Antibacterial Properties of *Cymbopogon martinii* essential Oil against *Bacillus subtillis* food industry pathogen

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Introduction

Bakery Industry common problem detected

Bacillus subtillis and Bread rope deseasse

B. subtilis is a gram-positive, aerobic, spore-forming soil bacterium. Bread **Rope** spoilage initially develops an unpleasant fruity odor followed by a stiky bread crumb especially noticiable when cutted in parts and pulled apart forming 'ropes' that can be seen stretching from one surface to the other.

Espores are **resistant to heat** and can survive baking temperatures and subsequently grow in the finished product under hot and humid conditions

Essential oils as natural preservatives opportunity

Essential oils as food preservatives

Essential oils (EO) are aromatic and volatile compounds extracted from plants material, such as flowers, aerial parts, roots, bark, leaves and fruits. The EOs are used in food industry for food preservation, due to aroma, flavors and natural antimicrobial contents against pathogenic bacteria.

Objective

The aim of this study was to evaluate the effect of essential oils on growth, spore production of *Bacillus subtillis* that could alternative synthetic chemical preservatives in bakery industry.

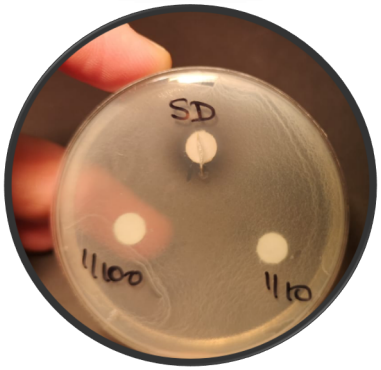
Materials and methods

1 Bacterial strains and media.

The *Bacillus subtillis* (Ehrenberg 1835) Cohn 1872, strain CECT 4522 was used in this study. *B. Subtillis* was maintained on Nutrient Broth medium and solidified. Growth temperature was 30ºC and the incubation time was 48h. Nutrient Broth medium (Beef extract, 0,5%; Peptone, 1%; NaCl, 0,5%) was adjust pH to 7.2.

2 Essential oils (EO)

Essential oils chemotyped are extracted from different plants by steam distillation (Pranarôm, S.A.). The twenty four EO used for the study are: *Citrus sinensis, Citrus reticulata, Elettaria cardamomum, Laurus nobilis, Cymbopogon martinii var.motia, Zingiber officinale, Eugenia caryophyllus, Cinnamomum camphora, Rosmarinus officinalis, Melaleuca quinquenervia, Chamaemelum nobile, Melaleuca alternifolia, Thymus vulgaris CT LINALOL, Citrus paradisi, Citrus junos, Origanum compactum, Mentha x piperita, Myrtus communis, Curcuma longa, Cinnamomum cassia, Thymus satureioides, Eucalyptus radiata ssp radiata, Cinnamosma fragrans and Mentha arvensis*



3 Antibacterial activities of essential oils

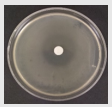
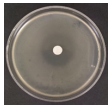
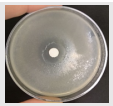
The assessment of the antibacterial activities of essential oils was performed by the diffusion method. The disc absorption capacity was 5 µl/disc only The disk diffusion assay with EOs was conducted to detect antimicrobial activity. Sterile disks were impregnated with 5 ul of EO at different concentrations by serially diluted in vegetal oil; (100%, 10%, 1%) (V/V)), and each disk was placed on a Nutrient Broth Agar plate smeared with *B. subtillis*. The plates were incubated for 48 h at 30 ºC to determine the antimicrobial effect. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each EOs (Table 1). Each reported was realized in two different experiments. As sterile vegetal oil without EO was used as a negative control

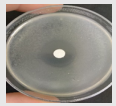
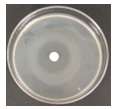
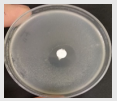
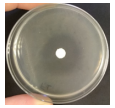
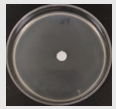
Results and Discussion

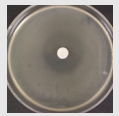
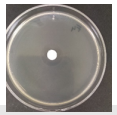
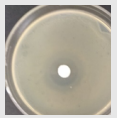
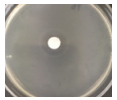
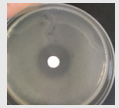
1

Essential Oils against *B. subtillis*

EOs and their constituents play a key role in exerting antimicrobial activity, the result of screening of twenty-four EOs was evaluated against *B. Subtillis*

EOs		Molecule
Origanum compactum		Carvacrol 57.6% Thymol 8.21% γ Terpineno 14.1%
Cymbopogon matinii var.motia		Geraniol 80.5% Geranyl acetate 8.95%
Eugenia caryophyllus		Eugenol 79.9% Eugenyl acetate 12.3%
Mentha arvensis		Menthol 71.1% Mentone 5.88% Isomentone 3.85%
Mentha x piperita		Menthol 44.5% Mentone 18.2% 1.8 cineole 4.64%

EOs		Molecule
Thymus vulgaris CT Linalol		Linalool 68.4% Linalyl acetate 6.19 β- myrcene 3.32%
Thymus satureioides		Borneol 33.4% Thymol 10.6% Carvacrol 7.85%
Chamaemelum nobile		Methylamine angelate 20.2% Metalyl angelate 15.4% Hexil isobutyrate 8.31%
Citrus sinensis		Limonene 95.3%
Citrus reticulata		Limonene 71.1% γ Terpineno 18.3%

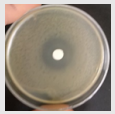
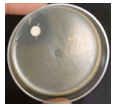
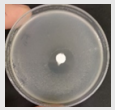
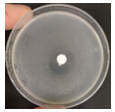
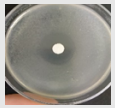
EOs		Molecule
Elettaria cardamomum		Terpenyl acetate 35.3% 1.8 Cineole 32.3% Linalyl acetate 5.35% Linalool 3.35%
Laurus nobilis		1,8 cineole 44.9% Terpenyl acetate 10.5% Sabinene 8.86% Linanool 4.43%
Zingiber officinale		α-zingibereno 28.2% α-Curcumin 7.93% Camphene 7.9% β-sesquifelandrene 7.56%
Cinnamomum camphora		1,8-cineole 56.8% Sabinene 13.4% α-terpineol 7.33%
Rosmarinus officinalis		α Pinene 38.8% Canfeno 8.88% Camphor 6.96% Bornyle acetate 6.94%

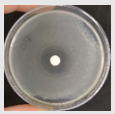
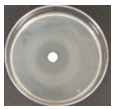
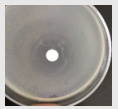
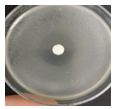
Results and Discussion

1

Essential Oils against *B. subtillis*

EOs and their constituents play a key role in exerting antimicrobial activity, the result of screening of twenty-four EOs was evaluated against *B. Subtillis*.

EOs		Molecule
Melaleuca quinquenervia		1.8 -cineole 50.6% α-terpineole 8.91% Limonene 7.48%
Melaleuca alternofolia		Terpinene 4-ol 40.6% ɣ terpinene 21%
Citrus paradisi		Limonene 94.5%
Citrus junos		Limonene 75.6% ɣ-terpinene 8.49% β- felandreno 3.29%
Myrtus communis		α Pinene 51% 1,8 cineole 22.8% Limonene 8.34%

EOs		Molecule
Curcuma longa		α- turmerone Mw218 34.8% β- turmerone Mw218curlone 16.2% AR-turmerone Mw 216 13.4%
Cinnamomum cassia		E-Cinamaldehyde 81% Cinnamyl acetate 3.25%
Eucalyptus radiata ssp radiata		1.8 cineole 66.6%% α- terpineol 11.2% Limonene 6.5%
Cinnamosma fragrans		1.8 cineole 37.7% Linanool 8.03% Limonene 7.83%

Results and Discussion

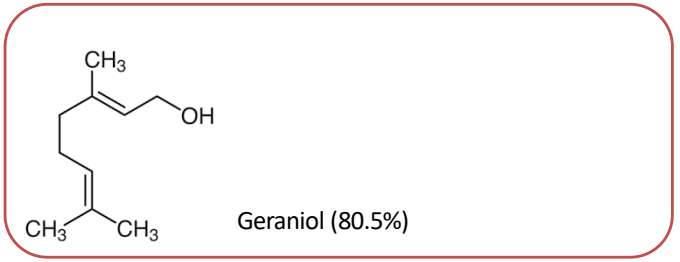
2

MOST EFECTIVE ESSENTIAL OILS:
five oils showed the highest antimicrobial activity at higher concentrations:
Cymbopogon Martinii var. motia (palmarosa oil), *Thymus vulgaris QT Linanol* (Thyme oil), *Thymus satureioides (Moroccan* thyme oil*), Mentha piperita (*peppermint oil*)* and *Eugenia caryophyllus (*clove oil*)*.

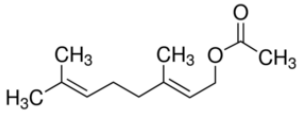
Cymbopogon Martinii var. motia
Palmarosa

Essential Oil

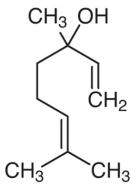
GC/MS
Characterization



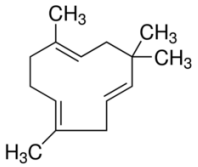
Geraniol (80.5%)



Geranyl acetate (8.95%)



linalool (2.45%)



β-caryophyllene (1.87%)

Results and Discussion

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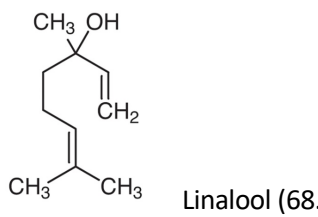
five oils showed the highest antimicrobial activity at higher concentrations:
Cymbopogon Martinii var. motia (palmarosa oil), *Thymus vulgaris QT Linanol* (Thyme oil), *Thymus satureioides (*thyme oil*)*, *Mentha piperita (*peppermint oil*)* and *Eugenia caryophyllus (*clove oil*)*.

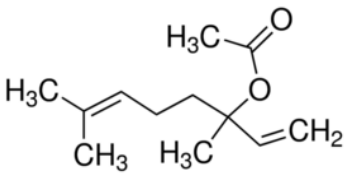
Thymus vulgaris QT Linanol
(Thyme oil)

Essential Oil

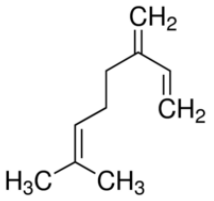
GC/MS
Characterization



Linalool (68.4%);



Linalyl acetate (6.19%)



β-myrcene (3.32%)

Results and Discussion

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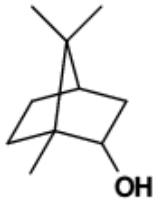
Cymbopogon Martinii var. motia (palmarosa oil), *Thymus vulgaris QT Linanol* (Thyme oil), *Thymus satureioides (*moroccan thyme oil*)*, *Mentha piperita (*peppermint oil*)* and *Eugenia caryophyllus (*clove oil*)*.

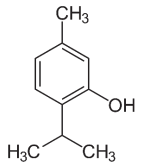
Thymus satureioides
(Moroccan thyme oil*)*

Essential Oil

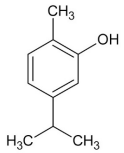
GC/MS
Characterization



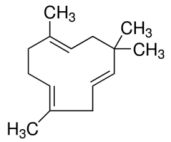
Borneol (33.4%)



Thymol (10.6%)



Carvacrol (7.85%)



β-caryophyllene (5.82%)

Results and Discussion

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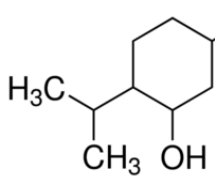
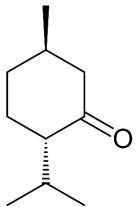
*Mentha piperita (*peppermint oil*)*

Essential Oil

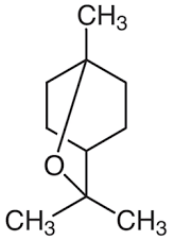
GC/MS
Characterization



Menthol (44.5%)



Mentone (18.2%)



1,8 cineole (4.64%)

Results and Discussion

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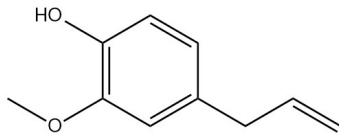
Cymbopogon Martinii var. motia (palmarosa oil), *Thymus vulgaris QT Linanol* (Thyme oil), *Thymus satureioides (Moroccan* thyme oil*)*, *Mentha piperita (*peppermint oil*)* and *Eugenia caryophyllus (*clove oil*)*.

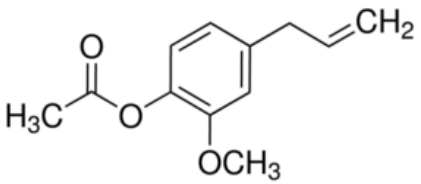
*Eugenia caryophyllus (*clove oil*)*

Essential Oil

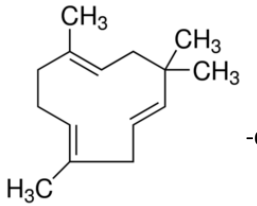
GC/MS
Characterization



Eugenol (79.9%)



Eugenyl acetate (12.3%)



-caryophyllene (5.39 %)

CONCLUSIONS

1. The results obtained in this study confirm the antibacterial and antioxidant activities of five essential oils: *Cymbopogon Martinii* var. motia, *Thymus vulgaris QT Linanol*, *Thymus satureioides*, *Mentha piperita* and *Eugenia caryophyllus*. All of them contain compounds with antioxidant activity (phenolic compounds), that may be used to prevent the growth of bacteria by damaging their membrane.

2. In food industry, the products must be supplied without any microbial contamination. The possible use of EOs to increase the shell life and safety of bakery products, raises new technological solutions despite some limitations, such as altered sensory parameters, may limit its applications.

3. Development of techniques such as nano-encapsulation for bakery doughs, active packaging of baked products or surface disinfectants is required. A choice to introduce *Cymbopogon Martinii* essential oil formula in bakery industry. However, further research is needed to evaluate the safety and the effectiveness of this EO in bakery doughs.