IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 14, Issue 8 Ser. IV (August 2020), PP 43-49
www.iosrjournals.org

Efficacy of *Pimentaracemosa* and *Syzygiumaromaticum* essential oils in the post-smoking conservation of fish in South Benin

Brice C. Atrevy*, René G. Dègnon, Brice Kpatinvoh, Modesty C. Gangbe, Euloge S. Adjou, AymardAllagbe
Laboratory of Research and Study in Applied Chemistry, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O.B: 2009 Cotonou, Bénin

Abstract
Smoked fish in South Benin deteriorate very quickly because of their nutritional richness and the climatic conditions that do not favour their conservation for a long time. The aim of this work is therefore to evaluate the effectiveness of essential oils in preserving the quality of smoked fish in South Benin. It was carried out on two species of fish with the use of two essential oils through two different application methods. The results obtained showed that the two oils used exerted an antibacterial activity on Total Mesophilic Aerobic Flora, Coliforms and Staphylococcus spp in the fish. They also have an antifungal effect on strains of yeast and mould. The two oils tested had similar activity on fish and fish kept in bags degraded much faster. The essential oils preserved the quality of the smoked fish for 5 days.

Keywords: *Smoked fish, preservation, essential oil.*

Date of Submission: 20-08-2020 Date of Acceptance: 06-09-2020

I. Introduction

Fish and fishery products are an important dietary component for a large part of the world's population, especially in developing countries, where fish is a cheap and available source of protein (Amusan*and al.*, 2010). Fish is a highly nutritious but highly perishable food (FAO, 2000). It contributes about 60% of the world's protein supply and more than 30% of the annual protein intake of developing countries (FAO, 2000). Despite its importance, fish deteriorates very rapidly in tropical countries due to climatic conditions and the non-use of cold storage systems in production areas, resulting in significant post-harvest losses of up to 40%. In Benin and following the example of other countries in the sub-region, post-harvest losses are estimated at around 20% (Anihouvi*and al.,* 2005). To limit post-harvest losses, different conservation techniques such as frying, fermentation, drying, salting and smoking are used individually or in combination (Yacoubou, 2009; Issa*and al.,* 2012). Of these conservation techniques, smoking aims to prevent the proliferation of spoilage microorganisms, but also to slow down certain biochemical reactions (ANSES, 2010). Despite the numerous efforts made in fish conservation through smoking, post-smoking losses of fish are still recorded because of the still perishable nature of smoked fish following extensive microbial proliferation and the risks of lipid oxidation (Agossou, 2012). The work of Fatou*and al.* (2014) reported the predominant role played by moulds in this contamination; they are mostly due to airborne contamination due to the exposure of smoked fish for sale. Indeed, essential oils are now known to have demonstrated their effectiveness in the fight against the alterative factors of food products. The work of Adjou*and al.* (2012) reported the efficacy of essential oils of *Menthapiperita* and *Cymbopogoncitratus* in the control of post-harvest peanut adulterating factors, as well as microorganisms that degrade the quality of local beers marketed in central Benin. Kpatinvoh, 2017 showed the bioefficiency of jute bags impregnated with plant extracts in the post-harvest protection of cowpea. Thus, the present work aims to evaluate the effectiveness of essential oils of *Pimentaracemosa* and *Syzygiumaromaticum* in the post-smoking conservation of fish.

II. Material and Methodology

Material

The equipment used to carry out this work consists of biological and laboratory materials. The biological material consists of fish species (Tilapia, Catfish) and essential oils of *Pimentaracemosa* and *Syzygiumaromaticum*. The laboratory material consists of the apparatus and glassware used in microbiological diagnosis.

Figure 1 : Sample of smoked fish

Figure 2: Photo of *Pimentaracemosa* leaves
Common Name: Laurier

Figure 3: Photo of the floral buds of *Syzygiumaromaticum*
Common Name: Atinkingbadota

Microbiological analyzes

The samples taken were evaluated by searching by standard methods reported by Joffin and Joffin (2003) quality microbiological parameters. These are total mesophilic flora at 30 ° C (total germs, NF V08-051), total coliforms, thermotolerant coliforms and Escherichia coli (NF ISO 4831) of Staphylococci spp at 37 ° C (NF EN ISO 6888) and yeasts and molds (ISO 7954). The culture media and reagents used come from LaboratoiresBioMérieux and Diagnostics Pasteur. The interpretation of the results was made according to a two-class plan with reference to the microbiological criteria for fresh animal products (French legislative and regulatory guide, No. 8155 of 12 December 2000), setting the tolerance threshold at M = 103 CFU / g or ml for total flora; at 10 CFU / g or ml for faecal coliforms; 2 CFU / g or ml for sulphite-reducing bacilli and the absence in 25 g of product analyzed for salmonella.

- **Preparation of the mother suspension:** 25 g of each sample were removed and aseptically ground. 225 ml of buffered peptone water were added to the mash and the mixture was homogenized with stomacher. From this suspension, decimal dilutions were carried out.
- **Total flora count:** It was carried out by seeding in the mass. One (1) ml of the stock suspension and its decimal dilutions in duplicate were seeded in the Plate Count Agar (PCA) Supercooling Agar. The incubation was carried out at 30 ° C. for 72 h, then the count and the average of the colonyforming unit (CFU) / g of sample analyzed were made according to the method specified by the NF V08-051 standard.
- **Yeast and Mold Count:** 0.1 ml aliquots of the stock suspension and its decimal dilutions were surface seeded on PDA agar thoroughly prepared and cast in 9 cm diameter dish. The enumeration of white or colored colonies, smooth and creamy yeast and powdery mold was performed after 5 days of incubation at 25 ° C according to ISO 7954.
- **Search for total and faecal coliforms:** Total coliforms are searched according to the MPN method described by standard NF ISO 4831. The search for thermotolerant coliforms is carried out by counting colonies obtained at 44 ° C according to the method specified by standard NF V 08 -060.
- **Staphylococci Investigation:** The surface spread technique of 0.1ml inoculum (sample and decimal dilutions) on Baird Parker medium was used. Incubation of the inoculated media was done at 37 ° C / 48H. The method used is that described by standard NF EN ISO 6888-1.

Statistical analyze:In order to evaluate the efficiency of essential oils in the real conservation of smoked and smoked dried fish by diffusion, addition and according to the type of fish, several types of models are explored. These are essentially the Generalized Linear Models (GLM) of the Poisson family and its extensions. The methodology used consisted of running several models and choosing the best one. The best model was selected using the Akaike Information Criterion' (AIC); the best model being the one with the lowest AIC value.

Conducting conservation tests

In order to evaluate in vitro the efficacy of essential oils of *P. racemosa*, and *S. aromaticum*, in the post-smoking conservation of smoked fish (Tilapia, Catfish), conservation tests were carried out. Thus, two types of catfish were used. These are smoked catfish and dried smoked catfish. The incorporation of the essential oil was done at the end of the technological treatment by the method of addition. Taking into account the results obtained during the evaluation of the antifungal properties of the essential oils, the essential oils of *Pimentaracemosa* and *Syzygiumaromaticum* were used. Different doses of essential oil were tested, these were 0.25µl/g, 0.30µl/g and 0.35µl/g of fish. Periodic inspections followed by sampling and analysis made it possible to monitor the quality of smoked catfish and dried smoked catfish preserved with essential oil.

Figure 4: Experimental set-up for smoked fish preservation tests (Addition and Diffusion)

III. Results and discussion

Results

In Table I and Figures 5, 6, 7, 8 and 9 the results of the effect of the type of fish, the method of preservation, the shelf life, the essential oil, its dose and the method of application on the different microbial flora of the smoked fish were presented.

The results showed the presence of total mesophilic aerobic flora, Staphylococcus spp, coliform, yeast and mould in all samples. It is also noted that the number of germs on smoked fish was significantly (prob< 0.05) dependent on the fish species, method of preservation, storage time, method of oil application and dose (Table I). Indeed, the results also revealed that apart from yeast, other germs were more abundant on the smoked catfish species than on the smoked Tilapia species. The smoked fish kept in the bags showed a higher microbial contamination than the smoked fish kept in the basket. This microbial contamination increased with storage time and was more rapid in fish kept in bags than in fish kept in baskets. The high rate of germ growth in the bagged fish and the observed putrefaction could be due to the accumulation of heat in the bags. Finally, the results showed that smoked fish treated with added oil had fewer germs than smoked fish treated by diffusion. It should be noted that the higher the dose of oil, the less the presence of germs was found on the smoked fish. The physical manifestation of the presence of mould as well as signs of fish degradation (odour) was observed from the seventh day of storage, unlike the control where these signs were already observed on the third day in some samples.

IV. Discussion

The results thus obtained corroborate with those obtained by Faton*and al.* (2014), who also had an increase in shelf life of two days in smoked horse mackerel fish (*Trachurustrachurus*) preserved with essential oils of *Pimentaracemosa* in Benin. These results highlighted the proven antifungal potential of this essential oil (Alitonou*and al.*, 2012; Yèhouénou, 2012) and its efficacy in preserving the fungal quality of smoked fish. However, the growth resumptions observed in the total mesophilic aerobic flora could be linked to the drop in the residual effect of the essential oil. The results thus obtained are different from those of Özlem*and al.* (2012) who demonstrated the effectiveness of rosemary, thyme and clove essential oils in increasing the shelf life and quality of rainbow trout (*Oncorhynchus mykiss*) fillets preserved hot under vacuum for 112 days. This difference in the shelf life of the fish is believed to be due to the use of vacuum in the preservation process. A vacuum is a space where molecules contained in the atmosphere are evacuated, thereby lowering the pressure. Thus vacuum allows powders to be dried without heating them. Vacuum can also be used to distil a fragile

substance at low temperature in order to avoid chemical degradation of the molecule. By combining vacuum and heating, it is possible to accelerate the drying of complex circuits such as refrigeration circuits or certain circuits from which water must be banished (Châles, 2012).

Table I: Effect of type of fish, method of storage, shelf life, essential oil and its dose and method of application on the different microbial flora of smoked fish

Factors	Df	Chisq	Pr(>Chisq)
Coliform			
Fishs	1	0,009	0,923
Preservationmethod	1	9,205	0,002**
Storage duration	3	88,232	<0,001***
Oil	2	2,831	0,243
Method of application	1	2,984	0,084
Dose	1	3,061	0,08
FMAT			
Fishs	1	5,377	0,02*
Preservationmethod	1	15,039	< 0,001***
Storage duration	3	485,788	< 0,001***
Oil	2	0,502	0,778
Method of application	1	27,35	< 0,001***
Dose	1	3,2	0,074
Yeast			
Fishs	1	10,67	0,001**
Preservationmethod	1	7,01	0,008**
Storage duration	3	236,223	< 0,001***
Oil	2	7,198	0,027*
Method of application	1	180,371	< 0,001***
Dose	1	0,862	0,353
Mould			
Fishs	1	0,353	0,552
Preservationmethod	1	0,023	0,881
Storage duration	3	99,751	< 0,001***
Oil	2	7,067	0,029
Method of application	1	27,143	< 0,001***
Dose	1	13,336	< 0,001***
Staphylococcus Spp			
Fishs	1	9.349	0.002**
Preservationmethod	1	7.001	0.008**
Storage duration	3	139.415	<0,001***
Oil	2	0.042	0.979
Method of application	1	12.290	<0,001***
Dose	1	4.383	0.036*

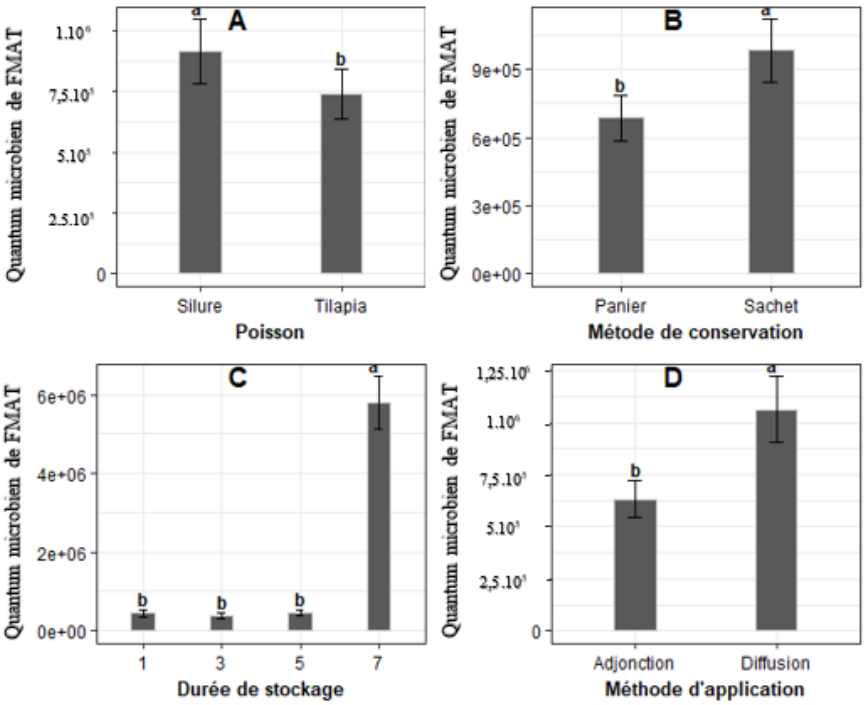

Figure 5: Microbial Quantum of Total Aerobic Mesophilic Flora on Smoked Fish by Fish Species, Conversation Method, Storage Time and Method of Application

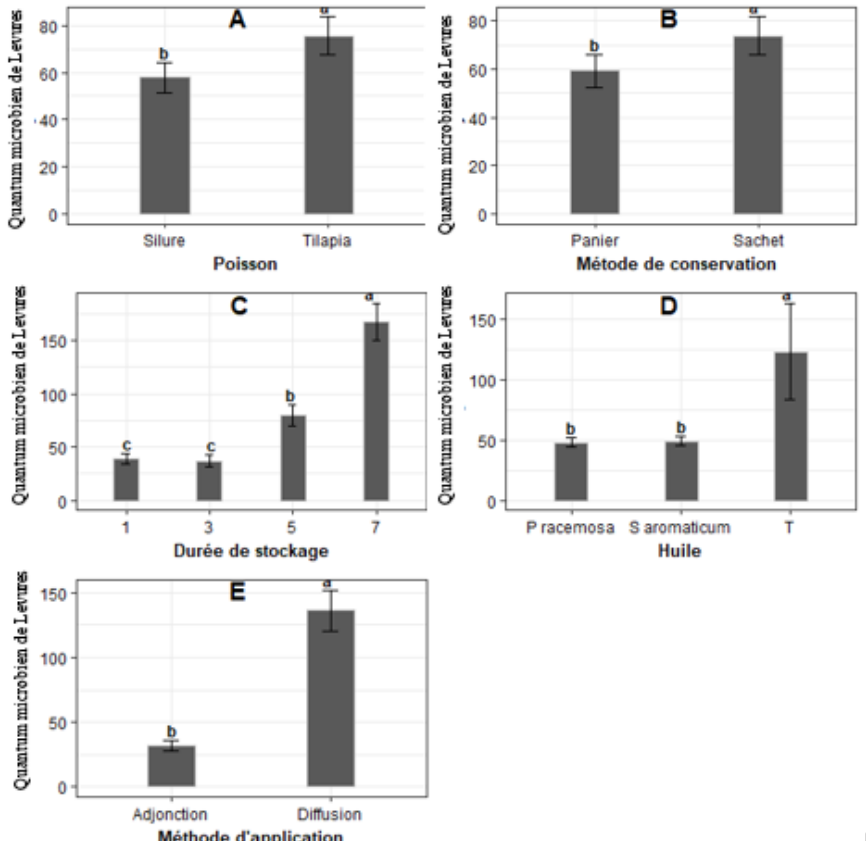

Figure 6: Yeast microbial quantum on smoked fish by fish species, conversation method, storage time, oil and application method

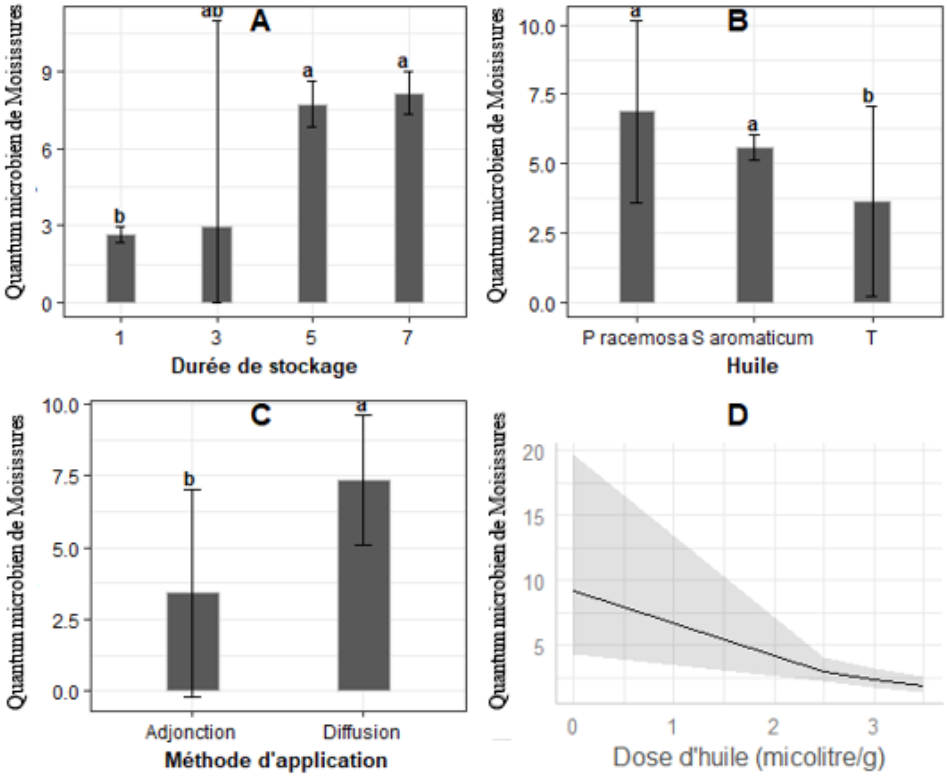

Figure 7: Microbial Mould Microbial Quantum on Smoked Fish by Shelf Life, Oil, Application Method and Dose

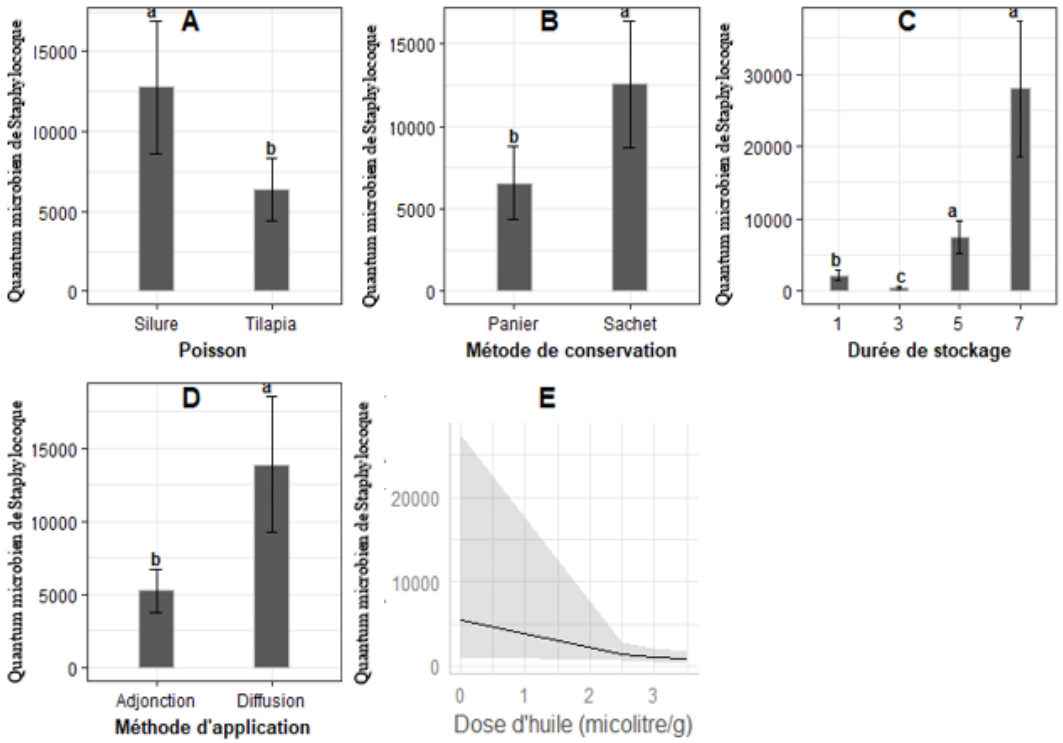

Figure 8: Microbial quantum of Staphylococcus on smoked fish according to fish species, conversation method, storage time, application method and dose.

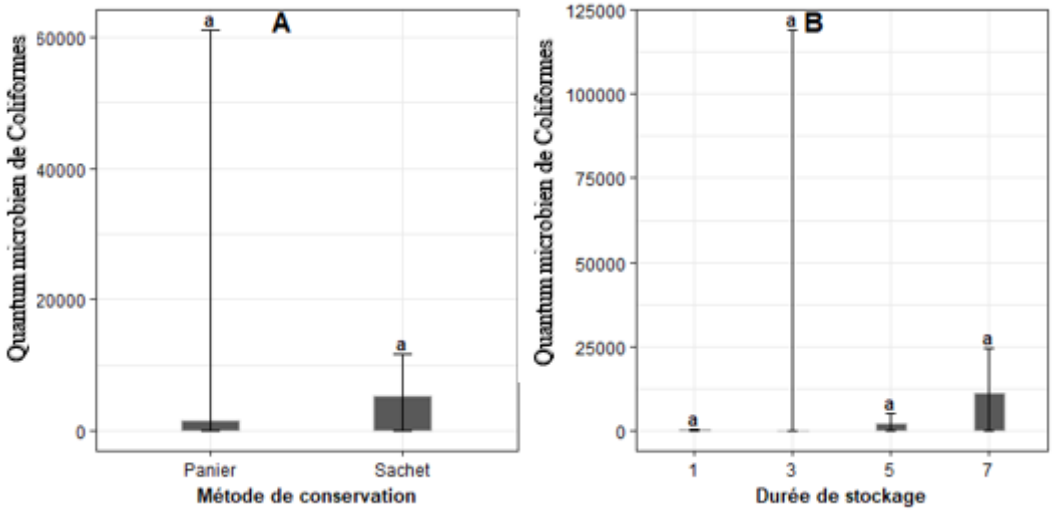

Figure 9: Microbial coliform microbial quantum on smoked fish by fish species and shelf life

V. Conclusion

The present work to evaluate the efficacy of two essential oils in the conservation of post-harvest smoked fish was carried out on two species of fish. At the end of this work we can conclude that the essential oils used exerted an antibacterial activity on Total Mesophilic Aerobic Flora, Coliforms and Staphylococcus spp in fish. They also showed an antifungal activity on strains of yeast and mould. The two oils tested had similar activity, however, with fish kept in sachets having degraded much faster. The essential oils preserved the quality of the smoked fish for 5 days.

Bibliographical reference:

[1]. AdjouE.S.,KoutonS.,Dahouenon-AhoussiE.,SohounhloueD.C.K.&SoumanouM.M.,2012.Antifungalactivity of*Ocimumcanum*EssentialoilagainstToxinogenicFungiisolatedfromPeanutSeedsinpostharvestinBenin,*Int.Res.J.Biol.Sci.*,1(7): 20-26.

[2]. Agossou, D. S. M., Tossou, C. R., Vissoh, V. P., et Agbossou, K. E., 2012. Perception des perturbations climatiques, savoirs locaux et stratégies d'adaptation des producteurs agricoles béninois. *AfricanCrop Science Journal*, *20*(2), 565-588.

[3]. Alitonou G.A., Noudogbessi J-P., Sessou P., Tonouhewa A., Avlessi F., Menut C., Sohounhloué D.C.K., 2012. Chemical composition and biological activities of essential oils of *Pimentaracemosa* (Mill.) J. W. Moore. from Benin. *International Journal of Biosciences*, 2(9): 1-12.

[4]. Amusan, E., Oramadike, C.E., Abraham-Olukayode, A.O., &Adejonwo, O.A. (2010).

[5]. Anihouvi, V.B., Hounhouigan, J.D., &Ayernor, G.S. 2005. La production et la commercialisation du lanhouin, un condiment à base de poisson fermenté du golf du Bénin, Cahiers agricultures 14(3): 323–330.

[6]. ANSES, 2010. Consommation des poissons, mollusques et crustacés : Aspects nutritionnels et sanitaires pour l'Homme. p. 190.

[7]. Châles Jaques, 2012. A quoi sert le vide, fédération Francaise de société scientifiques, p1.

[8]. FAO, 2000. United Nations Food and Agriculture Organization. FAO yearbook. *Fishery statistics capture production*, 86 (1), 99-100.

[9]. Faton, A.N.N. 2014. Etude comparée de la conservation du poisson chinchard (tachirus, trachirus) fumé à l'aide des huiles essentielles ocimumgratissimum, pimenta racemosa et syzygiumaromaticum au Bénin. Mémoire de fin de formation pour l'obtention du Diplôme d'Ingénieur de Conception en Technologie Alimentaire, p 50-57.

[10]. Kpatinvoh Brice, AdjouEuloge S., Dahouenon-Ahoussi Edwige, Konfo T. R. Christian, Atrevy Brice C., Soumanou M. Mohamed, Sohounhloue Dominique 2017. Efficacité des huiles essentielles de trois plantes aromatiques contre la mycoflores d'altération du Niébé (ViguaUnguiculataWalp) collecté dans les magasins de vente de Sud-Bénin. Journal of Appled. Biosciences, (2017) 109 : 10680-10687.

[11]. Özlem Emir Çoban&BahriPatir&Ökkeş Yilmaz, 2012. Protective effect of essential oils on the shelf life of smoked and vacuum packed rainbow trout (Oncorhynchus mykiss W.1792) fillets. J Food SciTechnol DOI 10.1007/s13197-012-0795-8.

[12]. Yacouba I, 2009. Analyse des techniques traditionnelles de transformation de la viande en poutasou). *International Journal Food Safety*, 12:122-126

[13]. Yehouenou Boniface, Sessou Philippe, Houinsou Rose de Lima, Noudogbessi Jean Pierre, Alitonou Guy Alain, ToukourouFatiou, Sohounhloue Dominique (2012). Chemical composition and Antimicrobial activities of C*innamomumzeylanicum* Blume dry Leaves essential oil against Food-borne Pathogens and Adulterated Microorganisms. *International Research Journal of Biological Sciences* :Vol.1(6),18-25.

Brice C. Atrevy, et. al. " Efficacy of Pimentaracemosa and Syzygiumaromaticum essential oils in the post-smoking conservation of fish in South Benin." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 14(8), (2020): pp 43-49.