International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 9, p. 20-28, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Chemical composition and *in vitro* antifungal activity of *Zingiber officinale* essential oil against foodborne pathogens isolated from a traditional cheese wagashi produced in Benin

Sessou Philippe[1,2*], Farougou Souaïbou[1], Noudogbessi Jean-Pierre[2], Fanou Brice[1], Azokpota Paulin[3], Youssao Issaka[1], Sohounhloué Dominique[2]

[1]*Laboratoire de Recherche en Biologie Appliquée, Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, 01 BP 2009 Cotonou, Bénin*

[2]*Laboratoire d'Etude et de Recherche en Chimie Appliquée, Ecole Polytechnique d'Abomey-Calavi, 01 BP 2009 Cotonou, Bénin*

[3]*Laboratoire de Microbiologie et de Biotechnologie Alimentaire, Faculté des Sciences Agronomiques, 01 BP 526 Cotonou, Bénin*

Received: 02 August 2012
Revised: 15 August 2012
Accepted: 16 August 2012

Key words: *Zingiber officinale*, volatile extract, antifungal activity, wagashi.

Abstract

The aim of the present work was to assess some antifungal activity parameters as mycelial growth inhibition, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of *Zingiber officinale* essential oil from Benin against *Aspergillus* (*flavus* and *tamarii*), *Fusarium* (*poae* and *verticillioides*) and *Penicillium* (*citrinum* and *griseofulvum*) species isolated from traditional cheese wagashi. The chemical composition of the essential oil extracted by hydrodistillation with Clevenger apparatus from fresh rhizomes of ginger was studied by GC-FID and GC-MS and showed α-zingiberene (40.7%) as major component with minor compounds in significant percents such as geranial (8.9%), elemol (5.9%), neral (4.5%), camphor (4.3%), limonene (3.7%) and (E, E)-α-farnesene (3.6%). The evaluation of antifungal activity of this oil has shown a less activity against all the species tested with mycelia growth inhibition not exceeding 79.66% excepted *Penicillium griseofulvum* on which the oil had fungistatic activity with MIC equal to 1000 mg/L. Results obtained in the present study reveal that *Zingiber officinale* essential oil is not a good antifungal agent against moulds species responsible for wagashi deterioration. Its activity may be improved by its combination with other natural active products.

*Corresponding Author: Sessou Philippe ✉ sessouphilippe@yahoo.com

Introduction

The microbiological quality of a food constitutes is one of the essential bases of its aptitude to satisfy the safety of the consumer. A food, exposed to deterioration by the fungus can have a decreasing in its sensory, nutritive and medical characteristics. Fungi are also responsible for the formation of taste and the production of made up and allergenic mycotoxins (Barkat and Bouguerra, 2012). In Benin, traditional cheese wagashi obtained without ripening and often coloured with red sorghum, is an important source of animal proteins especially for rural populations. Wagashi is widely consumed not only by rural poor people but also in the main cities of Benin. Due to its proteins content, wagashi could efficaciously contribute to the resolution of nutritional problems due to the deficiency of proteins of these populations (Kèkè et al., 2008; Sessou et al., 2012). However, wagashi is produced and preserved using rudimentary methods under unsanitary conditions which may lead to the contamination of the product by toxinogenic or pathogenic microorganisms especially fungi (Aissi et al., 2009). The contamination of this product by fungi may contribute to the loss of its quality and safety. In fact, the fungal growth may result in several kinds of cheese spoilage: off-flavours, toxins, mycolytic enzymes and rotting (Filtenborg et al., 1996). Furthermore, fungi produce allergenic compounds and toxic metabolites which may penetrate the cheese and affect the consumer's health (Nasser, 2001; Nguefack et al., 2004). A better control measures to prevent spoilage of wagashi is necessary to avoid its contamination by mycoflora and minimize public health hazards. The use of synthetic fungicides to control cheese foodborne pathogens has been discouraged due to their effects on cheese, carcinogenicity, teratogenicity, high and acute residual toxicity, long-term degradation (Angelini et al., 2006). Alternative natural additives are therefore needed in order, to guarantee food safety in preserved wagashi. Aromatic plants are traditionally employed for seasoning and prolongation of shelf life of food. The majority of their properties are due to the essential oils produced by their secondary metabolism (Wang and Huang, 2010). Essential oils (EOs) as antimicrobial agents are recognized as safe natural substances to their user and for the environment and they have been considered at low risk for resistance development by pathogenic microorganisms (Burt, 2004). Among the aromatic plants, *Zingiber officinale* belonging to Zingiberaceae family and widely used as a food spice (Daudu et al., 2012) had shown strong antimicrobial activities against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Candida albicans*, *Trichoderma* spp, *Aspergillus niger, Pencillium* spp and *Saccharomyces cerevisiae* (Sasidharan and Menon, 2010), *Staphylococcus aureus, Escherichia coli* and *Listeria monocytogenes* (Krittika et al., 2007). According to da Silva et al. (2011), essential oil of ginger possessed antifungal activity against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus.* Studies of El-Baroty et al. (2010), Fawzi et al. (2009), Bansod and Rai (2008) showed that oil of *zingiber offinale* had significant inhibitory activity against strains of bacteria (*Bacillus subtilis* ATCC 6633; *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 27840, *Micrococcus luteus* ATCC 4698, *Klebsiella pneumoniae* ATCC 13883 and *Serratia marcescens* ATCC 13880) and pathogenic fungi (*Aspergillus niger*, *Penicillium notatum*, *Mucora heimalis, Alternaria alternaria* and *Fusarim oxysporum, Aspergillus fumigatus*)**.** The efficacy of this oil on cheese mycoflora has been few studied. Its efficacy on moulds isolated from wagashi must be verified in order to measure its potential biopreservation for the valorization of this product. The objective of this research was to assess *in vitro* antifungal activity of essential oils of *Zingiber officinale* against six mycotoxigenic moulds, *Aspergillus flavus*, *Aspergillus tamarii*, *Fusarium poae*, *Fusarium verticillioides*, *Penicillium citrinum* and *Penicillium griseofulvum* both isolated from wagashi produced in Benin for its potential use as wagashi biopreservative.

Material and methods

Plants material and extraction of the essential oils

Fresh rhizomes of *Zingiber officinale* were collected in Malanville in North Republic of Benin at October 2011 and were identified by Dr Yedomonhan of National Herbarium of Benin. The essential oil was extracted from fresh rhizomes (100 g) by hydrodistillation during 3 hours, using a Clevenger apparatus, in LERCA/Polytechnic School of Abomey-Calavi, University of Abomey-Calavi (LERCA/EPAC/UAC). Oil recovered was dried over anhydrous sodium sulfate and stored at +4 °C until it was used (Yehouenou et al., 2010).

Identification of chemical components of Zingiber officinale essential oil

Quantitative and qualitative analyses of the essential oil of *Zingiber officinale* were carried out by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS).

GC/FID analyses were performed using a Varian CP-3380 GC equipped with a HP-5 (100% dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program 50°-200°C at 5°C/min, injector and detector respectively at 220°C and 250°C, carrier gas N_2 at a flow rate of 0.5 mL.min^{-1}. Diluted samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode (1/100). The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes (C_9-C_{20}).

GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP-5 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70° to 200°C at 10°C/min; injector temperature was 220°C. Helium was used as carrier gas at a flow rate of 0.6 mL.min^{-1}, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode (1/100).

The identification of individual compounds was based on the comparison of their relative retention times with those of authentic samples on the HP-5 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/or the NBS75K.L and NIST98.L libraries and published data (Adams, 2007).

Strains of filamentous fungi tested

The fungi used in this study were: *Aspergillus flavus*, *A. tamarii*, *Fusarium poae*, *F. verticillioides*, *Penicillium citrinum* and *P. griseofulvum*. They have been isolated from a traditional cheese wagashi collected near its vendors. Colonies of these moulds isolated from DBRC medium by dilution method (ISO 21527-1: 2008) were purified by streaking onto Malt Extract Agar (MEA) and then three point inoculated onto MEA and Czapeck Yeast autolysate (CYA) agar before identification based both on macroscopic characters (colony growth, colony diameter) and microscopic characters using the identification schema of Samson et al (1995) and Pitt and Hocking (2009).

Antifungal assay

The test was performed by the agar medium assay (Tatsadjieu et al., 2009). Potato Dextrose Agar (PDA) medium with different concentrations of essential oil (200, 400, 600, 800 or 1000 mg.L^{-1}) were prepared by adding appropriate quantity of essential oil to melted medium, followed by addition of Tween 80 (100 µL to 100 mL of medium) to disperse the oil in the medium. About 20 ml of the medium were poured into glass Petri-dishes (9 cm x 1.5 cm). Each Petri-dish was inoculated at the centre

with a mycelial disc (6 mm diameter) taken at the periphery of a fungus colony grown on PDA for 48 h. Positive Control (without essential oil) plates were inoculated following the same procedure. Plates were incubated at 25°C for 8 days and the colony diameter was recorded each day. Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of essential oil in which no growth occurred. The MGI (Mycelia Growth Inhibition) percentage was calculated according to the equation:

MGI = (dc-dt)/dc x 100 where dc = mean diameter for control – 6 mm and dt = mean diameter for treated mycelium – 6 mm.

The Minimal Fungicidal Concentration (MFC) values were determined by the method described by Angelini et al. (2006). This was done by subculturing the inhibited fungal discs at MICs on PDA medium without essential oil. Observations were recorded after 7 days of incubation at 25 °C. Fungal growth on the seventh day was indicative of a fungistatic nature, while the absence of fungal growth denoted a fungicidal action of the oil.

Statistical analysis

Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0 (Statistica, 2010). Differences between means were tested using Z-test. Difference on statistical analysis of data was considered significant at $P < 0.05$.

Results and discussion

Chemical composition of ginger essential oil

The chemical composition of ginger essential oil with yield equal to 0.23 ± 0.7 % is given in the Table 1. Thirty two components which represented 98.8 % of the total oil were identified in the essential oil. Zingiberene (40.7%) was the major compound, followed by minor components in significant percent such as geranial (8.9%), elemol (5.9%), neral (4.5%), camphre (4.3%), limonene (3.7%) and (E, E)-α-farnesene (3.6%). Monoterpene hydrocarbons was 12.8% represented by camphor (4.3%), limonene (3.7%), (E)-β-ocimene (1.3%), α-phellandrene (1.2%), p-cymene (1%), sabinene (1%), β-pinene (0.3%).The content of oxygenated sesquiterpenes and monoterpenes compounds in the ginger oil were respectively 46.5% and 16.4%. The main oxygenated compound was geranial (8.9%) followed by elemol (5.9%) and neral (4.8%) and other compounds. Main sesquiterpene compound was zingiberene and other sesquiterpene hydrocarbons present was (E, E)-α-farnesene (3.6%). Main aromatic component was eugenol (2.2%). Phytol, in group of other component, was present in lesser quantity (0.6%).

Table 1. Chemical composition of *Zingiber officinale* essential oil from Benin.

N°	Noms des composés	RI (HP-5)	RI (Adams)	(%)
1	α-pinene	933	932	t
2	sabinene	973	969	1.0
3	β-pinene	978	974	0.3
4	myrcene	992	988	t
5	α-phellandrene	1005	1002	1.2
6	p-cymene	1024	1020	1
7	limonene	1028	1024	3,7
8	camphor	1145	1141	4.3
9	(E)-β-ocimene	1050	1044	1.3
10	linalool	1097	1095	t
11	terpinen-4-ol	1175	1174	t
12	α-terpineol	1189	1186	3.0
13	citronellol	1227	1223	t
15	neral	1246	1235	4.5
14	geranial	1277	1264	8.9
17	eugenol	1370	1356	2.4
18	α-copaene	1378	1374	1.2
19	geranyl acetate	1383	1379	2.2
20	β-cubebene	1392	1387	0.4
21	ar-curcumene	1485	1479	0.1
22	α-zingiberene	1496	1493	40.7
23	(E, E)-α-farnesene	1509	1505	3.6
24	eugenyl acetate	1526	1521	2.6
25	α-cadinene	1542	1537	0.5
26	elemol	1552	1548	5.9
27	germacrene-D-4-ol	1578	1574	2.7
28	spathulenol	1582	1577	2.1
29	α-muurolol	1651	1646	0.5
30	α-cadinol	1658	1652	2.1
31	(2E, 6E)-α-farnesol	1748	1742	2.0
32	phytol	1949	1942	0.6
	Hydrogenated monoterpenes			12.8

Oxygenated monoterpenes	16.4
Hydrogenated sesquiterpenes	46.5
Oxygenated sesquiterpenes	15.3
Aromatic components	2.4
Esters	4.8
Other components	0.6
Total	98.8

t: trace < 0.05%

Several studies have shown that ginger essential oil is very complex mixtures of compounds and many variations have been found in the chemical composition. The major components found in our essential are quite similar compared with data reported by Sasidharan and Menon (2010); they had found Zingiberene (28.6%) as major compound, followed by geranial (8.5%), ar-curcumene (5.6%) and β-bisabolene(5.8%) in fresh ginger oil and zingiberene (30.3%), followed by arcurcumene (11%), β-bisabolene (7.2%), β-sesquiphellandrene (6.6%) and germacrene-D (4.2%) in dry ginger oil of India. This oil contained major components also similar of those found, zingiberene (38.10%), beta phellandrene (12.00%), β- sesquiphellandrene (9.546%), α-curcumene (9.224%), camphor (5.94%), α-farnesene (4.573%), β-bisabolene (4.39%), citral (3.91%), α-pinene (2.33%), in Bangladesh ginger essential oil by Aziz et al. (2012) but quite different of those found, curcumene (26.54%), camphene (20.60%), citral (17.90%), α-pinene (6.63%), borneol (5.40%), β-isabolene (4.57%) in China ginger oil by the same authors. Wang et al. (2012) found in essential oil of ginger α-zingiberene (52.35%), β-pinene (14.20%) and β-sesquiphellandrene (12.11%) as major components in China. The essential oil composition of dried Nigerian ginger (*Zingiber officinale* Roscoe) was constituted of 64.4% sesquiterpene hydrocarbons, 6.6% carbonyl compounds, 5.6% alcohols, 2.4% monoterpene hydrocarbons and 1.6% esters with main compounds as zingiberene (29.5%) and sesquiphellandrene (18.4%) (Chidozie and Hashimoto, 1990). The chemical composition of our essential oil was quite different of those reported by Hussain Shahnaz et al. (2011) who found β-germacrene-D (25.4%), linalool (11.8%) and camphene (9.4%) in India ginger oil and those found, β-sesquiphellandrene (25.16%), cis-caryophyllene (15.29%), zingiberene (13.97%), α-farnesene (10.52%), α- (7.84%) and β-bisabolene (3.34%) by El-Baroty et al. (2010) in Egypt. Ginger essential oil of Cuba was characterized by the presence of ar-curcumene (22.1%), zingiberene (11.7%), β-bisabolene (11.2%) and cadina-1,4-diene (12.5%) (Pino et al., 2004). Such variations in the chemical composition of distilled oils is known to differ considerably not only due to the existence of different subspecies, but also might be attributed to the varied agroclimatic condition (climatic, seasonal, geographic) of the regions, stage of maturity, adaptive metabolism of plants, distillation conditions and some other factors (El-Baroty et al., 2010).

Biological activities of Zingiber officinale essential oil

Recently, the scientific interest in biological properties of essential oils has been increased. New researches about biological active secondary compounds present in essential oils of plants have been seen as a potential way to control fungal contamination (Burt, 2004). The antifungal activity of the essential oil of ginger against selected moulds isolated from wagashi was assessed. The MGI and fungistatic activity values of the essential oil extracted from fresh rhizomes of ginger against the tested fungi are reported in Table 2. The result showed that the percentages of mycelial growth inhibition are significantly ($p < 0.05$) influenced by incubation time and essential oil concentrations. Mycelia growth was reduced with increasing concentration of essential oil while their growth increased with incubation time (figure 1). Ginger essential oil showed weakness antifungal effect against *Aspergillus flavus, A. tamarii, Fusarium poae, F. verticillioides* and *Penicillium citrinum* according to the concentrations applied and the exposure time.

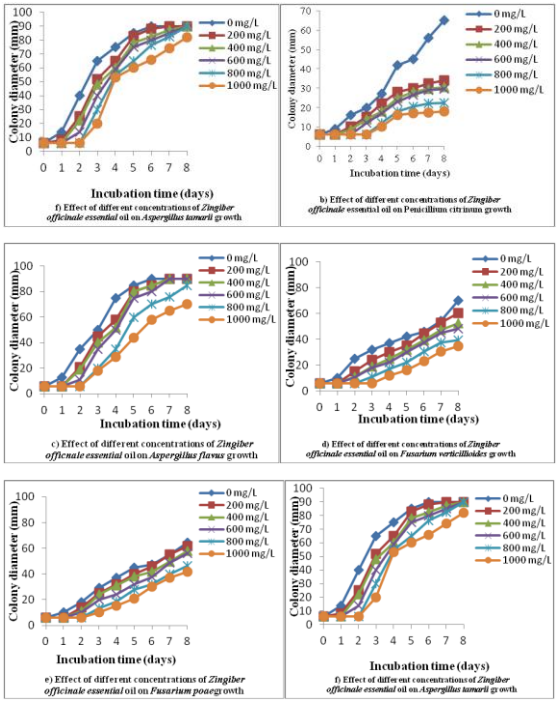

Fig. 1. Effect of different concentrations of essential oil of *Zingiber officinale* against tested moulds.

The mycelial growth inhibition observed in our work for these last five moulds didn't exceed 79.66%. *Aspergillus tamarii* was the most resistant strain with MGI equal to 11.90 ± 0.48 at eighth day of incubation at concentration of 1000 mg/L. Therefore, this oil completely inhibited (MGI = 100%) at 1000 mg/L *Penicillium griseofulvum* which was the most sensible specie. The sensitivity of *P. griseofulvum* to the oil in contrary to the others could be justified by the nature of its external membranous probably constituted of lipids which allows the permeability of cell membrane once at contact of essential oil of ginger.

The antifungal activity of ginger oil observed in our study is contrary to those reported in several studies. Indeed, El-Baroty et al (2010) reported that *Zingiber officinale* rhizomes essential oil possessed high antifungal activity against pathogenic fungi; this oil completely inhibited (MGI=100%) the growth of *Aspergillus niger, Penicillium notatum, Mucora heimalis and Fusarim oxysporum* at 100 µg/mL. This same effect was observed by da Silva et al. (2011) who reported that essential oil of ginger possessed antifungal activity against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. Sasidharan and Menon (2010) reported that *Zingiber officinale* essential oil was good antifungal agent against *Trichoderma* spp, *Aspergillus niger* and *Pencillium* spp. Bansod and Rai (2008) showed that oil of *zingiber offinale* had significant inhibitory activity against pathogenic fungi, *Aspergillus niger*, *Penicillium notatum*, *Mucora heimalis, Alternaria alternaria, Fusarim oxysporum* and *Aspergillus fumigatus***.** Sharma et al. (2011) showed that essential oil of ginger had a good antifungal activity against common causes of *Pityriasis versicolor* infections in humans**.** According to these authors, ginger essential oils rich in sesquiterpenes such as zingiberene possessed a wide spectrum of antimicrobial activity. The quite contrary results obtained in our studies to those of literature could be probably due to high resistance of our strains or to the antagonist effect of zingiberene with others compounds present in this oil.

Table 2. Mycelial growth inhibition, fungistatic and fungicidal activity of essential oil of Zingiber officinale on tested fungi.

EO (mg/L)	Mycelial Growth Inhibition (%)					
	A. flavus	*A. tamarii*	*F. poae*	*F. verticillioides*	*P. citrinum*	*P. griseofulvum*
200	0e	0e	5.12 ± 0.82d	14,84 ± 0.37c	52.54 ± 0.24a	22.54 ± 2.10b
400	0e	0e	11.11 ± 0.45d	27.34± 0.73c	58.47 ± 0.46a	38.23± 0.36b
600	0e	0e	14.52 ± 1.20d	34.37 ± 0.55c	60.16 ± 0.94b	79.41 ±1.74a
800	5.95 ± 2.38e	0f	30.76 ± 0.92d	47.65 ± 1.62c	72.03 ± 1.08b	94.11 ± 0.76a
1000	23.80 ± 0.95e	11.90 ± 0.48f	39.31 ± 0.69d	66.07 ± 14286c	79.66 ± 0.11b	100a (FS)

EO: Essential oil; FS: fungistatic activity; Data in the line followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions ± standard deviation.

Conclusion

The present work on chemical composition and antifungal activity of essential oil extracted from fresh rhizomes of *Zingiber officinale* revealed that α-zingiberene and accessorily geranial, elemol, neral, camphor, (E, E)-α- limonene and farnesene are the main components of this essential which

had weakness effect on the radial growth of *Aspergillus flavus, A. tamarii*, *Fusarium poae, Fusarium verticillioides and Penicillium citrinum* isolated from wagashi produced in Benin. Moreover, this oil exhibited a fungistatic activity only against *Penicillium griseofulvum*. *Zingiber officinale* essential oil could not be used alone as natural biopreservative of wagashi.

Acknowledgements

The authors acknowledge University Council of Development (CUD) for their financial support. They are also grateful to Mr. Ahounou Serge, Agniwo Bienvenu, Boniface Yèhouenou, Chabi-Egba Mispar, François Dossa and Mrs. Kaneho Sandrine for their technical assistance.

References

Adams RP. 2007. Identification of essential oil components by Gas Chromatography/Mass Spectrometry, 4th edition; Allured Publishing Corporation Carol Stream. IL**.**

Aïssi VM, Soumanou MM, Bankolé H, Toukourou F, de Souza CA. 2009. Evaluation of Hygienic and Mycological Quality of Local Cheese Marketed in Benin. Australian Journal of Basic and Applied Sciences **3(3),** 2397-2404

Angelini P, Pagiotti R, Menghini A, Vianello B. 2006. Antimicrobial activities of various essential oils against foodborne pathogenic or spoilage moulds. Annals of Microbiology **56(1),** 65-69.

Aziz S, Hassan MSM, Nandi S, Naher S, Roy KS, Sarkar PR, Hossain H. 2012. Comparative Studies on Physicochemical Properties and GC-MS Analysis of Essential Oil of the Two Varieties of Ginger (*Zingiber officinale*). International Journal of Pharmaceutical and Phytopharmacological Research **1(6),** 367-370.

Bansod S, Rai M. 2008. Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic *Aspergillus fumigatus* and *A. niger*. World Journal of Medical Sciences **3(2),** 81-88.

Barkat M, Bouguerra A. 2012. Study of the antifungal activity of essential oil extracted from seeds of *Foeniculum vulgare* Mill. for its use as food conservative. African Journal of Food Science **6(9),** 239-244.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in food-areview. Int. J.of Food Microbial 94 (2004) 223-253.

Chidozie Onyenekwe P, Hashimoto S. 1990. The composition of the essential oil of dried Nigerian ginger (*Zingiber officinale* Roscoe). European Food Research and Technology **209(6),** 407-410.

da Silva C.F., Chalfoun M S., de Siqueira M. V., Botelho D. M. dos S., Lima N., Batista RL. 2011. Evaluation of antifungal activity of essential oils against potentially mycotoxigenic *Aspergillus flavus* and *Aspergillus parasiticus*. Brazilian Journal of Pharmacognosy 1-9.

Daudu OM, Sekoni AA, Ogundipe SO, Erakpotobor GT, Olugbemi TS. 2012. The effect of supplementation of enzyme on performance and some blood chemistry parameters in broiler finisher chickens fed ginger by-product meal (*Zingiber officinale*). International Journal of Biosciences **2(7),** 59-65.

El-Baroty SG, Abd El Baky HH, Farag SR, Saleh AM. 2012. Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. African Journal of Biochemistry Research **4(6),** 167-174.

Fawzi EM, Khalil AA, Afifi AF 2009. Antifungal effect of some plant extracts on

Alternaria alternata and *Fusarium oxysporum*. African Journal of Biotechnology **8(11),** 2590-259.

Filtenborg O, Frisvad JC, Thrane U. 1996. Moulds in food spoilage. International Journal of Food Microbiology 33, 85-102.

Hussain Shahnaz S, Ali M, Ansari Shahid H. 2011. Composition of the volatile oil of *Zingiber officinale* Roscoe rhizomes and effects of physical factors on the oil. International Research Journal of Pharmacy **2(9),** 173-177.

ISO 21527-1, 2008 (F). 2008. Microbiologie des aliments-Méthode horizontale pour le dénombrement des levures et moisissures, Partie 1 : technique par comptage des colonies dans les produits à activité d'eau supérieure à 0,95, 1ère édition 2008-07-01.

Kèkè M, Yèhouénou B, Dahouénon E, Dossou J, Sohounhloué DCK. 2008. Contribution à l'amélioration de la technologie de fabrication et de conservation du fromage peulh waragashi par injection de *Lactobacillus plantarum*. Annales des Sciences Agronomiques du Benin **10(1),** 73-86.

Krittika N, Natta L, Orapin K. 2007. Antibacterial Effect of Five Zingiberaceae Essential Oils. Molecules **12,** 2047-2060.

Nasser AL. 2001. Fungal contamination of white cheese at the stage of consumption in Saudi Arabia. Pakistan Journal of Biological Sciences **4(6),** 733-735.

Nguefack J, Leth V, Amvam Zollo PH, Mathur SB. 2004. Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. Int. J. Food Microbiol. 94: 329– 334.

Pino AJ, Marbot R, Rosado A, Batista A. 2004. Chemical Composition of the Essential Oil of *Zingiber officinale* Roscoe L. from Cuba. Journal of Essential Oil Research **16(3),** 186-188.

Pitt JI, Hocking AD. 2009. Fungi and Food spoilage, Springer Dordrecht Heidelberg London New York, USA, p. 518.

Samson A, Hoekstra E, Frisvad JC, Filtenborg O. 1995. Introduction to food-borne fungi. Copyright 1995 Centraalbureau voor Schimmelcultures ISBN 90-70351-27-7, p 322.

Sasidharan I, Menon N. 2010. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (*Zingiber officinale roscoe*). International Journal of Current Pharmacy Research **2,** 40-43.

Sessou P, Farougou S, Noudogbessi JP, Yèhouenou Boniface, Azokpota P, Youssao I, Menut C, Sohounhloue D. 2012. Chemical composition and antifungal activity of essential oil of fresh leaves of *Cinnamomum zeylanicum* from Benin against six mycotoxigenic fungi isolated from a traditional cheese wagashi. Elixir Applied Biology **49,** 9787-9791.

Sharma R, Sharma G, Sharma M. 2011. Additive and inhibitory effect of antifungal activity of Curcuma longa (Turmeric) and Zingiber officinale (Ginger) essential oils against Pityriasis versicolor infections. Journal of Medicinal Plants Research **5(32),** 6987-6990.

Statistica. 2010. Statistica version 2010 monoposte 6.0.

Tatsadjieu NL, Dongmo Jazet PM, Ngassoum MB, Etoa F-X, Mbofung CMF. 2009. Investigations on the essential oil of *Lippia rugosa* from Cameroun for its potential use as antifungal agent against *Aspergillus flavus* Link ex Fries. Food Control **20**, 161-166.

Wang HF, Yih KH, Huang KF. 2010. Comparative Study of the Antioxidant Activity of Forty-five Commonly Used Essential Oils and their Potential Active Components. Journal Food and Drug Analysis **18(1),** 24-33.

Yèhouenou B, Noudogbèssi JP, Sessou P, Avlessi F, Sohounhloué D. 2010. Etude chimique et activités antimicrobiennes d'extraits volatils des feuilles et fruits de *Xylopia aethiopica* (DUNAL) A. Rich. contre les pathogènes des denrées alimentaires. Journal de la Société Ouest Africaine de Chimie **29,** 19-27.