

Full Length Research Paper

Biological control of spoilage and pathogens moulds in culture medium and Beninese traditional cheese wagashi by *Syzygium aromaticum* essential oil

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The investigation highlighted the antifungal effectiveness in culture medium and traditional cheese wagashi foodsystem of *Syzygium aromaticum* (*Eugenia caryophyllata*) essential oil (EO) against spoilage and toxinogenic moulds isolated from wagashi produced in Benin. The chemical composition of the EO obtained by hydrodistillation, characterized through GC-FID and GC-MS analysis, revealed eugenol (75.2%) and trans-caryophyllene (12.0%) as major components. The evaluation of *in vitro* antifungal activity of this oil showed a significant fungistatic activity against *Aspergillus* (*flavus*, *tamarii*, *niger*, *aculeatus*, *ustus*, *terreus*), *Penicillium* (*brevicompactum*, *citrinum*, *griseofulvum*), *Fusarium* (*poae*, *verticillioides*) and *Scopulariopsis brevicaulis* with MIC ranged from 200 to 600 mg/L due probably to its richness in eugenol. Moreover, this EO had fungicidal activity against *Aspergillus terreus* and *S. brevicaulis* which were the most sensitive respectively at 600 and 400 mg/L. The assessment of antifungal activity of the oil studied in wagashi foodsystem against the less sensible isolates at *in vitro* assay revealed high sporale reduction rate (55% at least at 1000 mg/L) on all species investigated above all on *Penicillium citrinum* and *Aspergillus aculeatus*, two harmful mycotoxins producers in cheese. Results obtained indicate the possibility of exploiting *S. aromaticum* EO to preserve wagashi against moulds contamination and probably mycotoxins inhibition during wagashi storage.

Key words: *Syzygium aromaticum*, essential oil, antifungal activity, wagashi, Benin.

INTRODUCTION

Cheese is highly nutritious food with many diverse flavor and texture and it can be used as a snack or as a part of

dish or prepackaged conveniences food (Elkhider et al., 2011). Soft unripened traditional cheese locally called

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Abbreviations: EO, Essential oils; GRAS, generally recognized as safe substances; PDA, potato dextrose agar; MIC, minimal inhibitory concentration; MGI, mycelia growth inhibition; MFC, minimal fungicidal concentration.

wagashi is an indigenous cheese produced in Benin. Due to its proteins content, it could efficaciously contribute to the resolution of nutritional problems due to the deficiency of proteins (Kèkè et al., 2008; Sessou et al., 2012a, b). However, this foodstuff is produced and preserved using rudimentary methods under unsanitary conditions which may lead to its contamination by spoilage and pathogenic microorganisms especially fungi (Aissi et al., 2009; Sessou et al., 2012a, b, c, d, e). The fungal growth in this product may result in several kinds of cheese spoilage: off-flavours, toxins, discolouration, mycolytic enzymes, rotting and formation of pathogenic or allergenic propagules. The deterioration of sensorial properties is often due to the production of exoenzymes during growth. Filamentous fungi can produce a vast number of enzymes: lipases, proteases and carbohydrase. Once inside the food, these enzymes may continue their activities independent of the destruction or removal of the mycelium (Filtenborg et al., 1996; Angelini et al., 2006). The production of mycotoxins, in particular, has a major negative impact of fungal growth in cheese (Nasser, 2001; ICSMF, 2005). Thus, a better control to prevent contamination and spoilage during the production, sale and distribution and to extend the shelf life time of wagashi is necessary to avoid its contamination by mycoflora and minimize public health hazards. Synthetic chemicals could be used to control these pathogens. However, there is a strong debate about the safety aspects of these chemical preservatives since they are considered responsible for many carcinogenic and teratogenic attributes as well as residual toxicity (Hsouna et al., 2011; Barkat and Bouguerra, 2012). Concurrently, modern society is looking for natural products that have less impact on the environment and that contain less synthetic antimicrobial food additives. In the meantime, a lower food salt content is also being promoted by the World Health Organization, in an attempt to reduce the incidence of cardiovascular diseases (WHO, 2002; Angelini et al., 2006). For these reasons, alternative methods to control cheese-borne fungi of wagashi and consequently to improve the safety of the product are needed to be performed (Goni et al., 2009; Lv et al., 2011). Essential oils (EO's) obtained from many plants have recently gained a great popularity and scientific interest. Attention is being paid to these compounds as a new alternative to prevent the proliferation of microorganism and protect food from oxidation (Hsouna et al., 2011; Varona et al., 2013). They have been recognized as bioactive components with antimicrobial activity and classified as generally recognized as safe substances (ESO GRAS - 182.20) by the Food and Drug Administration (FDA, 2005). Therefore they could be used to prevent growth of many pathogenic and spoilage microorganisms in foods (Velázquez-Nuñez et al., 2013). Several studies have reported results on their preservative action (Nielsen and Rios, 2000; Burt, 2004; Sessou et al., 2012g). Essential

oil obtained from spice *Syzygium aromaticum* also called *Eugenia caryophyllata* is effective against many strains (Lopez et al., 2005; Pawar and Thaker, 2006; Pinto et al., 2009). Based on our knowledge, its use as cheese preservative has been few studied. The efficacy of this essential oil on fungal isolates from wagashi must be verified in order to measure its potential biopreservation for the valorization of this product. The objective of this study was to investigate antifungal activity of *S. aromaticum* flowers buds oil against spoilage and toxinogenic moulds isolated from wagashi in culture medium and in this foodstuff for its potential use as preservative.

MATERIALS AND METHODS

Plants material and extraction of the essential oil

The dried flowers buds of clove (*Eugenia caryophyllata*) were collected at Malanville in North of Republic of Benin at November 2011 and were identified by Doctor YEDOMOHAN of National Herbarium of Benin. They were hydrodistilled for about 3 h, using a Clevenger apparatus. Oil recovered in a dark sterile glass was dried over anhydrous sodium sulfate and stored at +4°C until it was used (Yehouenou et al., 2012a, b).

Chemical analysis of *S. aromaticum* essential oil

Quantitative and qualitative analyses of the essential oil of *S. aromaticum* 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 DB5 (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 temperature 220°C, detector temperature 250°C, carrier gas N₂ 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 (C₉-C₂₀). GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadrupole 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 DB5 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 strains used in this study were constituted of spoilage and

pathogens moulds *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus tamarii*, *Aspergillus terreus*, *Fusarium poae*, *Fusarium verticillioides*, *Penicillium citrinum*, *Penicillium griseofulvum*, *Aspergillus aculeatus*, *Aspergillus ustus*, *Penicillium brevicompactum*, and *Scopulariopsis brevicaulis*. They have been isolated and identifying from a traditional cheese wagashi collected near its vendors. Colonies of these moulds isolated from Dichloran Rose Bengal Chloramphenicol medium by dilution method (ISO 21527-1: 2008) were purified by streaking onto malt extract agar and then three point inoculated onto malt extract agar and Czapeck yeast autolysate 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).

Preparation of conidial suspension

The strains isolated from cheese wagashi were cultured on potato dextrose agar medium for 10 -14 days at $25 \pm 1^\circ\text{C}$. Conidia were harvested by adding 10 ml of 0.05% Tween 80 solution to culture and gently scraping the mycelia with a sterile inoculating loop to free spores. Conidial concentration was determined by a haemocytometer and the suspension was diluted with 0.05% Tween 80 solution to give a final concentration of 108 spores/mL approximately (Gandomi et al., 2009, Sessou et al., 2012g).

Antifungal assay in culture medium

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 mycelia growth inhibition (MGI) percentage was calculated according to the equation:

$$\text{MGI} = (\text{dc}-\text{dt})/\text{dc} \times 100$$

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.

Antifungal assay in cheese wagashi foodsystem

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Test in cheese wagashi

The procedure was based on that of Smith-Palmer et al. (2001) and performed by Sessou et al. (2012g). 10 g of sterile cheese wagashi was added to 90 ml of 0.1% peptone (CM0009 Oxoid, LTD Basingstoke, Hampshire, England) in stomacher bags and homogenized for 2 min in a stomacher. Essential oil of *S. aromaticum* was added to the cheese mixture to achieve final concentrations wished (600, 800 and 1000 mg/L). The controls contained peptone but no plant essential oil. The cheese mixture was inoculated with 100 µl of sporale suspension culture, which had been prepared previously. The inoculum was mixed thoroughly with the cheese mixture by gently squeezing the bags by hand and the concentration of mould in the cheese determined at 0 h and 1, 2, 3, 4, 7, 10 and 14 days using the serial dilution and spread plate technique.

Statistical analysis

Data from three independent replicate trials were subjected to statistical analysis using Statistica version 6.0. Differences between means were tested using Z-test. Results were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of *Eugenia caryophyllata* (*S. aromaticum*) essential oil

The chemical composition of *S. aromaticum* essential oil with yield equal to $6.7 \pm 0.7\%$ is presented in Table 1. Fifteen components which represented 98.5% of the total oil were identified in the essential oil. The oil constituted mainly of eugenol (75.2%) and trans-caryophyllene (12.0%). The minor compounds presented in significant percents in clove oil studied were eugenol acetate (7.0%), and sesquiphellandrene (1.7%). The major components found in our essential were quite similar compared with data reported by Raina et al. (2001), Ranasinghe et al., 2002; Prashar et al. (2006), Pawar and Thaker (2006), Chaeib et al. (2007) and Pinto et al., 2009) but varied widely in proportions. In fact, Raina et al. (2001) found in essential oil of *E. caryophyllata* a high percentage of eugenol (94.41%) with 2% β -caryophyllene whereas Prashar et al. (2006) found 78% of eugenol with 13% β -caryophyllene. Pawar and Thaker (2006) found that the content of eugenol was 47.64%, with the concentration of benzyl alcohol at 34.10%. Chaeib et al. (2007) obtained a high concentration of eugenol (88.58%), eugenyl acetate (5.62%) in essential oil of *S. aromaticum* which contained 79.2 and 85% eugenol according, respectively, to studies of Ranasinghe et al. (2002) and Pinto et al., (2009).

Table 1. Chemical composition of *S. aromaticum* essential oil collected at Malanville (Benin).

IK	Chemical composition	Percentage (%)
831	Furfural	0.1
1370	Eugenol	75.2
1423	Trans- β -caryophyllene	12.0
1438	B-Duprezianene	0.2
1451	(Z)- β -farnesene	0.2
1473	α -Umulene	0.3
1494	γ -Uurolene	1.1
1514	Germacrene D	0.1
1517	Eugenol acetate	7.0
1537	β -Sesquiphellandrene	1.7
1560	δ -Cadinene	0.1
1569	10-epi- α -Cubebol	0.2
1616	Isolongifolanone	0.1
1697	Davanol acetate	0.3
1708	Heptadecane	0.1
	Hydrogenated monoterpenes	0.1
	Oxygenated monoterpenes	75.2
	Hydrogenated sesquiterpenes	22.5
	Oxygenated sesquiterpenes	0.7
	Total	98.5

***In vitro* antifungal activity of essential oil of *S. aromaticum* in culture medium**

This part of the work concerned the *in vitro* evaluation of antifungal activity of essential oil of *S. aromaticum* against twelve fungal isolates of wagashi belonging to *Aspergillus*, *Fusarium*, *Penicillium* and *Scopulariopsis*. The results obtained from this work indicated that the observed mycelial reduction rate of strains were significantly ($p < 0.05$) influenced by the incubation time and the concentrations of essential oil tested. Indeed, mycelial growth is reduced with increasing essential oil concentration while the mycelial growth increased with the duration of incubation (Figure 1). The percentage of mycelial growth inhibition, minimum inhibitory concentrations (MIC) and fungicidal (CMF) of the oil are shown in Table 2. Analysis of this table shows that the essential oil of *E. caryophyllata* present differentially fungistatic and fungicidal activities against of all strains investigated. The minimum inhibitory concentrations of this oil ranged from 200 to 600 mg/L and the more sensitive strains in decreasing order were *S. brevicaulis* (MIC = 200 mg/L; CMF = 400 mg / L) and *A. terreus* (MIC = 400 mg / L; CMF = 800 mg / L). The least sensitive strains, all entirely inhibited by the oil concentration at 600 mg/L, were *A. aculeatus*, *A. tamarii*, *F. poae*, *P. brevicompactum* and *P. citrinum*. The most pronounced activity of the essential oil of *S. aromaticum* is certainly

due to its major component, eugenol, a phenolic compound that is recognized as having potential antimicrobial properties. In fact, according to Burt (2004), essential oils with strong antibacterial and antifungal activities are generally those that are rich in phenolic compounds such as carvacrol, eugenol and thymol. Mechanisms of action of these phenolic compounds and their effects on the cell membrane are known. Their influence on cell permeability in terms of interference on membrane functions (electrons transport, synthesis of amino acids and nucleic acids and enzyme secretion) are certainly at the origin of this character for the observed antimicrobial activity of the oil against the strains investigated. One important characteristic of these phenolic compounds is their hydrophobicity that allows them to bind to the ergosterol, the majority of lipid cell membrane strains, creating a disturbance of the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents (Murray et al., 2003; Burt, 2004; Yèhouénou et al., 2012a and b). In the literature, several studies on the antifungal activity of the essential oil of *Syzygium* against food pathogens have been reported and their results are in accordance with those of the present work. Indeed, Lopez et al. (2005) showed that the essential oil of *S. aromaticum* possess antifungal properties on some fungal strains isolated from foods. Pawar and Thaker (2006) showed that the essential oil of

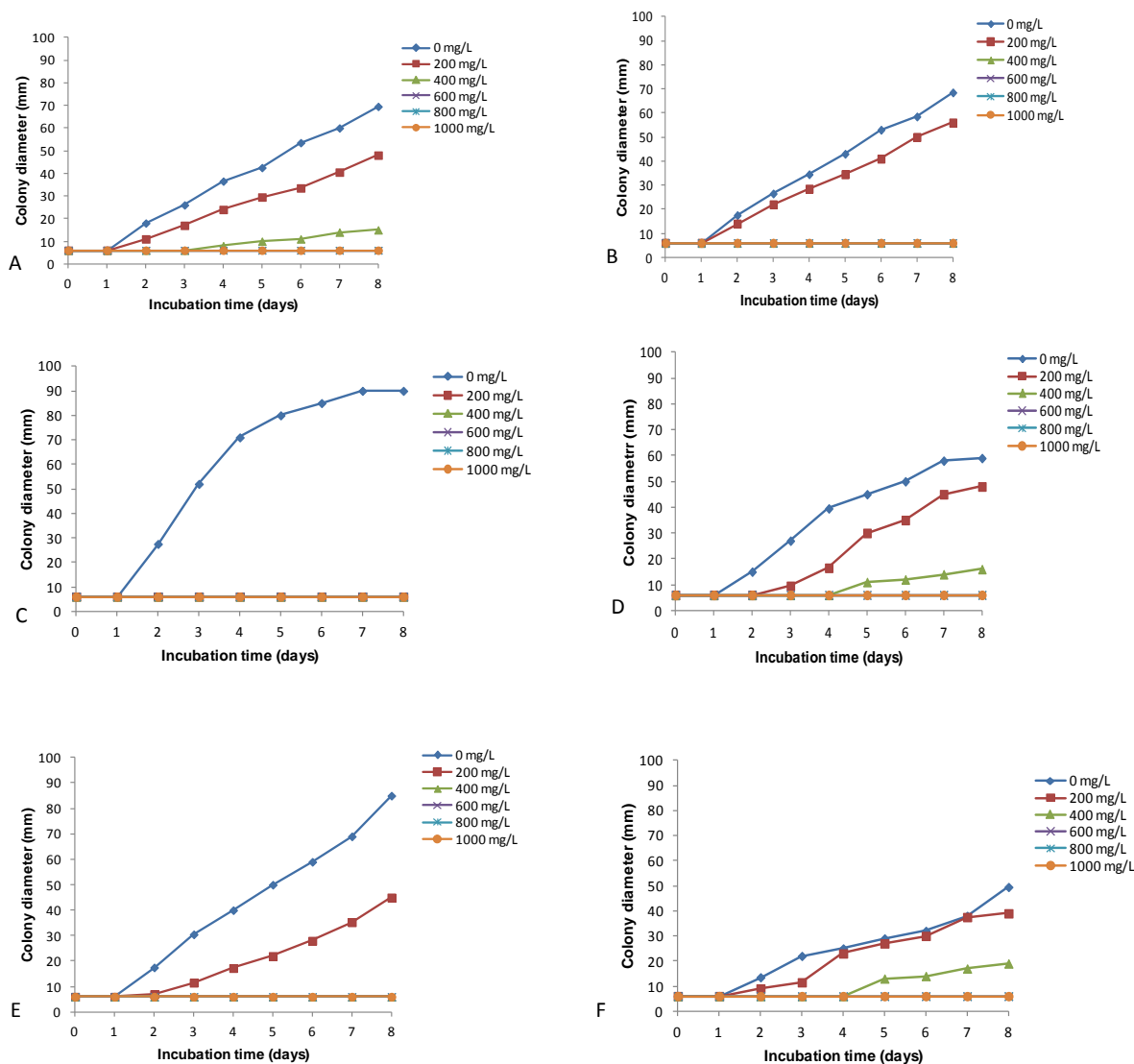


Figure 1. Effect of different concentration of *Syzygium aromaticum* essential oil on species investigated. A, *Fusarium poae*; B, *Fusarium verticillioides*; C, *Scopulariopsis brevicaulis*; D, *Penicillium citrinum*; E, *Aspergillus terreus*; F, *Penicillium brevicompactum*; G, *Aspergillus niger*; H, *Aspergillus ustus*; I, *Aspergillus tamari*; J, *Aspergillus aculeatus*; K, *Aspergillus flavus*; L, *Penicillium griseofulvum*.

clove has an inhibitory activity on *A. niger*. Studies of Pinto et al. (2009) also showed that the essential oil of *S. aromaticum* was active against some strains such as *A. flavus*, *Aspergillus fumigatus* and *Candida*. Considering the results obtained in our present studies, the tested essential oil can be used in the fight against pathogens and spoilage moulds of wagashi.

***In situ* effects of *S. aromaticum* against moulds in wagashi food system**

The data presented in Figures 2, 3 and 4 are related to the use of *S. aromaticum* oil as a substitute of synthetic

chemical preservatives in the fight against spoilage and pathogenic moulds *A. aculeatus*, *A. tamarii*, *F. poae*, and *P. brevicompactum*, *P. citrinum* in wagashi. These strains were chosen because of their less sensitivity to this extract when tested *in vitro* in culture medium. As mentioned before, to evaluate the antifungal activity of *S. aromaticum* essential oil in the food system, three distinct oil concentrations was adopted with respect to the full inhibitory concentrations (minimal and highest concentrations) found for all tested moulds. A blank test, without the essential oil was also conducted. The concentrations of essential oil tested *in situ* were then 600, 800 and 1000 mg/mL which had totally inhibited all the strains at *in vitro* experimentation. It should be noted

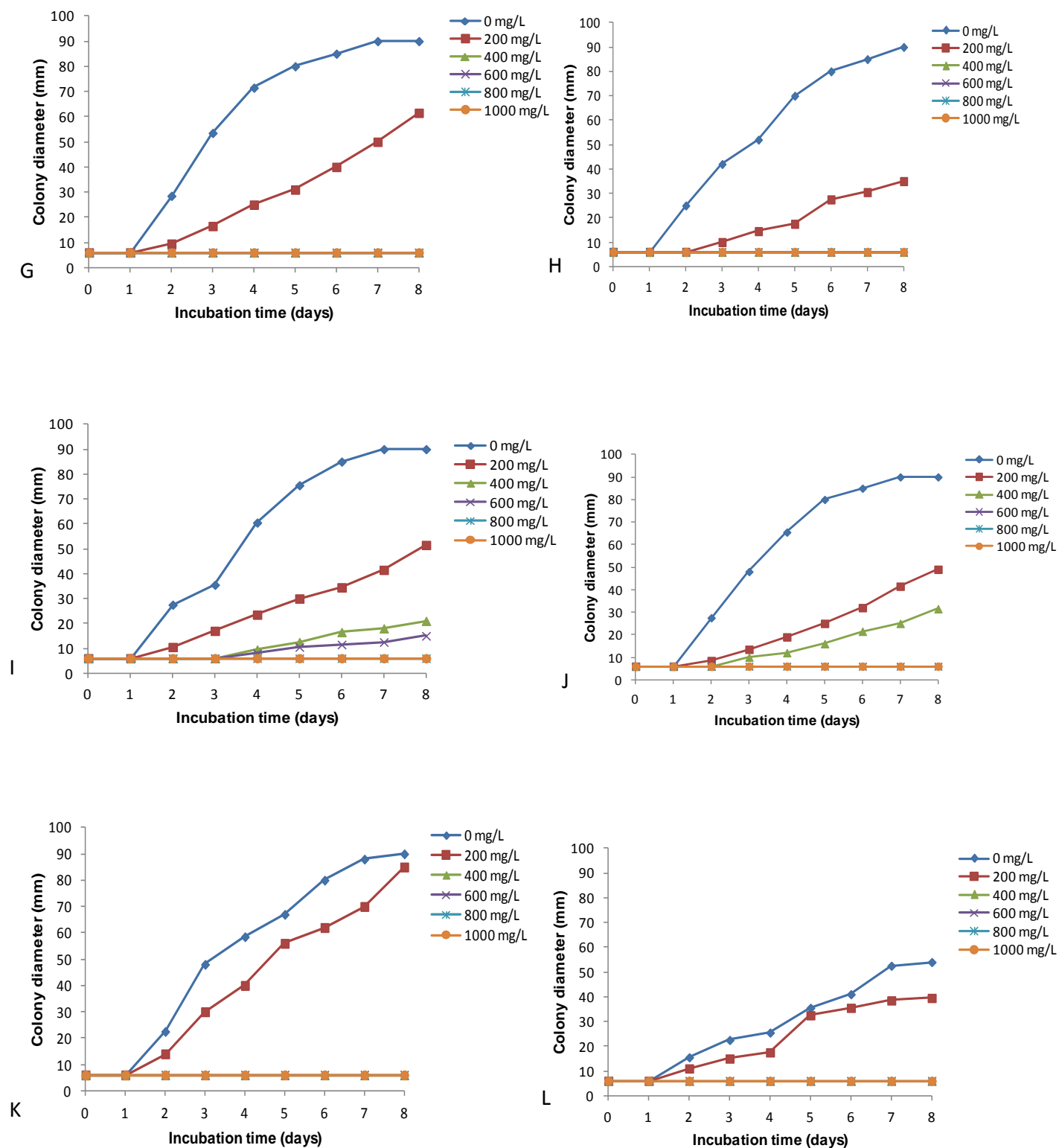


Figure 1. Contd.

that the effect of oil is reported here for wagashi tenth diluted in peptone water and is not necessarily a real

trend in the wagashi solid but nevertheless necessary for its practical importance. Despite this fact, the

Table 2. Mycelial growth inhibition, fungistatic and fungicidal activities of essential oil of *Syzygium aromaticum*.

Essential oil (mg/L)	Mycelial growth inhibition (%)											
	<i>A. acu</i>	<i>A. fla</i>	<i>A. nig</i>	<i>A. tam</i>	<i>A. ter</i>	<i>A. ust</i>	<i>F. poa</i>	<i>F. vert</i>	<i>P. brev</i>	<i>P. cit</i>	<i>P. gris</i>	<i>S. brev</i>
200	48,8±0.5d	5,9±0.9i	33.9±0.7e	45.8±1.3d	50.6±0.2c	65.4±0.7b	33.8±1.1e	20.0±0.3h	24.1±0.7g	20.7±2.5gh	30.2±0.8f	100a (FS)
400	69.6±2.8d	100a (FS)	100a (FS)	82.1±3.2bc	100a (FS)	100a (FS)	85.8±2.2b	100a (FS)	70.1±0.5d	81.1±0.4c	100a (FS)	100a (FC)
600	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FC)
800	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FC)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FC)
1000	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FC)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FS)	100a (FC)

FS, Fungistatic activity; FC, fungicidal activity; *A. acu*, *Aspergillus aculeatus*; *A. fla*, *Aspergillus flavus*; *A. nig*, *Aspergillus niger*; *A. tam*, *Aspergillus tamarii*; *A. ter*, *Aspergillus terreus*; *A. ust*, *Aspergillus ustus*; *F. poa*, *Fusarium poae*; *F. vert*, *Fusarium verticillioides*; *P. brev*, *Penicillium brevicompactum*; *P. cit*, *Penicillium citrinum*; *P. gri*, *Penicillium griseofulvum*; *S. brev*, *Scopulariopsis brevicauli*. Data in the line followed by different letters are significantly different ($p < 0.05$). The values are means of three repetitions \pm standard deviation.

Investigation showed the potential application of the essential oil of *S. aromaticum* as curator of wagashi. Indeed, after addition of 600, 800 and 1000 mg/L of essential oil in wagashi containing spores of strains, we observed a significant reduction in spore load of strains based on the retention time (storage) and the concentration of essential oil. In all cases, the inhibitory effects were dose-dependent; moreover, the concentration of the oil is higher, more the sporale reduction rate increases (Figures 2, 3 and 4). The three preparations showed a gradually decrease in the count of spores of these fungi with increasing essential oil concentrations. For example, the strain of *A. tamarii* was reduced to 4.39 log₁₀ cfu/g of its load at fourteenth day of storage at 600 mg/L whereas at 800 and 1000 ppm, the spore load of the same strain was reduced respectively to 4.06 and 2.67 log₁₀ cfu/g. At the same time, the spore load of the control without essential oil has increased to 8.11 log₁₀ cfu/g on the fourteenth day of storage. At 1000 ppm, strains of *P. citrinum*, *A. aculeatus*, *P. brevicompactum*, *A. tamarii* and *F. poae* were reduced to 1.02, 1.30, 1.40, 2.14 and 2.67 log₁₀ cfu/g, respectively, of

their sporale quantum the fourteenth days of storage. The same trend was obtained on the same strains at the concentration of 800 ppm where the spore loads of these strains were respectively reduced to 2.01, 1.89, 2.40, 2.96 and 4.06 log₁₀ cfu/g at the last day of storage. It is the same observation at 600 mg/L when the same strains were reduced to 2.21, 2.14, 3.31, 3.33 and 4.39 log₁₀ cfu/g, respectively, in the fourteenth day of storage. At the same time, there was an increase in spore loads 8.11, 8.35, 8.05, 8.27 and 8.11 log₁₀ cfu/g of the respective controls without essential oil of these five strains on the fourteenth day of storage. After all, the essential oil of *S. aromaticum* is active on all strains but preferably on *P. citrinum*, *P. brevicompactum* and *A. aculeatus*. It is important to notice that no strain has seen its initial quantum reduced to 100% during storage. The percentages of reduction of mold spores in the wagashi are lower than those observed in the culture medium. This may be related to more complex matrix (fat and protein) of wagashi than the culture medium. In general, concentrations of essential oils and their compounds necessary to inhibit microbial growth

in food are higher than in the culture media (Burt, 2004). This is due to interactions between the compounds and oils from the food matrix (Nuchas and Tassou, 2000; Sessou et al., 2012g). Several authors (Farbood, 1976) showed that the fat in the diet could form a protective layer around the micro-organisms, thereby protecting against antimicrobial agents (Sessou et al., 2012g). These researchers also showed that the lipid fraction of food absorbs the antimicrobial agent, thus reducing the oil concentration in the aqueous phase. The protein content of the diet may also have been a factor in the effectiveness of essential oil (Smith-Palmer et al., 2001; Sessou et al., 2012g). According to Juven et al. (1994), bovine serum albumin neutralized the antimicrobial action of thymol resulting in the formation of complexes between phenolic compounds in oil and protein foods (Omidbeygi et al., 2007; Sessou et al., 2012g). The addition of oil to the mixture of *S. aromaticum* and mold spores in wagashi exerted a considerable reduction in spore loads of all strains investigated which can lead to spoilage of this foodstuff and produce their metabolites in wagashi and could affect the health

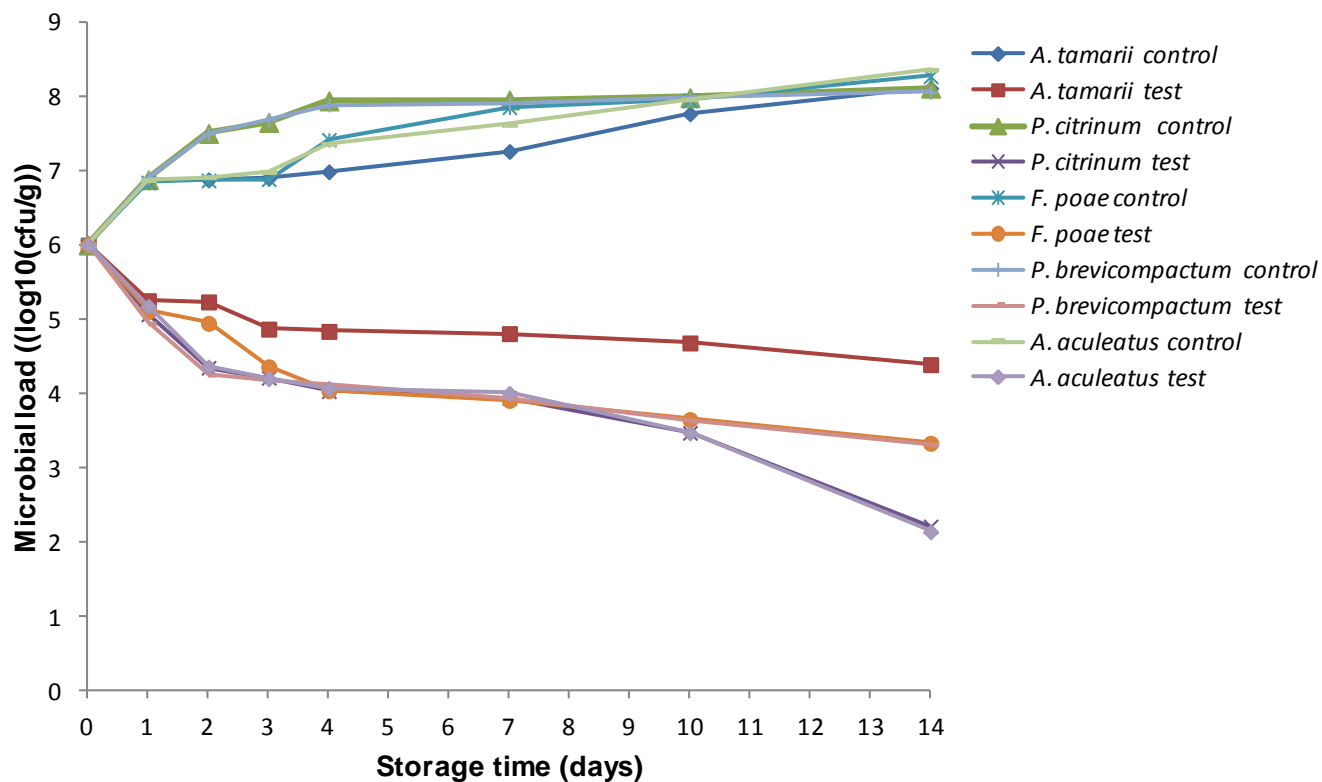


Figure 2. Inhibition of moulds investigated in traditional cheese wagashi by *S. aromaticum* essential oil at concentration of 600 mg/L.

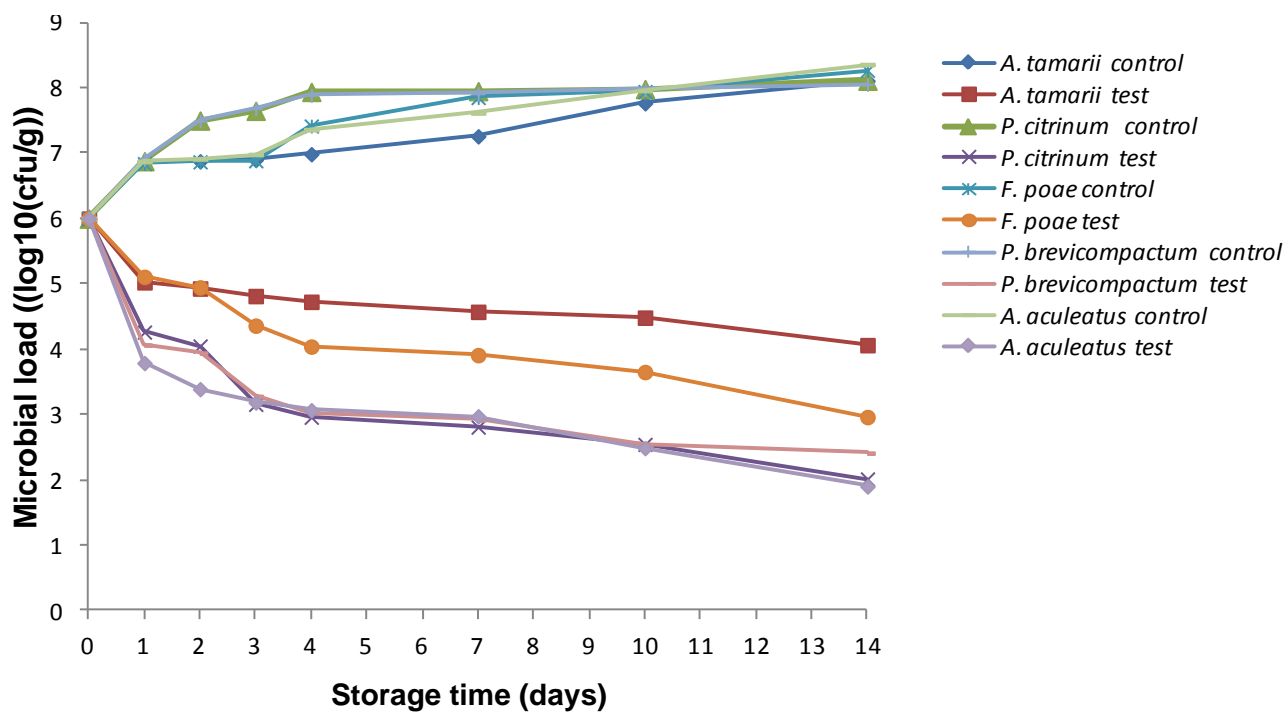


Figure 3. Inhibition of moulds investigated in traditional cheese wagashi by *S. aromaticum* essential oil at concentration of 800 mg/L.

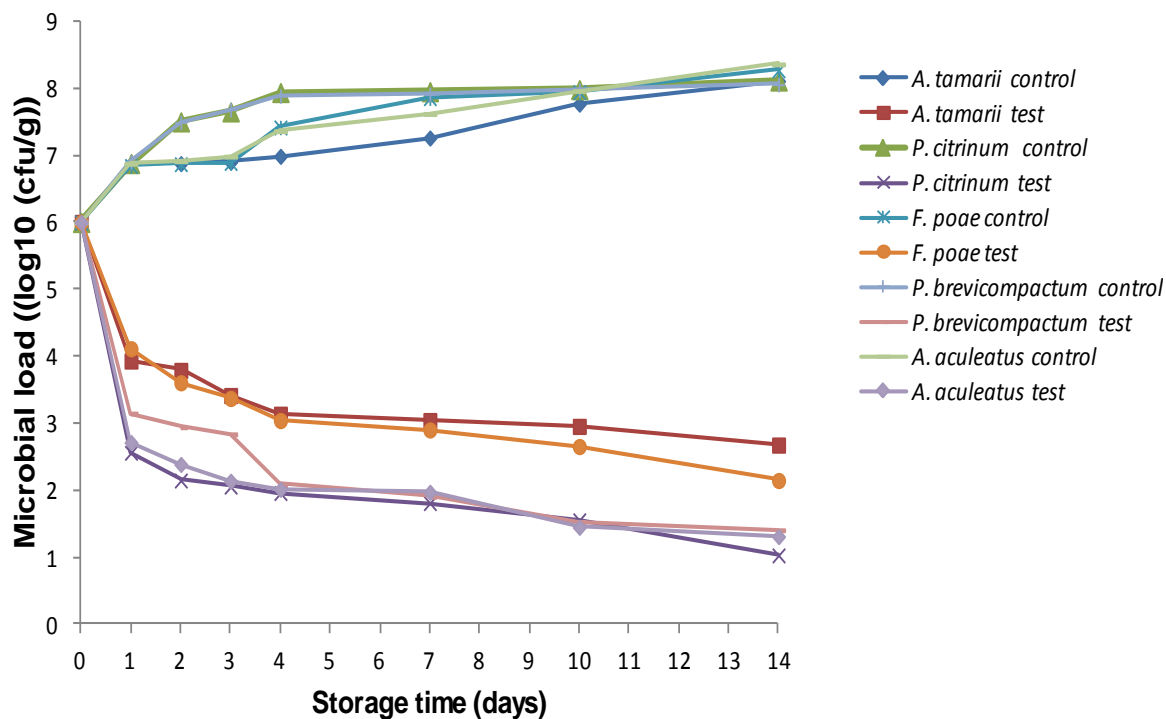


Figure 4. Inhibition of moulds investigated in traditional cheese wagashi by *S. aromaticum* essential oil at concentration of 1000 mg/L.

of consumers. This study showed that the essential oil of *S. aromaticum* has antifungal activity and can be used as an alternative of synthetic chemical preservatives to extend shelf life of wagashi. Antimycotoxigenic and toxicity for human tests of this oil are needed to be investigated for practical use of this extract for the valorization of wagashi, a food product much appreciated by the people of Benin and surrounding.

Conclusion

The *in vitro* and *in situ* antifungal activities respectively in culture medium and traditional cheese wagashi of *S. aromaticum* essential oil against twelve fungal isolates of wagashi belonging to *Aspergillus*, *Fusarium*, *Penicillium* and *Scopulariopsis* genera were evaluated in this work. Essential oil of *E. caryophyllata* constituted of eugenol and trans- α -caryophyllene as major components showed high effect against all the species tested at *in vitro* tests and especially high sporule reduction on *P. citrinum*, *A. aculeatus* in wagashi foodsystem. Results obtained in the present study allow the use of this oil to fight against moulds contamination of wagashi during storage. Further studies are needed to evaluate the antitoxigenic and toxicity for human of *E. caryophyllata* essential oil before its practical use as substitute of chemical preservatives of wagashi.

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