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Contemporary Agriculture, Vol. 73, No. 3-4 (2024): 145-156
DOI: https://doi.org/10.2478/contagri-2024-0018
UDC: 547.913

Original scientific paper

ISSN 2466-4774
https://www.contagri.info/

OPEN ACCESS

PRESERVATION PROPERTIES OF CITRUS ESSENTIAL OILS IN FRUITS AGAINST FOOD SPOILAGE MICROORGANISMS

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Submitted: 16.11.2023.
Reviewed: 29.12.2023.
Accepted: 31.01.2024.

SUMMARY

Pre- and post-harvest spoilage of fruits due to microbial contamination leads to significant economic losses. Therefore, this study was conducted to evaluate the antimicrobial and preservative potential of citrus essential oils (CEOs) extracted from the peels of lime (Citrus aurantifolia), lemon (Citrus limon), and grapefruit (Citrus paradisi). Microorganisms were isolated from apples, cucumbers, tomatoes and oranges using conventional microbiological methods. The in vivo antimicrobial activity of CEOs was assessed against microorganisms in apparently healthy oranges and apples. Bacillus cereus and Penicillium digitatum caused the largest lesions on fruits, with diameters ranging from 28 to 37 mm and 36 to 46 mm, respectively. Disease severity of 86.55% and 91.67 % was observed in the untreated oranges following inoculation with Bacillus cereus and Penicillium digitatum, respectively. The combination of CEOs, applied at a concentration of 100 mg/mL, effectively prevented disease occurrence in both apples and oranges. In contrast, the unpreserved apples and oranges had the highest fungal counts of 9.40 x 10^6 and 3.55 x 10^7 CFU/g, respectively. CEOs were found to extend the shelf life of fruits and preserve their quality without altering organoleptic properties. The bioactive compounds in CEOs can be extracted and utilized as preservative agents to mitigate microbial spoilage of fruits.

Key words: *post-harvest, preservation, citrus peels, essential oils, and shelf life*

Abbreviations:
CEOs - citrus essential oils

INTRODUCTION

The global demand for fruits is increasing due to their numerous health benefits. Fruits are a source of essential nutrients such as vitamins, soluble sugars, amino acids, and macro- and micro-minerals (Slavin & Lloyd, 2012). However, fruit availability is often seasonal due to climatic changes, and available quantities are frequently reduced by pests and microbial activity. Fruit spoilage has a direct impact on the economic development by causing undesirable quality deterioration such as rotting, discoloration, off-flavors, and odors, which reduce the market value of fruits (Erkmen & Bozoglu, 2016). The nutritional composition of fruits predisposes them to microbial spoilage, primarily caused by moulds, yeasts, and acid-tolerant bacteria (Shankar et al., 2021). Fruit spoilage by various molds poses a significant threat to human and animal health due to the production of mycotoxins, which are associated with a range of health issues (Fernández-Cruz et al., 2010).
The use of food additives and chemical preservatives has proven effective in preventing fruit spoilage caused by microorganisms (Oluk & Karaca, 2018). However, the potential negative impacts of chemical preservatives on

human health and the environment have prompted the food industry to reduce reliance on these compounds and explore plant-derived natural products as safer alternative preservative agents. Chemical preservatives such as sorbates, sulphites, and benzoates have been linked to carcinogenic, teratogenic, and neurotoxic effects (Sharif et al., 2017, Piper & Piper, 2017). Plant-derived natural products are gaining relevance in the food industry owing to their minimal health risks, cost-effectiveness, and environmental sustainability compared to synthetic compounds (Lourenço et al., 2019). Bio-preservation, as an alternative food preservation technology, not only ensures the hygienic quality of food but also extends the shelf life, minimizes alterations in organoleptic properties, and enhances the nutritional quality of final food products (Singh, 2018).

In this context, CEOs and other plant-derived essential oils have gained research interest due to their broad-spectrum insecticidal, antibacterial, and antifungal properties against spoilage microorganisms (Angane et al., 2022). CEOs play a crucial role in the food industry by extending the shelf life of food products, such as fruits juices, while ensuring the safety of consumers with a number of health benefits, including antioxidant, anti-inflammatory, anticancer, non-toxic, and hypoallergenic properties (Bora et al., 2020). The consumption of fruits and their products has increased in parallel with global population growth, driven by their health-promoting effects, such as antioxidant, anti-inflammatory, anticancer, anti-diabetic, and neuroprotective benefits, which are attributed to the bioactive compounds they contain (Dhalaria et al., 2020). To meet the growing consumer demand and ensure the availability of fresh fruits, the use of natural plant-based preservatives offers a healthy alternative to synthetic preservatives, effectively restricting the growth of spoilage microorganisms. Therefore, this study investigates the *in vivo* antimicrobial potential of CEOs against microorganisms associated with fruit spoilage and evaluates their preservative efficacy in fruits.

MATERIALS AND METHODS

Source of fruits

Fresh apples were purchased at King's market in Akure, whereas cucumbers, tomatoes, and oranges were harvested at maturity and obtained directly from farmers. The fruits were sorted based on size and absence of physical damage and diseases. Lime, lemon and grapefruit peels were collected immediately after harvest. All the fruits and vegetables considered were authenticated by the Department of Crops, Soil and Pest Management at the Federal University of Technology, Akure.

Extraction of citrus essential oils

Citrus essential oils were extracted from citrus peels via steam distillation for 5 hours using a Clevenger apparatus, as described by Saxena et al. (2012). In brief, citrus peels were loaded into the apparatus, resulting in a clear, light-yellow oily layer forming on the top of the aqueous distillate. This oil layer was separated, dried using anhydrous sodium sulphate, and stored in air-tight glass vials covered with aluminum foil at 4°C until further analysis.

Isolation and identification of microorganisms from fruits

Fruits and vegetables were surface sterilized using 70% v/v alcohol and 1% v/v sodium hypochlorite to eliminate potential contaminants, followed by rinsing with sterile distilled water. Tissue segments from the margins of the rotten areas were aseptically excised and transferred into sterilized peptone water medium (20 ml). Serial dilutions were performed, and an aliquot of 0.1 mL from the 10^4 dilution was aseptically transferred into a Petri dish. Nutrient agar was used for bacterial growth, whereas potato dextrose agar (PDA) was used for fungal growth. The plates were allowed to solidify and incubated at 37 ± 1°C for 24 h for bacteria, and at 25 ± 2 °C for 2-3 days for fungi. Bacterial isolates were grouped based on their morphological, cultural, and staining characteristics. Biochemical tests, including catalase, hydrogen sulphide (H_2S) production, indole, urease, methyl red, oxidase, coagulase, motility, Voges-Proskauer, starch hydrolysis, and sugar fermentation, were conducted following the methods of Cheesbrough (2006). The biochemical results were compared to Bergey's Manual of Systematic Bacteriology for bacterial identification (Krieg et al., 2000). Fungal isolates were identified based on macro- and micro-morphological properties, according to Barnett et al. (2000) and Samson et al. (2010).

Pathogenicity test of isolated microorganisms

The pathogenicity test was conducted on apparently healthy, mature fruits (apples, cucumbers, tomatoes, and oranges) using the inoculation method described by Sani et al. (2015) and Chukwuka et al. (2010), with minor modification. In brief, freshly harvested ripe fruits were surface sterilized with 75% ethanol and rinsed five times with sterile distilled water. The surface-sterilized fruits were wounded with a sterile cork borer (4 mm) and inoculated with a bacterial inoculum (x 10^4 CFU/ml) of a 24-hour-old culture, followed by incubation at 37 ± 1°C.

Fungal spores (x 10^4 CFU/ml) from a 48-hour-old inoculum were also inoculated and incubated at 25 $\pm$ 1°C. The control fruits were injected with sterile distilled water. The inoculated fruits and controls were incubated under laboratory conditions (22 $\pm$ 2 °C) for 7 days. The diameters of the resulting lesions (rot) on the fruits were measured.

In vivo antimicrobial evaluation of CEOs (Disease severity)

The *in vivo* antimicrobial study of CEOs was conducted using a bacterium and a fungus responsible for prominent lesions on two of the most affected fruit crops. The method described by Lopez-Reyes et al. (2010) was adopted with minor modifications. Briefly, the fruits were disinfected with a 2% sodium hypochlorite solution for 2 minutes, rinsed with sterile distilled water, and allowed to air-dry. The fruits were then randomly assigned to groups, each consisting of five fruits. Wounds were created on the fruits using a sterilized cork borer to a depth of 0.4 cm and a width of 6.0 cm. The wounds were inoculated with a spore suspension containing 10^4 spores/ml of fungi and 10^4 CFU/ml of bacteria. Subsequently, CEOs and their combinations were introduced into the inoculation site. As controls, five fruits were wounded without pathogen inoculation, and another group of five fruits was wounded, inoculated with pathogens, but not treated with any CEOs. An additional group of five fruits was wounded, inoculated, and treated with a sodium benzoate solution. All treatments were maintained in an insect-free, ventilated chamber for 14 days at room temperature. Disease severity was calculated using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum(\text{number of infected fruits x number scale}) \quad \text{x } 100}{\text{Total number of fruits x highest number scale}}$$

The disease severity scale was as follows: 0 = no infection, 1 = one-quarter fruit decay, 2 = half fruit decay, 3 = three-quarters fruit decay, and 4 = complete fruit decay.

Evaluation of the preservative effect of CEOs and consumer palatability

This evaluation was conducted on fresh, apparently healthy oranges and apples. All the fruits were disinfected with a 2% sodium hypochlorite solution, rinsed with sterile distilled water, and allowed to air-dry. The oranges and apples were then sprayed with the stock solution of CEOs and stored in sterile commercial packaging in a well-ventilated, insect-free chamber. The fruits were observed daily over a period of three weeks. For quality assessments, sensory attributes such as color, firmness, taste, and aroma were evaluated by a panel of 30 participants, consisting of 15 females and 15 males. The respondents rated their level of agreement with eight statements using a 7-point scale, where 7 indicated "like extremely," 6 "like very much," 5 "like moderately," 4 "neither like nor dislike," 3 "dislike moderately," 2 "dislike very much," and 1 "dislike extremely." The appearance and visual characteristics of the fruits were assessed under natural daylight conditions.

Microbiological assessment of fruits preserved with CEOs

The microbiological evaluation of fruits preserved with CEOs was performed using the methods described under "Isolation and Identification of Microorganisms from Fruits."

Statistical analysis

The experimental data were subjected to one-way analysis of variance (ANOVA), and the means were separated using Duncan's New Multiple Range Test. Statistical significance was determined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Isolated and identified microorganisms from fruits

Microbial spoilage of fruits, coupled with the side effects of chemical preservatives, has prompted significant efforts to identify natural preservative agents. This study presents the antimicrobial and preservative efficacies of CEOs derived from the peels of lime, lemon, and grapefruit. Table 1 shows the spoilage microorganisms and the diameters of the lesions caused on fruits and vegetables. The microorganisms identified as responsible for these lesions include *Aspergillus niger*, *Fusarium avenaceum*, *Penicillium digitatum*, *Rhizopus stolonifer*, *Alternaria alternata*, *Saccharomyces cerevisiae*, *Candida tropicalis, Pichia kluyveri*, *Geotrichum candidum*, *Zygosaccharomyces rouxii*, *Bacillus muralis, Micrococcus luteus*, *Pseudomonas aeruginosa*, among others.

These findings align with the report by Erkmen & Bozoglu (2016), who identified *Aspergillus*, *Penicillium*, *Mucor*, *Alternaria*, *Cladosporium*, *Botrytis*, *Saccharomyces*, *Hanseniaspora*, *Pichia*, *Kloeckera*, *Candida*, and *Rhodotorula* as the most common genera of moulds and yeasts contaminating fruits and vegetables. The studies by Abdullah et al. (2016), Oo et al. (2018), and Khodadadi et al. (2020) isolated the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Cochliobolus*, *Curvularia*, *Colletotrichum*, *Eurotium*, *Eupenicillium*, *Fusarium*, *Gliochladium*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma*, *Ulocladium*, and *Verticillium* from fruits such as apples, oranges, cucumbers, bananas, mangoes, and grapefruits. Erkmen & Bozoglu (2016) further reported that specific microorganisms, such as *Cryptosporiopsis malicorticis*, cause lenticel rot in apples, whereas *Ceratocystis paradoxa*, *Fusarium roseum*, *C. musae*, and *Verticillium theobromae* are responsible for crown rot and bitter rot in bananas. Alegbeleye et al. (2018) identified several potential routes and sources of microbial contamination in fruits, including irrigation water, air, animals, waste containing diverse microorganisms, improper pre-harvest fungicide application, inadequate washing and culling, fluid exudates, contamination by plant pathogens, manure, soil, and other factors that promote microbial growth.

Table 1. Lesion diameter (mm) caused by microorganisms inoculated onto apparently healthy fruits

Fungi	Orange	Cucumber	Tomato	Apple
Aspergillus niger	36	18	20	21
Aspergillus flavus	18	11	20	29
Penicillium expansum	16	15	15	15
Penicillium digitatum	46	38	36	44
Aspergillus fumigatus	15	14	15	13
Aspergillus parasiticus	10	12	13	14
Fusarium solani	31	22	18	29
Fusarium oxysporum	12	14	13	18
Fusarium avenaceum	21	23	15	16
Mucor piriformis	24	23	17	18
Mucor racemosis	17	16	24	23
Rhizopus stolonifer	19	21	27	20
Rhizopus oryzae	30	32	36	15
Alternaria alternata	16	17	14	13
Candida tropicalis	10	13	13	16
Saccharomyces cerevisiae	21	20	14	24
Candida krusei	17	18	16	17
Torulopsis fragaria	14	17	18	15
Pichia kluyveri	15	23	16	17
Pichia anomala	15	18	18	25
Pichia fermentans	27	13	12	32
Geotrichum candidum	21	17	19	18
Zygosaccharomyces rouxii	20	22	21	24
Zygosaccharomyces bailii	12	16	11	09
Bacteria				
Lactobacillus plantarum	32	18	23	27
Bacillus cereus	37	32	28	34
Micrococcus luteus	20	18	11	20
Streptococcus uberis	19	16	14	23
Bacillus subtilis	12	15	18	19
Proteus vulgaris	16	15	17	19
Serratia marcescens	25	11	14	15
Streptococcus agalactiae	21	24	19	31
Pseudomonas aeruginosa	15	27	20	21

Pathogenicity test of isolated microorganisms

The size of lesions varied depending on the microorganisms isolated and the type of fruits. The lesions ranged from 10 mm to 46 mm on the oranges, 11 mm to 32 mm on the cucumbers, 11 mm to 36 mm on the tomatoes, and 9 mm to 44 mm on the apples. *Penicillium digitatum* (fungus) and *Bacillus cereus* (bacterium) caused significantly larger lesions than other microorganisms on the oranges and apples. The presence and pathogenicity of *Penicillium* sp. and *Bacillus cereus* on the apples are congruous with the findings of Juhneviča et al. (2011), who reported the dominance of these microorganisms in stored apples. *Rhizopus oryzae* caused larger lesions, with diameters of 32 mm and 36 mm on the cucumber and tomato, respectively. Petrasch et al. (2019) demonstrated that *Botrytis cinerea, Fusarium acuminatum*, and *Rhizopus stolonifer* readily infect ripe tomatoes, rapidly leading to disease symptoms. The isolated microorganisms from the studied fruits are capable of colonizing fresh, healthy fruits and causing lesions, indicating that they are spoilage microbes. Fruits contain a suitable balance of nutrients (carbohydrates, minerals, vitamins, and proteins), making them an ideal substrate for the infestation of spoilage microorganisms. The development of lesions depends on both the type of fruit and the microbial species involved (Zhao et al., 2020). The microorganism colonization of and lesion development on fruits often occur more rapidly when external damage, such as bruising, cracks, or punctures, is present, accelerating cross-contamination by microorganisms during handling, washing, sorting, and packing (Barth et al., 2009). In addition to causing lesions, some of the isolated microorganisms have been associated with premature fruit dropping and infections that promote false ripening (Gong et al. 2019). Lanza et al. (2019) demonstrated that large lesion influence the attachment of fruits to the tree, with over 90% of dropped fruits exhibiting significant lesions. Microbial spoilage is often associated with alterations in fruit that reduce palatability and, in some cases, render the fruit toxic to consumers. Such spoilage is typically accompanied by changes in taste, odor, and appearance, negatively impacting market value. Therefore, there is a need for natural protective and preservative agents.

***In vivo* antimicrobial evaluation of citrus essential oils (disease severity)**

The antimicrobial effects of lime, lemon, and grapefruit essential oils, as well as their synergistic combinations, were evaluated against *Penicillium digitatum* and *Bacillus cereus*, prominent pathogens isolated from fruits. The oranges and apples were inoculated, and the results are presented in Table 2. The CEOs demonstrated significant effectiveness at various concentrations, reducing the disease severity in the inoculated and treated fruits compared to the untreated controls. The disease severity (%) was 86.55% and 91.67% in the untreated oranges inoculated with *Bacillus cereus* and *P. digitatum*, respectively. The disease severity was 82.22% and 88.33% in the untreated apples inoculated with *Bacillus cereus* and *P. digitatum*, respectively. The inhibitory potential of the CEOs increased significantly with higher concentrations. In the oranges, both *Bacillus cereus* and *P. digitatum* were completely inhibited by all the CEOs at 100 mg/mL, except for the fruits treated with grapefruit essential oil, which exhibited a reduced disease severity of 5.56% and 9.44%. The combination of two citrus peel oils proved more effective than their individual applications. The lime and lemon essential oils were more effective than the grapefruit essential oil in reducing infections caused by *P. digitatum* on both the oranges and apples. The combination of lime, lemon, and grapefruit essential oils at 50 mg/mL resulted in a disease severity of only 13.89% in the oranges infected with *P. digitatum*, compared to the highest disease severity (91.67%) in the inoculated, untreated oranges. Figures 1 and 2 illustrate the effects of the CEOs on the oranges infected with *P. digitatum* and the apples infected with *B. cereus*, respectively. At 100 mg/mL, the combination of CEOs from lime, lemon, and grapefruit peels showed no disease severity in either the apples or oranges. The findings by Abd-Elwahab et al. (2016) and Guo et al. (2018) revealed that the CEOs from orange, lime, mandarin, and grapefruit peels displayed pronounced antibacterial activity against *S. aureus*, *B. subtilis*, *E. faecalis*, *E. coli*, *Neisseria gonorrhoeae*, and *P. aeruginosa*. The antimicrobial efficacy of CEOs against microorganisms such as *Bacillus cereus*, *B. subtilis*, *Micrococcus luteus*, *Streptococcus uberis*, *Proteus vulgaris*, *Alternaria alternata*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, as well as species of *Aspergillus, Mucor, Fusarium, Penicillium, Rhizopus, Candida, and Pichia* isolated from fruits, has been largely attributed to the presence of phenolic compounds such as limonene, alpha-terpineol, beta-pinene, terpinen-4-ol, alpha-phellandrene, and other bioactive compounds, as identified by the gas chromatography-mass spectrometry (GC-MS) in the studies of Ajayi-Moses et al. (2019). The combination of essential oils was highly effective in limiting the growth rates of microorganisms on fruits. Additionally, the bioactive compounds in the essential oils of thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*), rosemary (*Rosmarinus officinalis*), and marjoram (*Origanum majorana*) were responsible for the *in vivo* antifungal activity against *Penicillium expansum* and *Botrytis cinerea* in the inoculated pears (Nikkhah et al., 2019).

Table 2. Synergistic effects of citrus essential oil on fruits infected with *Penicillium digitatum* and *Bacillus cereus*

Treatment	***Penicillium digitatum***			***Bacillus cereus***		
Oil	**Orange disease severity (%)**					
	25 mg/ml	**50 mg/ml**	**100 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
Lime	29.95 ± 0.87d	17.78 ± 0.55cd	0.00 ± 0.00a	28.33 ± 0.96d	16.11 ± 0.56cd	0.00 ± 0.00a
Lemon	40.56 ± 0.56f	21.11 ± 1.10e	0.00 ± 0.00a	31.67 ± 0.96e	17.77 ± 0.55d	0.00 ± 0.00a
Grapefruit	56.11 ± 0.56h	30.56 ± 0.56g	9.44 ± 0.56b	51.11 ± 0.56g	24.44 ± 0.56e	5.56 ± 0.06b
Lime/lemon	24.44 ± 0.56c	16.11 ± 1.11c	0.00 ± 0.00a	23.89 ± 0.56b	15.56 ± 0.50c	0.00 ± 0.00a
Lime/grapefruit	36.67 ± 0.96e	17.78 ± 1.11cd	0.00 ± 0.00a	26.67 ± 0.96c	16.11 ± 1.11cd	0.00 ± 0.00a
Lemon/grapefruit	42.22 ± 0.55f	18.89 ± 0.56d	0.00 ± 0.00a	28.89 ± 0.56d	17.22 ± 0.55d	0.00 ± 0.00a
Lime/lemon/grapefruit	18.89 ± 0.56b	13.89 ± 0.56b	0.00 ± 0.00a	22.22 ± 0.55b	10.56 ± 0.56b	0.00 ± 0.00a
Sodium benzoate	50.56 ± 0.56g	26.67 ± 0.96f	0.00 ± 0.00a	47.77 ± 0.55f	26.11 ± 0.56e	7.22 ± 0.55c
Non inoculated control	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Inoculated and not treated	91.67 ± 7.96i	91.67 ± 7.96h	91.67 ± 7.96c	86.55 ± 4.06h	86.55 ± 4.06f	86.55 ± 4.06d
	Apple disease severity (%)					
Lime	54.44 ± 0.55cd	28.89 ± 0.56d	10.56 ± 0.02cd	60.56 ± 2.41b	27.78 ± 0.65d	9.44 ± 0.06cd
Lemon	59.44 ± 0.26d	29.44 ± 0.56de	10.56 ± 0.02cd	58.89 ± 1.00b	24.44 ± 0.61c	8.89 ± 0.05cd
Grapefruit	68.33 ± 0.96f	40.56 ± 0.56g	14.44 ± 0.16e	70.56 ± 2.05de	38.89 ± 1.03g	12.22 ± 0.02e
Lime/lemon	52.77 ± 0.55c	24.44 ± 0.56c	6.11 ± 0.05b	59.44 ± 0.16b	22.78 ± 0.60c	5.56 ± 0.16b
Lime/grapefruit	55.00 ± 0.00cd	35.56 ± 0.56f	9.22 ± 0.39c	72.78 ± 0.55e	30.56 ± 0.72e	7.55 ± 0.08c
Lemon/grapefruit	56.11 ± 1.11cd	37.22 ± 0.55f	11.11 ± 0.06d	68.33 ± 0.96d	34.44 ± 0.56f	10.00 ± 0.16d
Lime/lemon/grapefruit	49.44 ± 0.56b	20.56 ± 0.56b	0.00 ± 0.00a	65.67 ± 1.39c	19.44 ± 1.16b	0.00 ± 0.00a
Sodium benzoate	66.11 ± 1.26e	31.11 ± 1.11e	5.00 ± 0.00b	66.67 ± 1.67c	26.67 ± 0.09d	0.00 ± 0.00a
Non inoculated control	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Inoculated and not treated	88.33 ± 0.96g	88.33 ± 0.96h	88.33 ± 0.96f	82.22 ± 7.55f	82.22 ± 7.55h	82.22 ± 7.55f

Legend: Data are presented as mean ± standard error (n = 3). Values within the same column for each fruit that share the same superscript are not significantly different ($p < 0.05$)

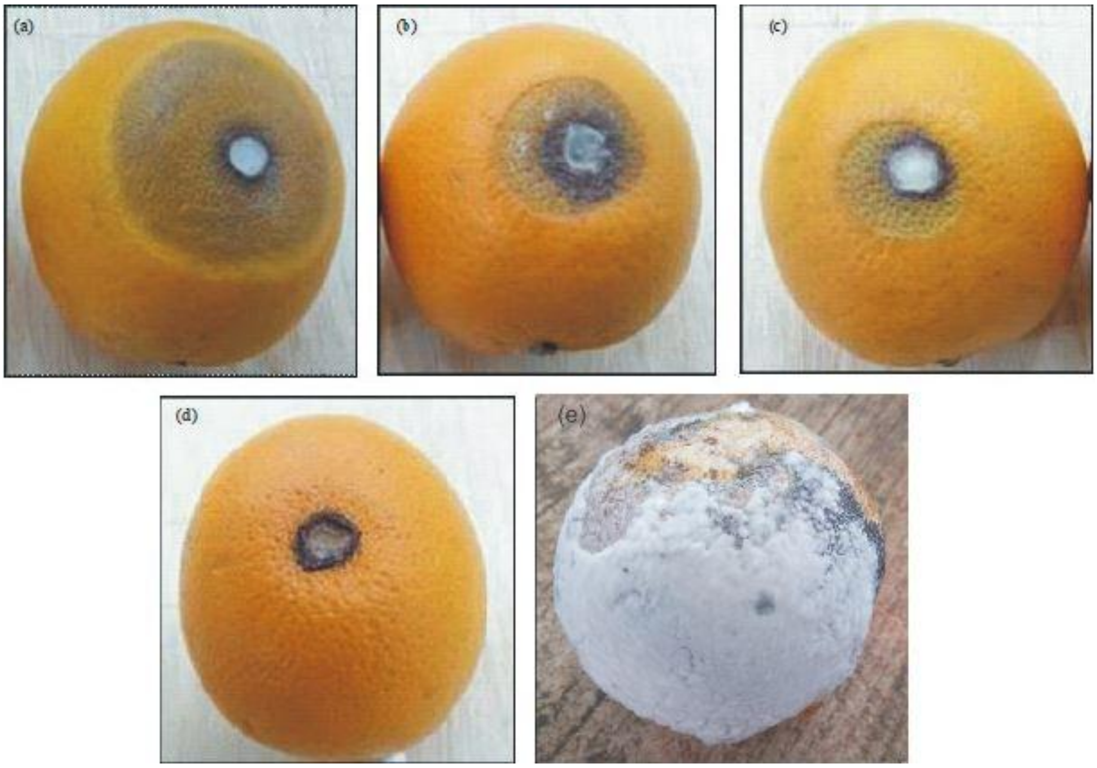

Figure 1. Orange infected with *P. digitatum* and treated with the CEOs of lime + lemon + grape at a- 25 mg/ml, b- 50 mg/ml, c-100 mg/ml, d- non-inoculated with *P. digitatum*, e- inoculated with *P. digitatum* and remain untreated

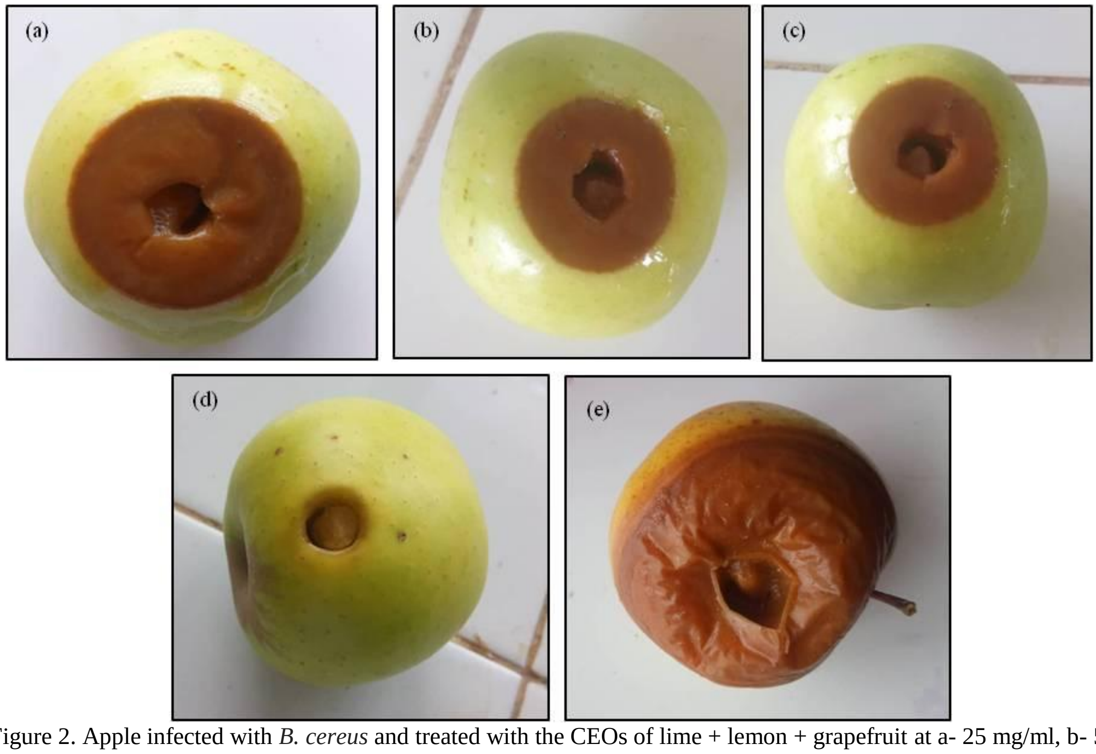

Figure 2. Apple infected with *B. cereus* and treated with the CEOs of lime + lemon + grapefruit at a- 25 mg/ml, b- 50 mg/ml, c-100 mg/ml, d- non-inoculated with *B. cereus*, e- inoculated with *B. cereus* and remain untreated

Evaluation of the preservative effect of CEOs and consumer palatability

Table 3 presents the organoleptic properties of fruits preserved with sodium benzoate and the CEOs from lime, lemon, and grapefruit peels, as well as the unpreserved fruit group. The fruits preserved with the CEOs maintained their quality and were preferred over those preserved with sodium benzoate and the unpreserved fruits. The color of preserved oranges ranged from 3.60 to 6.93, whereas the color of preserved apples ranged from 2.67 to 6.53. The aroma of the fruits (apples and oranges) ranged from 3.80 to 7.00, with significant differences ($p \leq 0.05$) compared to the fruits preserved with sodium benzoate (3.00 to 4.10). Overall, the color, taste, and aroma of fruits preserved with all the CEOs were better and more acceptable to the panelists. The CEOs from lime, lemon, and grapefruit peels exhibited a thin, watery viscosity, a pale greenish-yellow color, and a sharp yet fresh fragrance. These findings are consistent with those of Jafari et al. (2011) and Lourenço et al. (2019), who reported that the pleasant fragrance, color, flavor, and texture of essential oils can be largely attributed to their chemical constituents, which enhance the shelf life of food products and maintain the quality parameters desired by consumers. This study demonstrates that CEOs are more effective in maintaining the color, firmness, aroma, and texture of fruits than chemical preservatives. Plant-derived essential oils are Generally Recognized as Safe (GRAS) and can be considered a natural alternative to extending the shelf life of fruits by inhibiting the proliferation of spoilage microorganisms. These oils contain major bioactive components responsible for various bio-functional activities, such as antimicrobial, antioxidant, and preservative properties, with different mechanisms of action (Angane et al., 2022). The use of CEOs as flavoring agents, additives, antimicrobials, antioxidants, and preservatives in foods and beverages is accepted by the Food and Drug Administration, as they are considered GRAS within the food industry (Palazzolo et al., 2013).

Microbiological assessment of fruits preserved with CEOs

The microbiological quality of fruits preserved with the CEOs considered is presented in Table 4. The unpreserved oranges had the highest total bacterial count (8.9×10^5 CFU/g) and a fungal count of 3.55×10^7 CFU/g. In like fashion, the unpreserved apples had the highest bacterial count (3.69×10^5) and a fungal count of 9.40×10^6 CFU/g. No microbial growth was observed on the apples and oranges preserved with the combined CEOs after 5 days. However, the total microbial count increased as the storage period extended from day 5 to day 15. For the oranges treated with the CEOs, the total bacterial count ranged from 1.20×10^3 to 6.30×10^5 CFU/g, whereas for apples, it ranged from 0.97×10^4 to 3.10×10^5 CFU/g. The fungal count ranged from 1.03×10^4 to 11.40×10^6 CFU/g for the oranges and from 0.60×10^4 to 4.82×10^6 CFU/g for the apples treated with the CEOs. The application of the CEOs from lime, lemon, and grapefruit peels significantly reduced the microbial load in the preserved oranges and apples. The CEOs exhibited strong preservative activity against colonizing and spoilage microorganisms associated with these fruits. The antimicrobial and preservative effects of the CEOs are attributed to the presence of bioactive compounds, including α-/β-pinene, sabinene, β-myrcene, d-limonene, linalool, α-humulene, α-terpineol, phenolics, terpenes, and terpenoids (Bora et al., 2020). These bioactive compounds in the CEOs have potential as effective preservative agents for the food industry, offering a natural alternative to chemical preservatives, which have been associated with various toxicological concerns.

Table 3. Organoleptic properties of oranges and apples preserved with CEOs at 50 mg/ml

Treatments	Color			Firmness			Taste			Aroma		
	Day 5	Day 10	Day 15	Day 5	Day 10	Day 15	Day 5	Day 10	Day 15	Day 5	Day 10	Day 15
Orange												
LI	6.87±0.06^{a}	5.40±0.09^{ab}	3.60±0.09^{b}	6.63±0.09^{b}	5.33±0.09^{b}	3.73±0.08^{c}	6.97±0.03^{c}	6.20±0.10^{b}	4.43±0.09^{b}	7.00±0.00^{e}	6.17±0.07^{e}	4.70±0.09^{e}
LE	6.83±0.07^{a}	5.43±0.09^{ab}	3.67±0.09^{b}	6.70±0.09^{c}	5.60±0.09^{c}	3.70±0.09^{c}	6.93±0.05^{c}	6.17±0.10^{b}	4.50±0.09^{b}	6.27±0.08^{c}	4.70±0.09^{c}	4.20±0.07^{c}
G	6.93±0.05^{a}	5.53±0.13^{ab}	3.90±0.10^{c}	6.53±0.09^{a}	5.77±0.08^{c}	3.53±0.09^{b}	6.93±0.05^{c}	6.10±0.09^{b}	4.40±0.09^{b}	6.10±0.06^{c}	4.60±0.09^{c}	4.13±0.06^{c}
LI +LE	6.90±0.06^{a}	5.46±0.09^{ab}	3.70±0.09^{b}	6.83±0.07^{d}	5.83±0.07^{d}	3.86±0.09^{d}	6.97±0.03^{c}	6.27±0.11^{c}	4.50±0.09^{b}	6.57±0.09^{d}	5.47±0.15^{d}	4.47±0.09^{d}
LI+ G	6.90±0.06^{a}	5.43±0.09^{ab}	3.80±0.10^{bc}	6.67±0.09^{bc}	5.70±0.09^{c}	3.73±0.08^{c}	6.93±0.05^{c}	6.23±0.09^{b}	4.43±0.09^{b}	6.50±0.09^{d}	5.43±0.14^{d}	4.30±0.09^{cd}
LE+G	6.90±0.06^{a}	5.40±0.09^{ab}	3.83±0.11^{bc}	6.80±0.07^{d}	5.60±0.09^{c}	3.47±0.09^{b}	6.90±0.06^{c}	6.27±0.08^{c}	4.60±0.10^{b}	6.23±0.08^{c}	4.83±0.07^{c}	3.80±0.07^{b}
LI+LE+G	6.93±0.05^{a}	5.70±0.14^{b}	3.83±0.12^{bc}	6.90±0.06^{e}	5.97±0.08^{d}	3.97±0.11^{e}	6.97±0.03^{c}	6.30±0.11^{c}	4.53±0.09^{b}	6.50±0.09^{d}	5.47±0.13^{d}	4.17±0.07^{c}
SB	6.73±0.11^{a}	5.30±0.09^{a}	3.70±0.47^{b}	6.93±0.09^{e}	6.10±0.09^{e}	3.43±0.09^{b}	6.77±0.08^{ab}	6.00±0.07^{ab}	3.90±0.10^{a}	4.10±0.06^{a}	3.77±0.08^{a}	3.07±0.05^{a}
Unpreserved	6.80±0.07^{a}	5.23±0.08^{a}	3.50±0.09^{a}	6.53±0.09^{a}	4.87±0.06^{a}	3.27±0.13^{a}	6.73±0.08^{a}	5.83±0.08^{a}	4.10±0.06^{a}	4.60±0.09^{b}	4.07±0.07^{b}	3.20±0.07^{a}
Apple												
LI	6.17±0.07^{b}	3.93±0.12^{ab}	2.87±0.06^{c}	6.33±0.13^{b}	4.87±0.06^{c}	3.33±0.09^{bc}	6.93±0.05^{b}	6.07±0.11^{c}	3.80±0.07^{c}	7.00±0.00^{f}	6.03±0.08^{f}	4.60±0.09^{f}
LE	6.27±0.08^{c}	3.90±0.11^{ab}	2.77±0.08^{c}	6.33±0.14^{b}	4.93±0.08^{cd}	3.20±0.07^{b}	6.93±0.05^{b}	6.03±0.09^{c}	3.73±0.08^{bc}	6.17±0.07^{d}	4.63±0.09^{d}	4.29±0.08^{cd}
G	6.53±0.09^{cd}	3.97±0.12^{ab}	2.67±0.09^{bc}	6.17±0.14^{b}	4.80±0.09^{c}	3.10±0.06^{b}	6.90±0.06^{b}	6.10±0.10^{c}	3.60±0.09^{b}	6.03±0.03^{c}	4.43±0.09^{c}	4.23±0.08^{c}
LI +LE	6.26±0.08^{c}	4.00±0.13^{bc}	3.00±0.07^{e}	6.33±0.13^{b}	5.00±0.08^{d}	3.43±0.09^{c}	6.93±0.05^{b}	6.00±0.10^{c}	3.90±0.06^{d}	6.40±0.09^{e}	5.27±0.12^{e}	4.47±0.09^{e}
LI+ G	6.27±0.08^{c}	4.03±0.12^{c}	2.87±0.06^{cd}	6.30±0.14^{b}	4.80±0.09^{c}	3.50±0.09^{cd}	6.96±0.03^{b}	5.93±0.13^{bc}	3.86±0.06^{cd}	6.37±0.09^{de}	5.20±0.14^{e}	4.30±0.09^{cd}
LE+G	6.33±0.09^{c}	4.07±0.11^{c}	2.90±0.07^{cd}	6.80±0.07^{cd}	4.70±0.09^{b}	3.67±0.09^{e}	6.96±0.03^{b}	6.26±0.13^{d}	3.73±0.08^{bc}	6.07±0.09^{c}	4.30±0.09^{c}	3.80±0.07^{b}
LI+LE+G	6.30±0.09^{c}	4.10±0.12^{c}	2.97±0.08^{de}	6.37±0.10^{bc}	4.87±0.06^{c}	3.87±0.12^{f}	6.93±0.05^{b}	6.30±0.11^{d}	3.97±0.03^{e}	6.40±0.09^{e}	5.46±0.13^{e}	4.23±0.06^{c}
SB	5.83±0.07^{a}	3.77±0.79^{a}	2.63±0.09^{ab}	6.33±0.13^{b}	5.93±0.05^{e}	3.63±0.09^{e}	6.60±0.09^{a}	5.77±0.08^{a}	3.30±0.09^{a}	4.10±0.06^{a}	3.76±0.08^{a}	3.00±0.07^{a}
Unpreserved	6.10±0.06^{b}	3.87±0.06^{a}	2.53±0.09^{a}	5.93±0.13^{a}	4.60±0.09^{a}	2.90±0.06^{a}	6.89±0.02^{b}	5.73±0.04^{a}	3.53±0.09^{b}	4.60±0.09^{b}	4.07±0.07^{b}	3.03±0.03^{a}

Legend: Data are presented as mean ± standard error (n = 3). Values within the same column for each fruit that share the same superscript are not significantly different ($p < 0.05$). Keys: LI- Lime, LE-Lemon, G-Grapefruit, SB- Sodium benzoate

Table 4. Microbial load (CFU/g) of oranges and apples preserved with CEOs at 50 mg/ml for 15 days

Treatments	Bacterial load			Fungal load		
	Day 5 (x10^3)	Day 10 (x10^4)	Day 15 (x10^5)	Day 5 (x10^4)	Day 10 (x10^6)	Day 15 (x10^6)
Orange						
LI	1.70±0.14^c	5.30±0.18^e	4.10±0.10^b	2.80±0.20^{bc}	8.80±0.30^d	9.60±0.90^c
LE	1.90±0.17^d	5.40±0.06^e	5.40±0.13^c	3.10±0.10^d	8.00±0.50^d	10.80±0.20^d
G	1.90±0.08^d	6.30±0.02^f	5.70±0.16^d	3.80±0.10^e	10.10±0.50^e	11.40±2.50^d
LI +LE	1.30±0.11^b	4.70±0.13^d	5.30±0.15^c	1.80±0.10^b	6.50±0.30^c	8.30±0.50^c
LI+ G	1.20±0.11^b	4.20±0.10^c	6.30±0.21^e	2.10±0.20^b	6.40±0.40^c	9.40±0.30^c
LE+G	1.20±0.13^b	4.50±0.53^b	4.90±0.18^{bc}	2.70±0.20^{bc}	7.10±0.20^{cd}	9.00±1.20^c
LI+LE+G	0.00±0.0^a	3.10±0.11^a	3.00±0.18^a	1.03±0.10^a	2.00±0.20^a	3.30±0.50^a
SB	1.90±0.04^d	3.60±0.13^b	3.00±0.15^a	2.40±0.10^b	3.70±0.20^b	5.80±0.40^b
Unpreserved	3.70±0.15^e	7.60±0.28^f	8.90±0.25^f	7.80±0.10^f	14.60±0.90^e	35.50±1.30^e
Apple						
LI	0.00±0.00^a	1.41±0.23^b	2.30±0.12^b	2.12±0.06^d	3.07±0.09^b	4.19±0.10^b
LE	0.00±0.00^a	1.34±0.07^b	2.41±0.07^b	2.38±0.05^d	3.10±0.06^b	4.38±0.11^c
G	0.00±0.00^a	2.08±0.05^d	2.99±0.06^c	2.12±0.06^d	3.10±0.10^b	4.82±0.09^d
LI +LE	0.00±0.00^a	1.44±0.23^b	2.20±0.10^b	1.56±0.16^c	3.15±0.03^b	4.39±0.10^c
LI+ G	0.00±0.00^a	1.63±0.11^c	2.49±0.13^b	1.74±0.06^c	3.06±0.04^b	4.40±0.12^c
LE+G	0.00±0.00^a	1.94±0.04^{cd}	3.10±0.06^c	1.32±0.09^b	3.03±0.09^b	4.78±0.13^{cd}
LI+LE+G	0.00±0.00^a	0.97±0.00^a	1.04±0.04^a	0.60±0.05^a	1.14±0.10^a	2.04±0.04^a
SB	0.00±0.00^a	1.10±0.06^a	1.65±0.09^a	1.37±0.07^b	3.08±0.07^b	3.84±0.03^b
Unpreserved	1.06±0.00^b	2.61±0.08^e	3.69±0.12^d	4.49±0.23^e	4.34±0.03^c	9.40±0.26^f

Legend: Data are presented as mean ± standard error (n = 3). Values within the same column for each fruit that share the same superscript are not significantly different ($p < 0.05$). 0.0 = no microbial growth
Keys: LI- Lime, LE- Lemon, G-Grapefruit, SB- Sodium benzoate

CONCLUSION

Citrus fruit peels are widely available agricultural by-products globally. Essential oils extracted from these peels demonstrated significant antimicrobial activity, inhibiting microorganisms capable of causing lesions and other disease symptoms in selected fruits. The fruits coated (preserved) with CEOs were well-accepted by consumers, with no reported adverse effects. The application of CEOs as preservative agents on oranges and apples delayed, reduced, and in some cases, completely inhibited the colonization of spoilage microorganisms. The synergistic potential of the CEOs derived from citrus peels exhibited superior antimicrobial and preservative efficacy. The bioactive compounds in the CEOs responsible for this microbial inhibition and fruit preservation offer promising applications in the food industry, potentially reducing the need for chemical preservatives, which are associated with adverse side effects.

Authors' contributions: OCO, OBA and BJA designed the research. OCO and OBA conducted the experimental work. OCO and BJA supervised the study. OCO and OBA drafted the manuscript. All authors revised the manuscript. All authors reviewed and approved the final manuscript.

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Conflict of interest: The authors declare that they have no conflict of interest.

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