]	5.8±0.05[d]
Texure	7.99±.01[a]	7.43±0.02[a]	7.36±0.90[b]	6.21±0.19[c]	5.96±0.07[d]
Odor	7.95±0.06[a]	7.88±0.01[a]	6,89±0.58[b]	6.18±0.06[c]	5.77±0.02[d]
Appearance	7.77±0.02[a]	7.48±0.05[a]	7.30±0.53[b]	6.74±0.27[c]	6.00±0.06[d]
General taste	8.03±0.07[a]	7.39±0.06[a]	7.33±0.49[b]	6.58±0.69[c]	5.85±0.13[d]
Significance	0.00	0.00	0.00	0.00	0.00

*Values represent pooled means and standard deviations of triplicate determinations of wet weight.
**Values with different superscript letters vertically in columns are significantly different (p<0.05)

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