

Antimicrobial and Preservative Activities of *Lippia Multiflora* Essential Oil on Smoked Mackerel (*Scomber Scombrus*) Fish

Goly Kouassi Roselin Cyrille^{1,2}, Dadie Adjehi², Soro Yaya¹, Kouame N'zebo Désiré², Kassi Amian Brise Benjamin³ and DJE Marcellin²

¹Laboratory for Industrial Processes of Synthesis, Environment and New Energies, National Polytechnique Félix Houphouët-Boigny Institute, Yamoussoukro, Côte d'Ivoire

²Department of Food Science and Technology, Laboratory of Biotechnology and Food Microbiology, Nangui Abrogoua University, Abidjan, Côte d'Ivoire.

³Department of chemistry, Laboratory of Organic Chemistry of Natural Substances, Félix Houphouëtboigny Cocody University, Abidjan, Côte d'Ivoire

Corresponding author: Dadie Adjehi, Department of Food Science and Technology, Laboratory of Biotechnology and Food Microbiology, Nangui Abrogoua University, Abidjan, Côte d'Ivoire, Tel: 0022501879717; E-mail: thomasdadie@yahoo.fr

Received Date: January 12, 2017; **Accepted Date:** February 07, 2017; **Published Date:** February 14, 2017

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Citation: Goly KRC, Dadie A, Soro Y, Kouame ND, Kassi AB, et al. Antimicrobial and Preservative Activities of *Lippia Multiflora* Essential Oil on Smoked Mackerel (*Scomber Scombrus*) Fish. Arch Clin Microbiol. 2017, 8:1.

Abstract

Objectives: The present study aims to exploit antimicrobial potentialities of the essential oil of *Lippia multiflora* in preserving smoked mackerel fish.

Methods and findings: Essential oil extracted from *Lippia multiflora* was applied on smoked mackerel (*Scomber scombrus*) fish samples obtained from smoking and selling outlets in Yamoussoukro metropolis (Côte d'Ivoire). Microbiological quality determinations include microbial load counts, using enumerations of total aerobic mesophilic flora, total coliforms, yeasts & moulds and *Staphylococcus aureus*, before and after application of essential oil, and during fish samples preservation studies.

The contamination levels of market smoked fish samples were total aerobic mesophilic flora (4.3 log₁₀ CFU/g); total coliforms (1.42 log₁₀ CFU/g); yeasts & moulds (2.45 log₁₀ CFU/g) and *Staphylococcus aureus* (1.41 log₁₀ CFU/g). There were no microbial loads recorded for the freshly smoked mackerel fish samples. One ml of *L. multiflora* essential oil added to 70 g of fish samples inhibited the growth of total aerobic mesophilic flora by 100% in 24 h, while the smoked fish samples were preserved for 3 days without repeated smoking.

Conclusion: *L. multiflora* essential oil can serve as a bio-preservative for smoked mackerel fish instead of repeated smoking or chemical preservation.

Keywords: Essential oil; *Lippia multiflora*; Mackerel; *Scomber scombrus*; Food preservation

Introduction

Fish is a very important source of animal protein in West Africa [1] but easily perishable if not properly preserved. This very perishable foodstuff after its capture [2] has an impact not only due to lack of food hygiene but also on social, medical and economical characteristics, due to socioeconomic constraints hampering the development of industrial preservation of fish [3]. Smoking is one of the several methods of preserving easily-perishable sea-foods, such as fish; however, this method, which is mostly done as cottage food processing method at the local settings, can be responsible for easier contamination of smoked fish by microbial pathogens [4]. Microbial contamination may be through food handling [5], and sometimes, inadequate of ineffective fish smoking can constitute a risk factor for consumers, especially, due to harmful effects of smoke [6,7]. Thus, smoked fish can be of public health importance because of presence of pathogenic microorganisms, as well as chemical contaminants [8].

Materials and Methods

Materials

The study was carried out on the smoked mackerel fish (*Scomber scombrus*) (Figure 1). In Côte d'Ivoire, mackerel is frequently consumed in rural and urban area because of its low cost, its taste and availability. It is eaten fresh (fresh mackerel) and smoked (smoked mackerel). But, because of the difficulties of conservation of fresh fish by rural and some urban people, they use smoked fish [9-11].



Figure 1 Smoked mackerel (*Scomber scombrus*) sample on selling site.

Methods

Choice criteria and Sampling: The study has been carried out over a period of 1 month (July 2014). A sample constituted of 3 smoked fish of 100 to 300 g was taken on smoking and selling outlets in Yamoussoukro metropolis (Côte d'Ivoire).

Smoked fish were collected on two types of sites. Some are located on open squares and others on closed squares [12,13]. All these sites are unsafe with household garbage, domestic animals, insanitary environment and women dressed in dirty clothes. Dirty equipment, unhygienic environment of smoking and inappropriate fish handling justified the choice. All Samples were collected in a sterile manner, kept in an ice box (ESKIMO), maintained at 4°C and transported to the laboratory for analysis. A total of 72 samples smoked mackerel were randomly and aseptically collected at a week. So, smoked fish from 0.5 to 1 kg were taken during the study.

Extraction of essential oil: Fresh leaves of *L. multiflora* were collected from July to September 2013 around the National Polytechnic Institute Félix HOUPHOUËT BOIGNY of Yamoussoukro (Côte d'Ivoire). After identification by a botanist of the institute, leaves in a Herbarium were stained in the sun at room temperature ($27 \pm 2^\circ\text{C}$) for ten days before using [14]. A quantity of 300 g of dry *L. multiflora* leaves was introduced in Clevenger apparatus for oils extraction. The extraction was performed by steam distillation in a Clevenger type apparatus [15]. After two hours and half the recovered essential oils are dried and then stored at 4°C in a hermetically sealed flask [16].

Microbiological quality of smoked fish: The microbiological quality of the samples was evaluated by the standards methods of microbiology. The Total Aerobic Mesophilic Flora (TAMF) at 30°C (NF V08-051), Total Coliform (NF ISO 4831) at 44°C, *Staphylococcus aureus* at 37°C (NF IN ISO 6888-1) and yeasts and moulds at 25°C (ISO 7954). The enumeration of each germ mentioned above was carried out according to standard NF V08-010. Indeed, ten gram (10 g) of smoked fish sample was weighed aseptically into 90 ml of peptone water to obtain the initial dilution of 10^{-1} then serial dilution was done. The Total Aerobic Mesophilic Flora (TAMF) was determined by plating 1ml of the serially diluted sample on Nutrient agar (Scharlau, Gato

Perez, Spain) and incubating at $30 \pm 1^\circ\text{C}$ for 72 h. The presumptive Total Coliform (TC) counts were determined by plating 1 ml of the serially diluted sample on Violet Red Bile Lactose Agar (VRBL) (Ultimed, Castellar del Vallès, Spain) and incubating at $44 \pm 1^\circ\text{C}$ for 24 h. Fungi (yeasts and moulds) were determined by plating 0.1 ml of the serially diluted sample on Sabouraud (BIO-RAD; Marnes-la-Coquette; France) with Chloramphenicol and incubating at $25 \pm 1^\circ\text{C}$ for 72 h. *Staphylococcus aureus* was determined by plating 0.1 ml of the serially diluted sample on Baird Parker Agar (Ultimed; Castellar del Vallès, Spain) and incubating at $37 \pm 1^\circ\text{C}$ for 24 h.

Application of the essential oils

Evaluation of essential oil antimicrobial activity: Addition technic was used to determine the antimicrobial activities of essential oil according to the technic described by Dègnon et al. [17]. Volumes of 0.25 mL; 0.5 mL at 1 mL of the essential oil were applied on all the surface of 70 g of smoked fish with a swab to determine the low concentration which could inhibit the microflora on smock fish in 24 h. For each test, a control sample (without essential oil) was prepared. The samples tested and control samples were incubated during 72 h at 30°C for Total Aerobic Mesophilic Flora, 72 h at 25°C for yeasts and moulds; 30°C during 48 h for Total Coliforms and 48 h at 37°C for *Staphylococcus aureus*. Also, each concentration (0.25 mL/70g; 0.5 mL/70 g and 1 mL/70 g) have been tested for 24 h. These tests were done in order to determine the weakest oil concentration being able to neutralize the microflora and conserve smoked fish for one week.

Data processing

The results were analyzed by the variance method (ANOVA) using the STATISTICA software version 6.0 (treatment by ANOVA 1 factor). Comparison of the means was performed by the Tukey's test at 5%.

Results and Discussion

As shown the **Figure 2**, result of microbial analysis of smoked fish indicated that samples are contaminated by Total Aerobic Mesophilic Flora (TAMF) ($4.3 \log_{10}$ CFU/g), Total Coliform (TC), fungi (yeasts and moulds) and *Staphylococcus aureus*. But we note an absence of *Staphylococcus aureus*, yeasts and moulds in smoked fish just after smoking. The level of smoked fish contamination varied according to process of fish treatment. The results corroborate those of Dègnon et al. [17]. We observe that smoked fish took on selling sites was most contaminated in micro-organisms than those took just after smoking. This difference could be explained by the smoking temperature and smoke effect [18]. Maybe this happened because smoking can keep the fish uncontaminated for a special period of time. The temperature of hot smoking fish varies from 60 to 80°C. This evolution of temperature would contribute strongly to reduce level of microbial contamination. The highest contamination of smoked fish in selling could be explained by post-harvest handling, processing and marketing [19]. The exposure of fish smoked to ambient air, the contact of fish with the hands of the

vendor and/or the hands of the customer contributes also to smoke fish contamination. This contamination could also result from post process recontamination due to cross-contamination by dirty equipment or unhygienic environment. Indeed, the majority of people of smoked fish manufacturing and marketing do not observe elementary rules of hygiene. It was shown by the highest presence of Total Aerobic Mesophilic Flora (TAMF) in smoked fish as noticed by Djinou [5] and Oulaï et al. [4]. Dione [20] and Dègnon et al. [17] noted a high presence of Total Aerobic Mesophilic Flora (TAMF) in smoked fish. The high fungi contamination of smoked fish was already reported by Thiam and Ducommun and Oulaï et al. [21,4]. Total Coliforms are human and animal digestive tract hosts. Their presence is due to a fecal contamination [22]. The workshops of manuring and even the markets do not have a device for hands washing and disinfection. Thus, the requirement to wash the hands before each activity is not observed on smoking and selling sites.

Staphylococcus aureus was not into samples taken on smoking sites. This result is in conformity with that of Dègnon et al. [17]. *Staphylococcus aureus* are massively present in smoked fish taken on selling sites. This high presence of *Staphylococcus aureus* in smoked fish on selling sites is justified by recontamination. Indeed, according to Dègnon et al. [17], the high contamination of these samples would result from the low

level of hygiene. Studies of these authors carried out on shrimps showed that the exposure of the smoked product for selling could also constitute a source of contamination (Figure 2).

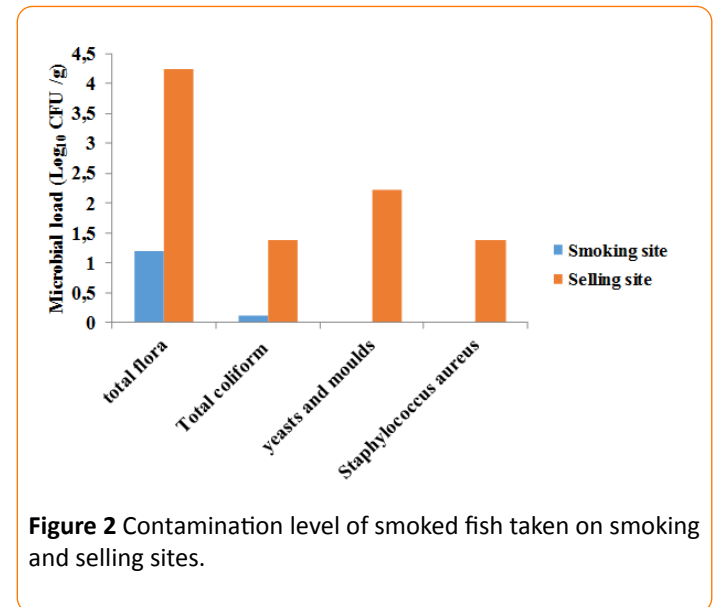


Figure 2 Contamination level of smoked fish taken on smoking and selling sites.

Table 1 Effect of application of *L. multiflora* essential oil on the contamination level of mackerel in 24 h.

Essential oil concentrations (mL/70 g of smoked fish)	Microbial load in smoked fish (Log ₁₀ CFU /g) in 24 h				
	Microbial Population and RR(%)	Total flora	Total Coliform	Yeasts and Moulds	Staphylococcus aureus
0.25	control	3.29 ± 1.12	2.30 ± 0.98	2.19 ± 0.21	2.38 ± 0.92
	Test	1.32 ± 0.36	<1	0.79 ± 0.42	<1
0.5	Test	<1	<1	<1	<1
	Reducing (%)	59.87a	100a	63.92a	100a
1	Test	<1	<1	<1	<1
	Reducing(%)	100b	100a	100b	100a

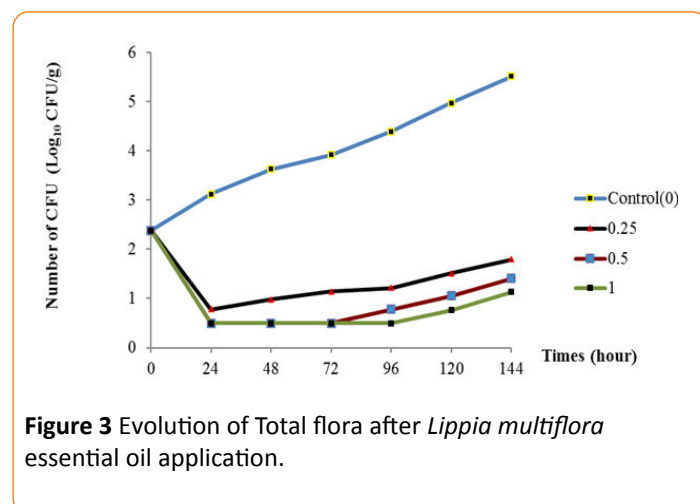


Figure 3 Evolution of Total flora after *Lippia multiflora* essential oil application.

Effect of essential oil concentrations are shown in Table 1. According to this table, concentrations of 0.5 and 1 mL per 70 g of essential oil inhibit completely microbial development on smoked fish during 24 hours. However, for 0.25 mL per 70 g *L. multiflora* inhibits completely *Staphylococcus aureus* and coliform development during 24 hours but not Total flora, Yeasts and Moulds which are inhibited respectively with 59.87% and 63.92% at the same time. This capacity of essential oil to inhibit the microbial development in smoked fish is explained by its strong content of antimicrobial composed [23,24].

The behaviors of each germ during the conservation period after application of the essential oil have been seen in Figures 3-6. The results show that until the first three day, any growth of germs has been noted in smoked fish. But, there were growth with a high number of germs on the control smoked fish

samples (without EO), that means deterioration of smoked fish. Studies of Dègnon et al. [17] confirm these results.

However, only concentration of 1 mL/70 g inhibited microbial growth. With 0.5 mL/70 g, the microbial growth is inhibited during the first three days during smoked fish conservation. This ability of essential oil to permit the preservation of smoked fish is explained by a high content of bio-actives components. This antimicrobial activity of essential oil of *L. multiflora* was already noted by Okpekon et al. and Goly et al. [25,24].

After this period, bio-protection of smoked fish leads by essential oil decrease progressively. This reduction in activity could be due to the remanence effect of essential oils because, they are very volatile substances. Their application and their exposure to ambient air involve a progressive disappearing of the volatile molecules of essential oil.

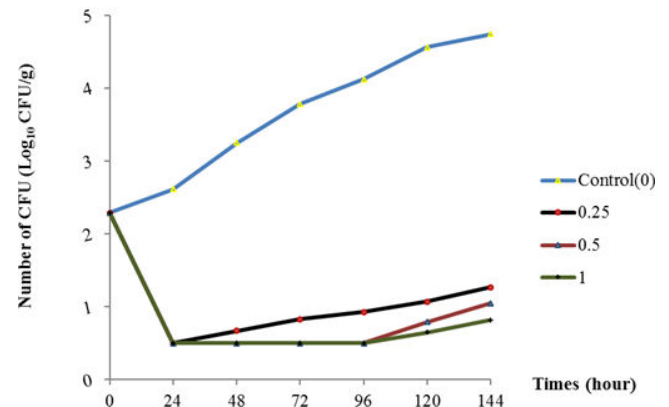


Figure 6 Evolution of *Staphylococcus aureus* after *Lippia multiflora* essential oil application.

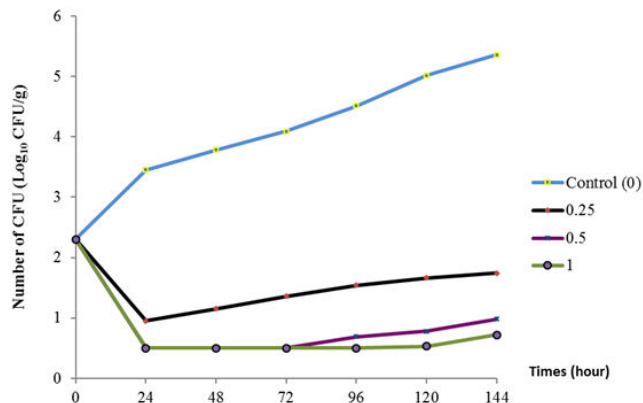


Figure 4 Evolution of Total Coliforms after *Lippia multiflora* essential oil application.

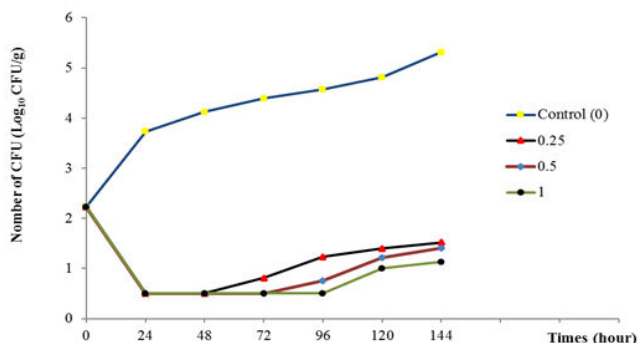


Figure 5 Evolution of Yeasts and moulds after *Lippia multiflora* essential oil application.

Conclusion

The present study revealed that there is a potential risk of recontamination of smoked fish, which is relation to the low level of hygiene applied in the production of that food. In vitro antimicrobial tests indicated that essential oil of *L. multiflora* has a pronounced antimicrobial activity. In order to seek natural antimicrobial products capable to extend preservation of smoked fish, this study has shown that *L. multiflora* essential oil could be used. Tests used to preserve smoked fish by addition of essential oil made it possible to prolong in a substantial way the shelf life of the product. At the fourth day of conservation, the fish is an acceptable microbiological quality. The results obtained show that the preservation of smoked fish by incorporation of essential oil increased the shelf life of the product without chemical conservative addition. However, this protection is not for a long time due to the volatile property of the essential oil.

Conflict of Interest

All authors have contributed equally to the realization of this work. None of the contributing authors has any conflict of interests relevant to the subject matter or materials discussed in the manuscript. No funding or other financial support was received.

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