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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF ESSENTIAL OIL FROM *MENTHA PIPERITA* L. LEAVES ON THE QUALITY OF MANGO PUREE DURING STORAGE

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ABSTRACT

The aim of this study was to evaluate the effect of essential oil (EO) from fresh leaves of *Mentha piperita* L. on the conservation of mango puree during storage. The results of physicochemical characterization of mango puree underlined its high nutritional potential, with carbohydrates, carotenoids and vitamin C contents of 9.5±0.4%, 20.05±0.03 mg/100g and 21.03±0.05 mg/100g respectively. Microbiological analyses revealed that Aspergillus and Mucor were the most common genera of fungi identified from mango puree in Benin by using a taxonomic schemes primarily based on morphological characters of mycelium and conidia. The isolated fungi include *Aspergillus parasiticus, A. versicolor* and *Mucor spp*. Antifungal assay, performed by the agar diffusion assay, indicated that essential oil exhibited high antifungal activity against the growth of fungi. The minimal inhibitory concentration (MIC) of the essential oil was found to be 2.0 µL.mL^{-1}for *A. parasiticus* and *A. versicolor;* and 1.0 µL.mL^{-1} for *Mucor spp.* The Minimal Fungicide Concentration (MFC)was recorded to be 2.5 µL.mL^{-1} for *A. parasiticus* and *A. versicolor*; and 1.5 µL.mL^{-1} for *Mucor spp*. Chemical analysis by GC/MS of the oil led to the identification of 15 components, characterized by menthol (46.7%), neomenthol (8.28%) and 1.8-cinéole (6.49%) as major components. Results obtained during the evaluation of the physicochemical characteristics of the mango puree stored by adding EO, indicated a significant decrease in the pH, carotenoids and vitamin C contents. However, with EO concentration of 2.5 µL.mL^{-1}, the pH of stored mango puree was 6.4±0.7 after 15 days of preservation, with a high carotenoids and vitamin C contents of 19.02±0.07 mg/100g and 21.01±0.03 mg/100g. The EO of *Mentha piperita* L., with high antimicrobial property, offers a novel approach to the management of fruits derivate products during storage.

Keywords: *Mentha piperita* L., essential oil, mango puree, conservation, Benin

INTRODUCTION

For a long times, the problems encountered in Africa by producers during the post-harvest period of agricultural products have been neglected, because they were combined with those related to production. Meanwhile, post-harvest losses are increasing, due to the fact that traditional storage technologies are generally inadequate (**Fandohan et *al.*, 2003**). Thus, to contribute to the reduction of food insecurity problems, agricultural production should be increased and local products should be valued through the judicious use of technical knowledge and biotechnological tools. For example, the valorization of tropical fruits through their biotransformation into better stabilized and value-added products should be promoted in low income countries.

African flora have a lot of tropical species including mango's tree (*Mangifera indica*), whose fruit is highly appreciated for its high content of carotenoids, flavonoids, vitamins and fibers (**Djioua *et al.*, 2009**). In Benin, the low valorization of this fruit causes a significant post-harvest loss due to the harvesting or storage conditions that attracted many microorganisms and parasites. Thus, one of the solutions envisaged is its transformation in a value-added products, such as mango puree.

However, the conservation of fruit products is a serious problem because of the rapid growth of micro-organisms. To overcome this problem, unspecified heat treatments or antimicrobials products from chemical synthesis are often used. Unfortunately, the nutritional value of fruit derivate products are often modified by heat treatments, and the application in high concentrations of synthetic chemicals products in food preservation, increases the risk of toxic residues in food products (**Hammer *et al.*, 1999**). Due to the increasing sensitivity of consumers to this residual pollution and the toxic effects of many antimicrobials from chemical synthesis, the importance of using natural products becomes necessary (**Bankole, 1997**). Similarly, the restriction imposed by the food industry and regulatory agencies on the use of some synthetic food additives has led to a renewed interest in the search for alternatives, such as natural compounds, (**Hammer *et al.*, 1999**).

Plant extracts have many properties whose one of the most important is the antimicrobial activity (**Yehouenou *et al.*, 2010**). Many researches have investigated the systematical study of the essential oils extracted from the aromatic plants commonly used in traditional pharmacopoeias in Benin (**Sohounhloué *et al.*, 1996; Alitonou, 2006**), and the importance of the use of essential oils in the food preservation are also reported by **Konfo *et al.* (2015)**, **Soumanou and Adjou (2016)** and **Adjou *et al.* (2017).** The use of essential oils as antimicrobial agents has two main advantages: the first is their natural origin which means more safety for the population and environment and the second is that they are considered to have a low risk of development of resistance by pathogenic microorganisms (**Tatsadjieu *et al.*, 2010**). Thus, the objective of our study was to evaluate the efficacy of the essential oil extracted from the fresh leaves of *Mentha piperita* L. against the spoilage flora of mango puree in Benin by physicochemical characterization and microbiological analysis.

MATERIAL AND METHODS

Collection of plant leaves

Plant materials used for essential oil (EO) extraction were fresh leaves from *Mentha piperita* L. Plants were collected at Abomey-calavi (6°26′54″ North /2°21′20″ East) (South Benin) and identified at the Benin national herbarium, where voucher specimens are deposited.

Essential oil extraction

The EO tested was extracted by the hydro-distillation method using Clevenger-type apparatus. The oil recovered was dried over anhydrous sodium sulfate and stored at 4 °C until it was used (**de Billerbeck *et al.*, 2001**).

Gas chromatography–mass spectrometry analysis

The EO were analyzed by gas chromatograph (Perkin Elmer Auto XL GC; Waltham, MA, USA) equipped with a flame ionisation detector, and the GC conditions were EQUITY-5 column (60 m x 0.32 mm x 0.25 µm); H_2 as the carrier gas; column head pressure 10 psi; oven temperature program isotherm 2 min at 70 °C, 3 °C/min gradient 250 °C, isotherm 10 min; injection temperature, 250 °C; detector temperature 280 °C. Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a Perkin Elmer Turbomass GC-MS. The GC column was EQUITY-5 (60 m x 0.32 mm x 0.25 µm); fused silica capillary column. The GC conditions were injection temperature, 250 °C; column temperature, isothermal at 70 °C for 2 min, then programmed to 250 °C at 37 °C/min and held at this temperature for 10 min; ion source temperature, 250 °C. Helium was the carrier gas. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with 70 eV ionisation energy. The sector mass analyzer was set to scan from 40 to 500 amu for 22 s. The identification of individual compounds is based on their retention times, retention indices relative to $C_5 - C_{18}$ n-alkanes, and matching spectral peaks available in the published data (**Adams, 2007**).

Collection of mango and puree production

Samples of mangoes used in this study were purchased at the large mango selling area of Dantokpa market in Cotonou (6° 21' 45" North/2° 25' 32" East) (South Benin). These samples are ripe mangoes of *Eldon* variety, characterized by a yellow-orange color and a strong aroma of turpentine. Samples of mangoes were washed and pulped. The pulp obtained is cut and then ground to obtain the mango puree.

Microbiological analysis

For microbiological analysis, 25 g of sample and 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count according to ISO 4833-1 (1) methods. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total coliforms count according to NF V08-050, and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using Eosine Methylene Blue (EMB) medium. Bair Parker medium was used for *Staphylococcus spp.* count according to NF EN ISO 6888-1/A1, and plates were incubated at 37°C for 24h. Tryptone sulfite neomycin agar was used for anaerobic sulfito-reducer (ASR) count, according to ISO 7937 methods and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked, using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, considering dilution factor. All media used for microbiological analysis were prepared as indicated by the manufacturer. After their isolation, bacteria were also controlled with API System (BioMérieux France).

Fungal isolation

The isolation of fungi from samples was performed using dilution plating method. Ten gram of each juice sample were added separately to 90 ml of sterile water containing, 0.1% peptone water. This was thoroughly mixed to obtain the 10^{-1} dilution. Further, 10fold serial dilutions up to 10^{-4} were made. 1 ml volume of each dilution was separately placed in Petri dishes, over which, 10 to 15 ml of potato dextrose agar amended with 60 µg/ml chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days. Fungal isolates from PDAC were sub-cultured on malt extract agar (MEA) (**Adjou *et al.*, 2017).**

Antifungal assay

Antifungal assay was performed by the agar medium assay (**Yehouenou *et al.*, 2010**). Yeast Extract Sucrose medium with different concentrations of essential oil (0.15, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50 µL/mL^{-1}) and Tween 20 (0.01%) were prepared by adding appropriate quantity of EO to melted medium, followed by manual rotation of Erlenmeyer to disperse the oil in the medium. About 20 ml of the medium were poured into glass Petri dishes (9 cm). Fungal isolates from mango puree on malt extract agar (MEA) are transplanted (subcultured), using a disc of 6 mm in diameter which carries spores from the anamorph mold, on the surface of a Petri dish containing the former medium Yeast Extract Sucrose (YES) and EO at different concentrations. Positive Control plates (without EO and inoculated following the same procedure) and negative control plates were also used. Plates were incubated at 25 °C for 5 days.

Determination of the fungiostatic or fungicidal activity

With the experimental concentrations where neither growth nor germination was observed, the fungiostatic or fungicidal activity was tested. This assay consisted by taking the mycelial disc not germinated at the end of the incubation of the Petri dish and reintroducing it in a new culture medium (former one) without EO. If the mycelial growth is always inhibited, the plant extract is fungicidal at this concentration and allows the determination of the minimum fungicidal concentration (MFC). In the contrary case, it became fungiostatic activity which is related to the minimum inhibitory concentration (MIC) (**Yehouenou *et al.*, 2010**).

Conservation of mango puree with essential oil

To evaluate the conservation potentiality of the EO of *Mentha piperita* L., on mango puree, five EO concentrations were tested. These are 0.50, 1.00, 1.50, 2.00 and 2.50 µL/mL^{-1}. These concentrations were chosen taking into account the high fragrant nature of the EO. A negative control (mango puree without EO) was also produced. Samples were placed at 25 °C. After 15 days of conservation at this temperature, microbiological and physicochemical qualities of conserved mango puree were then evaluated. The pH of the samples were determined in 10ml of mango puree using a digital pH-meter. Vitamin C (l-ascorbic acid) and carotenoids concentrations were determined using method described by **Adjou *et al.* (2013)**.

Statistical analysis

Experiments were performed in triplicate, and data analyzed are means ± SD subjected to one-way Anova. Means are separated by the Tukey's multiple range test when Anova was significant ($P<0.05$) (SPSS 10.0; Chicago, IL, USA).

RESULTS AND DISCUSSION

By hydrodistillation, fresh leaves of *Mentha piperita* L. yielded 0.45 % of EO. Chemical analysis by GC and GC-MS analysis of EO enabled the identification of 15 components, (Table 1) representing 94.2 % of the EO. In the volatile extract, different groups of terpene and terpenoid, such as hydrogenated monoterpenes (5.1%) and oxygenated monoterpenes (87.0%) were detected. The EO has chemical composition characterized by menthol (46.7%), neomenthol (8.28%) and 1.8-cinéole (6.49%) as major components.

The result of microbial analysis and isolation of fungi in pure culture revealed that the most contaminated microflora of mango puree were fungi (Table 2). Fungal isolates include *Aspergillus parasiticus*, *Aspergillus versicolor* and *Mucor spp.*

The results of physicochemical characterization of mango puree (Table 3) showed that the pH was 6.8±0.1, with carbohydrates, carotenoids and vitamin C contents of 9.5±0.4 %, 20.05±0.03 mg/100g and 21.03±0.05 mg/100g respectively.

EO exhibited pronounced antifungal activity against the growth of *A. parasiticus, A. versicolor* and *Mucor spp*. The MIC of the EO was found to be 2.0 µl/ml for *A. parasiticus* and *A. versicolor;* and 1.0 µl/ml for *Mucor spp.* The MFC was recorded to be 2.5 µl/ml for *A. parasiticus* and *A. versicolor; and* 1.5 µl/ml for *Mucor spp.*

The results obtained during storage tests of mango puree with the EO of *Mentha piperita* L. at different concentrations (Table 4) indicated a strong antimicrobial activity of the EO against the spoilage flora. Indeed, with the essential oil concentration of 2.0 µL.mL^{-1}, there was an important antifungal activity against A.*parasiticus, A. versicolor and Mucor spp.*

Table 5 presented the results of the evaluation of the physicochemical characteristics of the mango puree stored by adding EO after 15 days. These results indicated that, in mango puree stored with EO at the concentrations of 0.5 – 1.5 µL.mL^{-1}, there was a significant difference in pH, carotenoids and vitamin C contents, after 15 days of storage. However, at the EO concentration of 2.0 and 2.5 µL.mL^{-1}, there was no significant difference in pH, carotenoids and vitamin C contents after 15 days of storage.

Table 1 Chemical composition of *Mentha piperita* L. essential oil investigated

Components	Kovats Index (KI)	Percentage (%)
α-Pinene	939	0.8
β-Pinene	979	1.2
Limonene	1028	2.8
1,8-Cineole	1031	6.5
Terpinene	1060	0.3
Menthone	1153	7.4
menthofurane	1164	1.6
iso-menthone	1166	4.8
Menthol	1174	46.7
iso-menthol	1183	0.8
neo-menthol	1188	8.28
pulegone	1238	3.6
piperitone	1254	1.7
menthyl-acétate	1294	6.7
Trans β- caryophyllene	1408	2.1
Total		**94.2**

Edible fruits become an alternative source of food with high potential of vitamins, minerals and others interesting elements particularly during seasonal food shortage (**Umaru *et al.*, 2007**). They are also known to have nutritional and medicinal properties that can be attributed to their antioxidant effects and they can be used to fortify staple foods particularly for malnourished children (**Barminas, 1998**). The results of the proximate analyses revealed that mango puree are good source of carbohydrates, carotenoids and vitamin C. Carbohydrate content was similar to those reported in soursop fruit (**Degnon et *al.*, 2013**) and higher than those reported in palmyra juice (**Adjou *et al.*, 2013**) and in cashew apple juice (**Gbohaida *et al.*, 2015**). Carotenoids content was similar to those reported in palmyra juice (**Adjou *et al.*, 2013**). Vitamin C content is lower than those reported in oranges juice (**Adjou *et al.*, 2017**) and higher than those reported in palmyra juice (**Adjou *et al.*, 2013**). The high nutritional potential of mango puree, justified its uses as supplement in infant feeding in Benin. The results obtained from microbial analysis, showed that mango puree was contaminated with microorganisms. The most dominant flora was fungi, especially *Aspergillus parasituus*, *Aspergillus versicolor* and *Mucor spp.* These fungi species are known spore formers and their growth can result in the production and accumulation of mycotoxins. The moisture content of mango puree would also encourage microbial growth and so deterioration. The mango puree contamination by fungi does not only reduce its quality but may also could lead to mycotoxin production (**Sultan and Magan, 2010**). The present study also explores the bioefficacy of EO of *Mentha piperita* L. as the promising plant-based antimicrobial against mango puree-infecting fungal growth. This EO was found to be effective against all *Aspergillus* and *Mucor* strains tested. This bioefficacy may be due to the presence of some highly fungitoxic components in the oil such as terpenoids. Indeed, *Mentha piperita* oil has a chemical composition characterized by terpenes and terpenoids as the main chemical groups. Several studies have indicated that terpenes do not represent a group of constituents with a high inherent antimicrobial activity. For example, **Koutsoudaki *et al.* (2005)** compared the effect of α-pinene, ß-pinene, p-cymene, ß-myrcene, limonene, and γ-terpinene against *Escherichia coli, Staphylococcus aureus, and Bacillus cereus* and reported that their antimicrobial activity was low or absent. **Rao *et al.* (2010)** also reported that p-cymene and γ-terpinene were ineffective as fungicides against *Saccharomyces cerevisiae*. In contrast, terpenoids are a large group of antimicrobial compounds that are active against a broad spectrum of microorganisms (**Dorman and Deans 2000**). Their antimicrobial activities are linked to their functional groups and it has also been reported that the hydroxyl group of phenolic terpenoids and the presence of delocalized electrons are important for the antimicrobial activity (**Dorman and Deans 2000**). The antimicrobial activity of menthol, thymol and linnalol, against *Listeria monocytogenes, Enterobacter aerogenes, Eschericha coli, and Pseudomonas aeruginosa* were reported by **Bassole *et al.* (2010)**. These results confirm the high antimicrobial activity of a broad collection of terpenoids especially of menthol, which is the one of the major component of the EO of *Mentha piperita* L. A range of EO components (menthol, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral, and limonene) have been accepted by the European Commission for their intended use as flavorings in food products (**Hyldgaard *et al.*, 2012**). The United States Food and Drug Administration (FDA) also classify these substances as generally recognized as safe (GRAS). In our study, GC–MS data depicted remarkable variation in the earlier reports on the oils (**Sokovic *et al.*, 2007; Bassolé *et al.*, 2010**). The chemical profile of EO is reported to be influenced by the harvest period, and by climatic, seasonal, and geographical conditions, which can significantly affect the

Table 2 Microbiological quality of investigated mango puree (cfu/mL).

Microbiological parameters	Total bacterial count	Total coliforms count	*E.coli*	*S. aureus*	A.S.R. count	Yeast count	Fungi count
Mango puree sample	4.7×10^2	00	00	00	00	00	8.1×10^1

Table 3 Physicochemical quality of investigated mango puree.

Physicochemical parameters	pH	Carbohydrates (%)	Vitamin C (mg/100g)	Carotenoids (mg/100g)
Mango puree sample	6.8±0.1	9.5±0.4	21.03±0.05	20.05±0.03

Table 4 Microbiological quality of investigated mango puree after 15 days of conservation (ufc/ml).

Parameters	Concentrations of essential oil ***(µl/ml)***					
	0	***0.50***	***1.00***	***1.50***	***2.00***	***2.50***
Total bacterial count	$3.0 \times 10^{7a*}$	$2.0 \times 10^{5b*}$	10^{2c*}	10^{d*}	00^{e*}	00^{e*}
Fungi count	$1.0 \times 10^{5a*}$	$1.0 \times 10^{2a*}$	$1.2 \times 10^{1b*}$	10^{c*}	00^{d*}	00^{d*}

*Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 5 Physicochemical quality of investigated mango puree after 15 days of conservation

Physicochemical parameters	Mango puree 's characteristics at the beginning of the conservation tests	Characteristics of the mango puree after 15 days of conservation					
		Concentrations of essential oil (µl/ml)					
		0	***0.50***	***1.00***	***1.50***	***2.00***	***2.50***
pH	$6.80 \pm 0.10^{a*}$	$2.40 \pm 0.10^{b*}$	$4.90 \pm 0.40^{b*}$	$5.40 \pm 0.60^{b*}$	$5.70 \pm 0.20^{c*}$	$6.20 \pm 0.30^{a*}$	$6.40 \pm 0.70^{a*}$
Carbohydrates (%)	$9.50 \pm 0.40^{a*}$	$1.80 \pm 0.20^{b*}$	$5.70 \pm 0.30^{c*}$	$6.20 \pm 0.10^{c*}$	$8.10 \pm 0.60^{d*}$	$9.40 \pm 0.10^{a*}$	$9.10 \pm 0.30^{a*}$
Carotenoids (mg/100g)	$20.05 \pm 0.03^{a*}$	$4.51 \pm 0.07^{b*}$	$9.06 \pm 0.04^{c*}$	$11.53 \pm 0.02^{d*}$	$14.03 \pm 0.06^{e*}$	$18.03 \pm 0.05^{a*}$	$19.02 \pm 0.07^{a*}$
Vitamin C (mg/100g)	$21.03 \pm 0.05^{a*}$	$1.06 \pm 0.03^{b*}$	$4.07 \pm 0.09^{c*}$	$10.01 \pm 0.04^{d*}$	$12.08 \pm 0.07^{d*}$	$20.04 \pm 0.07^{a*}$	$21.01 \pm 0.03^{a*}$

*Values are means. The means followed by same letter in the same line are not significantly different according to ANOVA and Tukey's multiple comparison tests

amount and composition of the active constituents (**Kpadonou-Kpoviessi *et al.*, 2012; Soumanou and Adjou, 2016**). Thus, the biologically active EOs should be qualitatively standardized before their recommendation for practical exploitation as has been done in the present investigation.

CONCLUSION

This work underlined the bioactivity of EO of fresh leaves of *Mentha piperita* L. from Benin as a fungal growth suppressor in mango puree. Different major components such menthol (46.7%), neomenthol (8.28%) and 1.8-cinéole (6.49%) were present in the volatile extract. Based on its antifungal potential, this natural plant product may successfully replace synthetic chemicals and provide an alternative method to protect mango puree as well as other fruit derivate products with high nutritional significance against the contamination and the growth of bacteria and fungi.

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