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Study of the antibacterial and antioxidant properties of essential oils of plants *Cymbopognon citratus* (Poaceae), *Ocimum gratissimum* (Lamiaceae) and *Zingiber officinale* (Zingiberaceae) consumed in Gabon

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Abstract

Most food products are perishable in nature and require protection against lipid oxidation and antimicrobial spoilage during preparation, storage and distribution. Our work therefore consists in studying these two activities from three plants, *C. citratus, O. gratissimum and Z. officinale*, widely consumed in Gabon, with the aim of improving the qualities of food preservation, in this case fermented milk: the Milk of Canaan. The plant material for the study consists of the leaves of *C. citratus* (Poaceae) and *O. gratissimum* (Lamiaceae), and the rhizomes of *Z. officinale* (Zingiberaceae). Hydrodistillation is a simple extraction method that was used following the model adapted from Clevenger. The culture and identification of Canaan Milk bacteria strains were performed on culture media, such as agar, EMB, Soja Tryptique and Muller Hilton. Antibacterial tests were carried out using the disc method. The antioxidant activity was evaluated by measuring the reduction possibility of antioxidants in the presence of DPPH (2,2'diphenyl-1-picrylhydrazyl). The extraction yield and color of the EOs of *C. citratus* is light yellow in color and obtained the best yield, i.e. 0.67% (± 0.07), followed by the EO of *gratissimum*, colorless, i.e. 0.65% (± 0.05). The lowest yield is that of *Z. officinale*, light yellow in color, or 0.12% (± 0.007). We see a purple stain under the microscope, so we have Gram + bacteria. The test on the "api staph" gallery reminds us of an enterobacterium, in particular Micrococcus spp, among lactic acid bacteria. The results show that HE *O. gratissimum* has a larger inhibition diameter than *C. citratus* and *Z. officinale*. The diameters are respectively 35; 25 and 20mm. In view of these results, we can be allowed to think that these three essential oils are good natural preservatives of food and more specifically of dairy products such as yogurt / Canaan milk. In particular *C. citratus* and *O. gratissimum* which show good results of the antibacterial test.

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Introduction

Most food products are perishable in nature and require protection against lipid oxidation and antimicrobial spoilage during preparation, storage and distribution in order to extend their shelf life or use by date (BBD). This deterioration or alteration often due to exogenous pathogenic microorganisms has become a priority concern for public health (Sandri *et al*., 2007). In this context, the identification of appropriate preservation measures and techniques for the control of microbial populations and the quality of food products is essential and can be seen as a major challenge for the food industry.

Likewise, in response to new consumer trends demanding high quality products, free of synthetic preservatives and moderately processed, there is a great interest in developing more innovative and natural preservation approaches (Rajkovic *et al*., 2010; Hyldgaard *et al*., 2012).

Many alternative technologies and methods have been developed or are being researched to replace the synthetic preservatives and intense heat treatments most commonly used but which can cause changes in the organoleptic and nutritional properties of foods (Pfulg and Gould, 2000).

Oxidation of foods in particular lipid peroxidation is a complex process that reflects the interaction between molecular oxygen and polyunsaturated fatty acids (Yadegarinia *et al*., 2006). It is a mechanism that causes food spoilage but also serious health problems through free radicals. Synthetic antioxidants are widely used but can exhibit toxic side effects (Cornwell *et al*., 1998). Hence the growing interest in the use of natural molecules with similar properties. Among the natural molecules used, there are essential oils (EO). These are volatile aromatic compounds contained in the different parts of the plant.

Since ancient times, men have used essential oils for their cosmetic, nutritional and therapeutic needs (Ruberto, 2000). Today, many volatile compounds are common ingredients in pharmaceutical preparations. Our work therefore consists in studying these two activities from three plants, *C. citratus*, *O. gratissimum* and *Z. officinale*, widely consumed in Gabon, with the aim of improving the qualities of food preservation, in this case fermented milk: the Milk of Canaan.

Material and methods

Study framework

We carried out our work in three different laboratories, the Life and Earth Sciences Laboratory (LaSciViT) of the Ecole Normale Supérieure de Libreville/Gabon for the extraction of essential oils; the microbiology laboratory of the Institute for Research in Tropical Ecology (IRET) for antimicrobial testing and finally the multidisciplinary science laboratory (Le LAPLUS) of the Ecole Normale Supérieure for antioxidant testing.

Plant material

The plant material for the study consists of the leaves of *C. citratus* (Poaceae) and *O. gratissimum* (Lamiaceae), and the rhizomes of *Z. officinale* (Zingiberaceae). These plants are used in traditional medicine all over the world and more particularly in Gabon. Leaves of *C. citratus* were collected in the morning from a resident of the Sotéga district, in the second district of Libreville, while the leaves of *O. gratissimum* were collected in the morning from a resident of the Nzeng-ayong district, in the sixth district of Libreville. They were then cleaned and weighed before extracting the essential oils. The rhizomes of *Z. officinale* were bought at the Libreville "come and see" market, located in the third district of Libreville. It is important to specify that this product is mainly imported from Cameroon.

Biological material

Rationale for choosing Canaan milk (LDC Society)

The preservation of foods, in particular yogurt, using essential oils from plants is already well

known to the scientific community. However, in Gabon the field is not very experienced and moreover the common local plants available on the various markets are not yet exploited. Thus, the rather practical and economical choice of these products, available on the market.

Description of yogurt

The properties of our essential oils will be tested on yogurt. Yogurt is a fairly common product on the market, it is also a good testing ground for preservation tests. Indeed, the activity of lactic acid bacteria is essential for the production of yogurt, but this activity must be controlled and mastered. Since essential oils generally have antimicrobial and antioxidant properties, our three (essential) oils will be tested on the yogurt that we have chosen to identify these two properties and to see a possible preservation property of our yogurt.

Le yaourt utilisé est ''Le Lait de Canaan'', produit accessible sur le marché. Le contrôle de la qualité et même de la durée de conservation sont des domaines qui nécessitent encore qu'on se penche là-dessus. En effet, pour garantir un meilleur rendement ou profit, la donnée de conservation est plus qu'indispensable à maitriser.

Extraction of essential oils

Hydrodistillation is a simple and easy to perform extraction method. The oils are extracted through heat and steam entrainment. The heat bursts the secretory cells and the cells release the oil they contain which is carried away by the water vapor. Separation is done by density, because essential oils have a lower density than water.

The leaves of *C. citratus*, those of *O. gratissimum* and the rhizomes of *Z. officinale* from each plant, fresh extracts, were used for the extraction of essential oils by hydrodistillation in a still with a volume of water of 4 liters for 2 hours maximum and depending on the plant material, to using a suitable Clevenger device.

Although the extraction of essential oils from *O. gratissimum* and *C. citratus* is fairly easy, that of *Z. officinale* is more difficult. Indeed, the duration of the extraction is longer 3 hours and it is necessary to add cyclohexane to the hydrolyzate to allow the separation of the aqueous phase and the organic phase.

The plant material is poured into the still after being weighed, then set on fire with distilled water. The oil is entrained by water vapor and condensed in a suitable refrigerant. A water-oil mixture is obtained which must be decanted after having left to stand for at least 15 minutes.

The essential oils were then stored in sterile airtight containers, and then kept in a 4 ° C refrigerator, protected from light until use.

Antimicrobial properties

The antimicrobial properties test on Canaan Milk was carried out at the IRET microbiology laboratory. The yogurt was first observed fresh. A small amount of yogurt is removed using a handle, and then observed on slide and coverslip under a microscope.

Culture and identification of bacterial strains in Canaan Milk

Isolation of the bacterial strain

The culture media (or agar) EMB, Soja Tryptique and Muller Hilton are prepared beforehand. A small amount of fresh milk is removed using a loop and inoculated onto the specific EMB medium to isolate and identify Escherichia coli and Enterobacter, as well as Gram-negative intestinal bacteria in pharmaceuticals, dairy products and other food products. Culture is carried out for 24 hours in an incubator at 37°C.

24 hours later, bacterial colonies are observed. The bacterial colonies are then picked and cultivated on Tryptone-soya agar, which is used as a base medium intended to be supplemented with blood, is prepared with selected raw

materials which do not brown it. It is specially designed to highlight hemolytic reactions and to promote the growth of particularly demanding aerobic and anaerobic germs. Culture is carried out for 24 hours in an incubator at 37° C.

The colonies are taken from a slide using a loop, for observation under an electron microscope. The result after observation of Canaan's milk shows us bacteria of the shell type, mobile (Fig. 1).

Identification of the strain

Next, the Gram stain test is performed. Bacteria are fixed with a flame. A few drops of gentian violet solution (crystal violet) are placed on the fixed smear and left to stand for one minute. Rinse very briefly by running water on the slide above the smear (not directly on the smear). Place a few drops of lugol on the smear. Lugol (iodine compound) is a mordant which fixes violet in bacteria. Leave to stand for a minute. Decolorize by running alcohol on the slide over the smear. Rinse very briefly by running water on the slide above the smear (not directly on the smear). Apply a few drops of fugine solution for a minute and rinse with water. Dry with a paper towel. It is observed under a microscope (100x magnification, with a drop of oil immersion). We then perform the test on an api staph gallery to learn more about our bacterial colony.

Antimicrobial tests

Antibacterial tests were carried out using the disc method. Indeed, the FDA (Food and Drug Administration) has approved this method as a standard for the national clinical laboratory committee (Tajkarimi, Ibrahim, & Cliver, 2010). This technique, reliable and reproducible, is often the most used for the assessment of antibacterial activity (Fig. 12). It is similar to the antibiogram that tests antibiotics. It constitutes a preliminary step to more in-depth studies because it gives qualitative results. It consists of placing a sterile disc, soaked in essential oil, on the surface of the agar seeded with bacteria at the very start of its growth, and then the agars are incubated under the optimum temperature conditions of the microorganism studied for 24 to 48 hours.

Obtaining a clear halo around the disc shows the area where the bacteria could not grow. The diameter of the zone of inhibition, which depends on the sensitivity to HE, is measured inmm reflecting the antibacterial activity of the essential oil (Benkeblia, 2004)

The recommended culture medium for antibacterial testing is Mueller-Hinton (MH) medium. A disc of Whatman paper 6mm in diameter is placed in the middle of each box. The results will be presented in the next part, results and discussion.

Antioxidant properties

The antioxidant activity was evaluated by measuring the reduction possibility of antioxidants in the presence of DPPH (2,2'diphenyl-1-picrylhydrazyl). In this test, the antioxidants reduce the DPPH having a purple color to a yellow compound, the color intensity of which measured at 517 nm, using a spectrophotometer, is inversely proportional to the capacity of the antioxidants present in the medium to donate protons (Bouchet *et al*., 1998; Sanchez-Moreno, 2002).

Measuring antioxidant activity could help us understand the cytotoxic potential of the essential oil of our plants. The antioxidant activity of our essential oil was expressed by taking ascorbic acid (vitamin C) as a standard reference solution. The percent inhibition (I%) was calculated using the following formula:

Briefly, 0.05g of DPPH was dissolved in 100mL of pure 70° ethanol. The mixture was stirred vigorously and left in the dark at laboratory temperature for 30 min. A range of volumes of our HE was prepared (200, 250, 500 and 1000µL) in four tubes. 1.5 mL of the DPPH solution and 10

mL of ethanol are added to each tube. The mixture is incubated in the dark for 1 hour. The absorbances are measured at 517nm, on a spectronic 20d + type spectrophotometer against a control (1mL of DPPH + 10mL of ethanol) and the blank (10mL of ethanol) (Sanchez-Moreno, 2002; Kim *et al*., 2003). Ascorbic acid is used as a reference.

The concentration reducing 50% of DPPH (IC50) is obtained from the regression line of the percentage inhibition as a function of the concentration of EO (Tepe *et al*., 2005). The antioxidant activity index was calculated according to the following formula:

With [DPPH] the final concentration of DPPH and IC 50 the concentration inhibiting 50% of DPPH.

Results

HE efficiency

The extractions were carried out for several weeks for our three ET. Several kilograms were used to extract our EOs.

According to the AFNOR standard (1986), the yield of essential oil (RHE) is defined as the ratio between the mass of essential oil obtained after extraction (M') and the mass of the plant material used (M).

The EO of *C. citratus*, light yellow in color, obtained the best yield, 0.67% (± 0.07), followed by the EO of gratissimum, colorless, or 0.65% (± 0.05). The lowest yield is that of *Z. officinale*, which is light yellow in color, i.e. 0.12% (± 0.007) (Table 1).

Identification of bacterial strains

Analysis of fresh and turned yoghurt allowed us to identify three bacterial strains isolated from milk, including lactic acid bacteria (lactobacillus and streptococcus) and micrococcus spp. The presence of micrococcus *spp* in our milk is not a source of danger. Indeed, micrococci are mainly found on the skin of mammals and in this case, we could have a contamination during the preparation of our yogurt or even during manipulations in the laboratory. They are also found on meat, dairy products, soil and water. Generally non-pathogenic, micrococci can behave as opportunistic pathogens in immune compromised people.

We see a purple stain under the microscope, so we have Gram + bacteria. The test on the "api staph" gallery leads us to suspect an enterobacterium, in particular Micrococcus spp, among the lactic acid bacteria (Fig. 1).

Antimicrobial tests

The results are obtained 24 hours after incubation. The diameters of the halos formed, evidence of antimicrobial activity, are measured with a ruler. The results are shown in the following table.

Antioxidant activity

The antioxidant activity of the essential oils of *C. citratus*, *O. gratissimum* and *Z. officinale* was evaluated by the methods of DPPH. It resulted in the determination of the antioxidant activity indices (IAA) through the determination of the concentration which inhibits 50% of our DPPH (Table 2). The results in Table 2 show that the DPPH radical exhibits an intense purple coloration which disappears on contact with a proton donor substance followed by the appearance of the yellow coloration. This change in color highlights the antioxidant power of our oils by their ability to trap free radicals. Also these results show a proportional increase in the percentages of inhibition of the free radical DPPH as a function of the different concentrations of the total oil, which made it possible to obtain logarithmic curves. This indicates that our essential oils have strong anti-oxidant activities.

The lowest index of antioxidant activity is obtained for ascorbic acid (IAA = 0.738). This result shows that ascorbic acid has moderate

antioxidant activity, this is the benchmark. Then comes the HE of *Ocimum gratissimum* with an index of IAA = 1.367. The antioxidant activity is therefore high; but it is the EOs of *Zingiber officinale* and of *Cymbopognon citratus* which exhibit the best antioxidant activities with respectively indices of IAA = 8.358 and IAA = 7.445 which exhibit the best results and therefore very high antioxidant activity. Indeed, the antioxidant activity is considered weak if IAA <0.5, moderate if 0.5 <IAA <1, high if 1 <IAA <2 and very high if IAA> 2 (Scherer & Godoy, 2009), Table 2.

The results show that HE *O. gratissimum* has a larger inhibition diameter than *C.citratus* and *Z.officinale*. The diameters are respectively 35; 25 and 20mm (Fig. 2).

Table 1. Extraction performance and color of essential oils of *Cymbopogon citratus, Ocimum gratissimum and Zingiber officinale.*

	Rendement en pourcentage	Color
HE *C. citratus*	0,67 ± 0,07	Yelow
HE *O. gratissimum*	0,65 ± 0,05	Incolore
HE *Z. officinale*	0,12 ± 0,007	Yelow

Table 2. Results of measurements of the antioxidant activity of essential oils of *C. citratus, O. gratissimum and Z. officinale.*

Species	Material	Equation	R^2	IC_{50}	IAA
Ascorbique Acid		y=5.38x+0.81	R^2=0.99	6.80	0.74
Cymbopognon citratus	Leaves	y = 74.17x + 0.19	R^2 = 0.98	0.67	7.45
Ocimum gratissimum	Leaves	y=13.45x+0.83	R^2 = 0.08	3.66	1.37
Zingiber officinale	Rhizome	y=82.96x+0.37	R^2 = 0.75	0.60	8.36

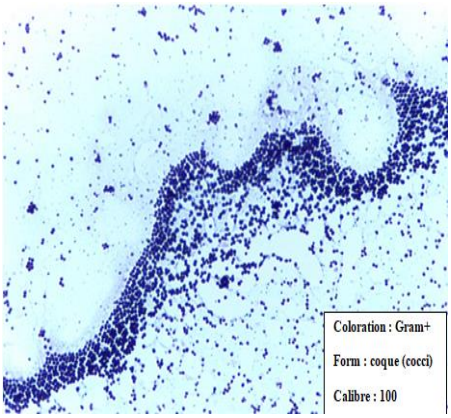

Fig. 1. Gram stain of LDC bacterial strains.

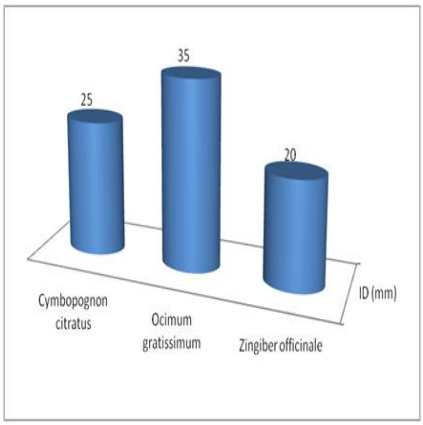

Fig. 2. Representation of the inhibition diameter of essential oils of three species. ID : inhibition diameter (mm)

Discussion

These results partly reflect those of the studies carried out by Réné Dossoukpevi, (2016) Degnon G. *et al*., (2013) which give the EO yields of *C. citratus* and *O. gratissimum* greater than yield of *Z. officinale*, respectively 1.10%; 1.07% and 1.00%. However, overall, the yields of our oils are much lower than the different theoretical yields for the same extraction method. According to the study conducted by Kouamé, (2012), we note that the yield of essential oil differs with the period and the harvest area. This is because the plant material can first be dried before proceeding with the actual extraction. This has the effect of increasing the yield.

Two hypotheses seem to explain these results. The first hypothesis is the harvest period and the geographical location of the different extractions. Indeed, the hydrodistillation of our HEs took place between the months of October to January 2018, during the rainy season. Theoretically, we know that the concentration of EOs is greater in the dry season and in regions of low rainfall. The second assumption is that of the quality of the distillation device.

In fact, we had to deal with leaks in the equipment and this would have resulted in a loss of water vapor and EOs.

Differences in essential oil yield from one species to another have been reported. According to several authors, the origin of the species' harvest, the harvest period, the plant organ, the drying time and the extraction method are among other factors that can also have a direct impact. on oil yields (Russo *et al*., 1998; Tonzibo, 1998; Vekiari *et al*., 2002, Karousou *et al*., 2005; Kouamé, 2012).

Antibacterial tests have given us very good results. With strong activities deduced from the inhibition diameters. The bibliography gives us better information on this observation. According to the classification of Duraffourd *et al*. (1990), essential oil is considered inactive if it produces inhibition diameters less than or equal to 8mm, intermediate for diameters between 8 and 14mm. It is moderately effective for a diameter between 14 and 20mm. For a diameter greater than or equal to 20mm, the oil is very effective.

The EO of *Z. officinale*, *C. citratus* and of *O. gratissimum* showed strong antibacterial activities with respective diameters of 20, 25 and 35mm for pure EO, on our lactic acid bacteria. It is therefore *O. gratissimum* that has the strongest antibacterial activity, ahead of *C. citratus* and *Z. officinale*.

We can therefore say that our EOs would have an antibacterial activity on lactic acid bacteria, in particular those present in the LDC, by having a bacteriostatic activity and could certainly constitute good natural preservatives of the LDC and lengthen its DLC.

Our results are confirmed by the work of Amari Sihem, (2016) and Stoilova *et al*. (2007) and which show that *Zingiber officinale* has considerable antioxidant activity with an IC50 between 0.64mg/ml and 1.29mg/mL, Localized mainly in the total oil of their rhizomes. This activity is due to the oleoresins which are present in the extract of ginger with an activity of 90%. However, our IC_{50} is lower than theory.

Concerning *O. gratissimum* and *C. citratus*, our results are confirmed by the work of Kporou *et al*., 2017 [24]. The results of his work on the antioxidant activity of the essential oil of *O. gratissimum* showed that this oil is a powerful antioxidant because the antioxidant activity was evaluated at F = 187 ± 1.57mm Trolox / ml or (I = 38 ± 0.74%). This activity is clearly better than that obtained under the same experimental conditions with the ethanolic and methanolic extracts of *Cymbopogon citratus* respectively at F = 178.07 ± 1.57mm Trolox / 1ml (I = 34 ± 0.74%) and F = 173, 93 ± 0.87mm Trolox / ml or (I = 33 ± 0.26%) (Kporou *et al*., 2017). The antioxidant activities of essential oils from the Ocimum genus have been well described in the literature and these activities are very interesting (Bozin *et al*., 2006; Pereira and Maia, 2007).

Conclusions

In the course of our work, we have extracted essential oils, demonstrated the antibacterial activity and antioxidant activity of the essential oils of *C. citratus*, *O. gratissimum* and *Z. officinale*. The EOs of *C. citratus* and *O. gratissimum* exhibited the best extraction yields. *Gratissimum* EO exhibited the greatest diameter of inhibition of bacterial growth and therefore the greatest antibacterial activity while *Z. officinale* exhibited the least antibacterial activity. The best antioxidant activity is to be attributed to the EO of *Z. officinale* followed by *C. citratus*.

In view of these results, we can be allowed to think that these three essential oils are good natural preservatives of food and more specifically of dairy products such as yogurt / Canaan milk. In particular *C. citratus* and *O. gratissimum* which show good results of the antibacterial test. However, *Z. officinale* is also not left out. By determining the minimum inhibition concentration (MIC) and the minimum

bactericidal concentration (CMB), we can compare the oils with the standard chemical preservatives used for the manufacture of reference milk.

References

Amari Sihem. 2016. Étude phytochimique et évaluation de l'activité antibactérienne et antioxydante de deux extraits de la plante Zingiber officinale. Mémoire Master En Sciences Biologiques p. 40-43.

Benkeblia N. 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Food Science and Technology **37(2),** 263-268.

Bouchet N, Barrier L, Fauconneau B. 1998. Radical scavenging activity and antioxydant property of tannins from *Guiera senegalensis* (Combretaceae), Phytoth. Res **12,** 159-162.

Bozin B, Mimica Dukic N, Samojlik I, Jovin E. 2007. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus offinalis* L. and *Salvia officinalis* L. Lamiaceae) essential oil. J. Agric. Food Chem **55,** 78-79.

Cornwell DG, Jones KH, Jiang Z, Lantry LE, Southwell KP, and Kohar I. 1998. Cytotoxicity of tocopherols and their quinones in drug-sensitive and multidrug-resistant leukemia cells. Lipids **33,** 295-301.

Duraffourd C, D'Hervicourt L, Lapraz JC. 1990. Cahiers de phytothérapie clinique. 1. Examens de laboratoires galénique. Eléments thérapeutiques synergiques. 2ème éd. Masson, Paris.

Hyldgaard M, Mygind T, Meyer RL. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology **3,** 1-24.

Karousou R, Koureas DN, Kokkini S. 2005. Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of Crete. Photochemistry **66,** 2668-2673.

Kim KS, Lee S. 2003. Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*, Jour. Ethnoph **85,** 69-72.

Kouamé-Bi KFP. 2012. Valorisation de quatre plantes médicinales Ivoiriennes: étude phytochimique. Thèse de doctorat, chimie organique, Université de Nantes et de l'Université de Cocody-Abidjan. 180

Kporou KE, Coulibaly I, Rodica P, Pintea A, Ouattara S, Odagiu A. 2017. HPLC Phenolic Compounds Analysis and Antifungal Activity of extract's from *Cymbopogon citratus* (DC) Stapf against *Fusarium graminearum* and *Fusarium oxysporum* sp tulipae**,** Jour. Sc. Res. Rep **15(1),** 1-11.

Matuci-Péreira CA, Ferreira-Maia J. 2007. Study of the antioxidant activity and essential oil from wild basil *(Ocimum gratissimum* L,) leaf, *Cienc. Tecnol. Aliment* 27, 624.

Pflug I, Gould GW. 2000. The Microbiological Safety and Quality of Food, Heat Treatment. In B. M. Lund A. Baird-Parker and G.M. Gould (Eds.). Maryland: Aspen Publishers Inc pp. 36-64.

Rajkovic A, Smigic N, Devlieghere F. 2010. Contemporary strategies in combating microbial contamination in food chain. International Journal of Food Microbiology **141,** S29-S42.

René D. 2016. Incidence des plantes régénérées in vitro sur les huiles essentielles de deux espèces de Ocimum cultivées au Bénin. Journal of Applied Biosciences Vol. 99.

René G Degnon, Abed-Négo, Euloge Faton, Adjou, Edwige Ahoussi-Dahouenon, Soumanou Mohamed, Dominique KC Sohounhloue. 2013. Efficacité comparée des huiles essentielles de deux plantes aromatiques dans la conservation post-fumage du Chinchard (*Trachurus trachurus*). Journal of Animal and Plant Sciences **19(1),** 2831-2839.

Ruberto G, Baratta MT. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chemistry **69,** 167-174.

Russo M, Galletti GC, Bocchini P, Carnacini A. 1998. Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. hirtum (Link): a preliminary evaluation of their use in chemotaxonomy by cluster analysis. Journal of Agricultural and Food Chemistry **46,** 3741-3746.

Sanchez-Moreno C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Int. Jour. Foods Sc. Tech **8,** 121-137.

Sandri IG, Zacaria J, Fracaro F, Delamare APL, Echeverrigaray S. 2007. Antimicrobial activity of the essential oils of Brazilian species of the genus Cunila against foodborne pathogens and spoiling bacteria. Food Chemistry **103,** 823-828.

Scherer R, Godoy HT. 2009. Antioxidant activity index (AAI) by 2, 2-diphenyl-1-picrylhydrazyl method. Food Chemistry **112,** 654:658.

Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. 2007. Antioxidant activity of a ginger extracts (*Zingiber officinale*). Food Chemistry **102,** p 764 -770.

Tajkarimimm, Ibrahim SA, et Cliver, DO. 2010. Antimicrobial herb and spice compounds in food. Food control **21,** 1199-1218.

Tepe B, Sokmen M, Akpulat H, Sokmen A. 2005. In vitro antioxidant activities of the methanol extracts of five species from Turkey. Food Chemistry **92(1),** 89–92.

Tonzibo ZF. 1998. Contribution à l'étude des huiles essentielles des espèces acclimatées en Côte d'ivoire. *Eucalptus citrodora, Ocimum gratissimum* et Ocimum basilicum. Thèse de3eme cycle, chimie organique, Université de Cocody-Abidjan, Côte d'Ivoire 136 p.

Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M. 2002. Composition and seasonal variation of the essential oil from leaves and peel of a lemon variety. Journal of Agrcultural and Food Chemistry **5(1),** 147-153.

Yadegarinia D, Gachkar L, Bagher Rezaei M, Taghizadeh M, Astaneh SA, Rasooli I. 2006. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochemistry **67,** 1249-1255.