

Efficacy of *Cinnamomum cassia* essential oil as a natural preservative and flavoring agent to improve orange juice quality and safety

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Abstract

Essential oils (EOs) are promising safe and natural alternatives for pasteurization and synthetic preservation of orange juice. The current research investigates the efficacy of *Cinnamomum cassia* essential oil (CCEO) as a natural food preservative and flavoring agent in orange juice. We evaluated the physicochemical, microbiological, and sensorial qualities of the juice treated with CCEO over 28 days of storage at 4°C, with measurements taken on every day 7. Results showed that CCEO significantly improved quality indicators by reducing browning reactions and inhibiting pectin methyl esterase activity ($p < 0.05$). However, potential hydrogen and titratable acidity remained unaffected ($p > 0.05$). CCEO significantly ($p < 0.05$) enhanced the microbiological and sensory properties of orange juice by reducing microbial load and enhancing taste, color, flavor, and the overall acceptability parameters. These findings demonstrated the potential of CCEO as a natural food preservative and flavoring agent for orange juice.

Keywords: *Cinnamomum cassia*; essential oil; food safety; orange juice; preservative

Introduction

Orange juice, because of its pleasant taste, health benefits, rich nutritional content, and energy uptake, is a widely consumed global drink (Cristiny de Oliveira Vieira *et al.*, 2020). It is a known valuable source of bioactive compounds, such as antioxidants, phenolic compounds,

folic acid, and vitamin C with pleasant sensory characteristics (de Prado *et al.*, 2019; Paravisini and Peterson, 2019). Apart from flavor, color is one of the most significant factors to determine consumer acceptance, because color is the first sensory indication experienced by consumers. Recent technological advancements in storage extension have revealed that the development of a brown

color in the juice is the primary cause of quality degradation (Paravisini and Peterson, 2016). In addition, the cloud stability of the juice is affected by pectin methyl esterase (PME) activity (Aghajanzadeh *et al.*, 2017). Fruit juice is a preferred substrate for the growth of microorganisms; hence, producers commonly use either pasteurization or chemical synthetic preservatives to prevent growth of the same. However, pasteurization affects the nutritional quality and flavor of juice (Charfi *et al.*, 2019), while excessive use of synthetic chemical preservatives may pose health risks (Charfi *et al.*, 2018).

Recently, enhancing food safety has become a major concern, with an increased emphasis on natural food preservatives (Elsharawy, 2018). Essential oils (EOs) have demonstrated strong antimicrobial properties against spoilage and pathogenic microorganisms in food, especially at low potential hydrogen (pH) levels. These EOs and their primary active constituents are utilized for food stabilization (Pandey and Negi, 2018).

Cinnamomum cassia is a plant belonging to the *Lauraceae* family and is considered one of the 50 fundamental herbs in traditional medicine (Shahrajabian *et al.*, 2021). *Cinnamomum cassia* essential oil (CCEO) has antimicrobial and antioxidant properties and is useful in food preservation (Kačániová *et al.*, 2021). According to scientific research, CCEO is effective in controlling anthracnose and crown-rot postharvest diseases of banana fruit (Kulkarni *et al.*, 2021). Minozzo *et al.* (2023) revealed that the encapsulated CCEO could be used as a natural alternative to control fungi in maize flour. Similarly, Liu *et al.* (2024) reported that CCEO could be used as a preservative against *Penicillium oxalicum* on rice noodles. In addition, Zhao *et al.* (2025) proved that CCEO could be used as a natural food alternative to prevent fungal contamination of *Rhizopus stolonifer* in soft rot of peaches. Considering the above findings, the current research aimed to investigate the effectiveness of CCEO as a natural alternative to pasteurization and synthetic preservatives for the preservation of orange juice. The study evaluated this by monitoring physicochemical, microbiological, and sensory modification of orange juice supplemented with CCEO.

Materials and Methods

Extraction of *Cinnamomum cassia* essential oil

Cinnamomum cassia barks (Chinese cinnamon) was used as a source material for extracting EO, and the bark was purchased from a reputable local herbalist in Bordj Bou-Arréridj, Algeria. Before the extraction process, the bark was thoroughly cleaned to eliminate surface contaminants. Then it was crunched to increase its surface

area for extraction. CCEO extraction was carried out by hydro-distillation in a Clevenger-type apparatus. Briefly, 100 g of crushed *C. cassia* bark was hydrodistilled for 3 h with 500 mL of distilled water. After extraction, the EO was dried with anhydrous sodium sulfate, filtered, and stored in an opaque bottle at 4°C for analysis (Ramazani *et al.*, 2020).

Organoleptic characterization of CCEO

Organoleptic characteristics have a crucial role in determining the quality and potential uses of EO. The organoleptic quality of an EO and its potential for scientific and economic use are determined by its appearance, color and odor.

Physical characterization of essential oils

Density index

Density measurement was carried out using the pycnometer method. Two pycnometers, one containing distilled water (m1) and the other containing CCEO (m2), were weighed alongside an empty tube (m0). The relative density (D) of CCEO was then calculated, providing a way to assess the purity and concentration of EO using Equation (1) according to Mayo *et al.* (2010):

$$D = \frac{m2 - m0}{m1 - m0} \quad (1)$$

Refractive index

The refractive index was analyzed to determine the optical properties of CCEO using an Abbe refractometer. This method involved applying drops of CCEO on a refractometer prism and taking multiple measurements to determine its refractive index, which offered valuable insights into the purity of EO (Mayo *et al.*, 2010).

Preparation of orange juice samples

Fresh orange (*Citrus sinensis*) fruits of equal maturity were purchased in bulk from a local market (Bourj Bou Arrerij, Algeria). The fruit was delivered and inspected in the laboratory for physical damage or visible insects before being washed and gently wiped to remove contaminants. Oranges were sectioned and fresh juice was extracted using a domestic extractor (Condor JC115, Algeria). The juice was filtered through a fine mesh strainer (with a filter pore size of 10 µm) to remove seeds. Finally, the prepared orange juice samples were stored in clean and airtight containers and kept in a refrigerator at 4°C to prevent oxidation and microbial contamination.

Preservation of natural orange juice with CCEO

To assess the effectiveness of CCEO in preserving orange juice, different doses (5, 10, 15, and 20 $\mu\text{L/L}$) were added to unpasteurized orange juice samples. These doses were selected based on the *in vitro* antioxidant and antifungal activity of CCEO. A negative control sample was also prepared (in all, five samples). The samples were homogenized using a vortex mixer. The samples were preserved at 4°C for 28 days to observe temporal variations. During this period, sensory analysis was performed to assess the juice's flavor, fragrance, hue, and general acceptability. Furthermore, physicochemical and microbiological characteristics were evaluated during the entire storage period. Simultaneously, a sensory analysis was conducted to determine the effect of CCEO on the organoleptic properties (such as taste, aroma, color, and the overall acceptability) of orange juice samples.

Physicochemical and microbiological parameters

pH determination

To determine the pH of orange juice samples, a pH meter (Model DC12000500D; HANNA Instruments, China) was used. Commercial buffer solutions (Sigma-Aldrich, Dublin, Ireland) of pH 7.0 and 4.0 were used to calibrate pH meter. Samples (10 mL) were placed in a beaker with a magnetic stirrer and measured at $20 \pm 0.5^\circ\text{C}$ (Altemimi *et al.*, 2021).

Titrateable acidity (TA)

To determine titrateable acidity, 20-mL sample was placed in a 250-mL beaker with 80-mL distilled water. This solution was then titrated with standardized 0.1-N NaOH (Sigma-Aldrich) up to the phenolphthalein endpoint ($\text{pH} = 8.2 \pm 0.1$). The volume of NaOH was converted into grams of citric acid per 100 mL of juice (Redd *et al.*, 1986), and the TA was calculated using Equation (2) (Barbosa-Canovas and Gould, 2000):

$$\text{TA\%} = \frac{V \times 0.1N_{\text{NaOH}} \times 0.067 \times 100}{m} \quad (2)$$

where V is the titer volume of NaOH, m is the mass of orange juice (in grams), and N is the normality of NaOH.

Total soluble solids (°Brix)

Total soluble solids (TSS) content (°Brix) was measured using a digital refractometer (Abbe, Atago Co. Ltd., Japan). The refractometer was calibrated using distilled water. The measurements were determined by placing a few drops of orange juice sample on the refractometer prism at $20 \pm 0.5^\circ\text{C}$. The refractometer prism was cleaned with distilled water after each analysis. The TSS content was recorded in degrees °Brix (Altemimi *et al.*, 2021).

Determination of Vitamin C (ascorbic acid)

Vitamin C was measured using the redox titration technique with iodine (Association of Official Analytical Chemists [AOAC], 2010). Pipette 20-mL aliquot sample into a 250-mL conical flask, followed by 150-mL distilled water and 1-mL starch solution indicator. The sample was titrated with 0.005-M iodine solution. The first lasting trace of a dark blue-black color because of the starch-iodine complex marked the end of titration. To establish an average titre value, the titration was repeated with additional aliquots of the sample. The amount of vitamin C in the sample was calculated in mg/100 mL.

Browning index (BI)

Browning Index was determined by centrifuging 10-mL sample of orange juice at $756 \times g$ for 10 min using a centrifuge machine (SIGMA 3-30KS, Germany). Ethanol (95%), 5 mL, was added to 5 mL of supernatant and centrifuged again. The absorbance of the supernatant was read at 420 nm using a UV-Visible spectrophotometer (Unicam UV-Vis 1800; Shimadzu, China) with a cell path length of 1 cm, with distilled water used as a blank (Meydav *et al.*, 1977).

Cloud value (CV)

Orange juice samples (5 mL) were centrifuged (SIGMA 3-30KS) at $756 \times g$ for 10 min at room temperature ($20.0 \pm 0.5^\circ\text{C}$). A UV-Visible spectrophotometer (Unicam UV-Vis 1800; Shimadzu) was used to evaluate cloud value as the absorbance of supernatant at 660 nm, with distilled water serving as a blank (Versteeg *et al.*, 1980).

Yeast and mold Count

In a laminar flow hood, ten-fold serial dilutions of juice samples were prepared by transferring 1 mL of each juice sample in 9 mL of sterile phosphate-buffered saline. Aliquots of 100 μL of each dilution were spread, using a sterile L-shaped glass rod (rake) on the surface of potato dextrose agar (PDA) with penicillin-streptomycin incubated for 5 days at 25°C . After incubation, the visible colonies on PDA plates were counted in units per milliliter (CFU/mL) (Starek *et al.*, 2019).

Sensory parameters

A total of 21 trained panelists (aged 20–35 years) were recruited based on availability, prior sensory experience, and confirmed sensory acuity (Larmond, 1977). All panelists were selected based on the following criteria: trained panelists; no known allergies or health conditions affecting taste or smell; and successful completion of a sensory acuity screening, including basic taste and odor recognition. Taste perception is controlled by the gustatory cortex. Taste and smell determine flavor, which is the sensory impression of food or other materials.

Food flavor can be modified by natural or artificial aromas that affect the senses. These differences in taste and flavor were discussed with panellists.

Orange juice samples were presented to panelists in a randomized manner. The samples were evaluated for color, taste, flavor, and the overall acceptability, because these parameters directly influence how consumers perceive and enjoy the product. Color is the first visual cue that affects consumer expectations and indicates freshness. Taste is critical for balance; orange juice should be sweet enough to be pleasant but also have enough acidity for freshness. A distinct flavor profile is indicative of high-quality juice. The overall acceptability integrates all sensory impressions (appearance, taste, flavor, mouth feel, etc.). The panelists were provided with a 9-point hedonic scale ranging from “like extremely” (9)

to “dislike extremely” (1) to rate the orange juice samples for acceptability of four sensory attributes. The sensory sheet model is presented in Table 1. The panelists were instructed to evaluate each sample individually based on specified sensory attributes using the provided hedonic scale. The process also involved facilitating discussion among the panelists to identify differences in the acceptability of four sensory attributes of orange juice samples. Discussions encouraged the panelists to share their sensory perceptions and feedback on the evaluated attributes.

In this study, individuals participated on a voluntary basis and did not receive any compensation. Moreover, the sensory evaluation did not require formal ethical approval from Algerian National Committee on Health Research Ethics. However, consents from all the participants were

Table 1. Sensory sheet model.

First and last name: Age: years Date: / / Gender: Female ☐ Male ☐ Assign a score on a scale of 1 to 9 for each sample A, B, C and D: 9 → Like extremely 8 → Like very much 7 → Like moderately 6 → Like slightly 5 → Neither like nor dislike 4 → Dislike slightly 3 → Dislike moderately 2 → Dislike very much 1 → Dislike extremely										
Assessment	Sample	1	2	3	4	5	6	7	8	9
Color	A									
	B									
	C									
	D									
	E									
Taste	A									
	B									
	C									
	D									
	E									
Flavor	A									
	B									
	C									
	D									
	E									
Overall acceptability	A									
	B									
	C									
	D									
	E									

obtained and appropriate protocols for protecting their rights and privacy were followed before and during the execution of the research.

Statistical analysis

Statistical analysis was performed using SPSS IBM Statistics 29.0.10 version. One-way ANOVA test followed by Tukey’s *post hoc* multiple comparison was performed to determine significant values ($p < 0.05$). All experiments were performed in triplicate and the results of statistical analysis were computed as mean $\pm$ standard deviation (SD).

Results and Discussion

Organoleptic and physical characterization of *Cinnamomum cassia* essential oil

The results of organoleptic and physical characterization of EO are presented in Table 2. The EO obtained was a clear, pale yellow liquid at room temperature, with a spicy sweet taste and pleasant characteristic odor and an aromatic flavor. Its density was 1.054 ± 0.004 , which was higher than distilled water (0.99), and was within the Association Française de Normalisation (AFNOR), 1999, standard range of 1.045–1.063. The refractive index was 1.6034, also higher than distilled water (1.33) and within the Association Française de Normalisation (AFNOR), 2000 range of 1.6020–1.6060. These results confirmed the purity of cinnamon EO. The yield obtained was 2.8% (w/w); which was relatively high, compared to our previous study (Boubrik *et al.*, 2025).

Our findings were aligned with the results of the literature. Koroch *et al.* (2007) found that the extracted EO

from commercial cinnamon barks was yellow with a spicy sweet taste and a relative density ranging from 1.019 to 1.026, and a refractive index ranging from 1.5817 to 1.5909. Similarly, Huang *et al.* (2019) obtained a pale yellow EO, with pleasant odor and strong or mild aroma spicy flavor, with a relative density of 0.998–1.067. In addition, Quyen and Quoc (2023) revealed that the extracted CCEO was a yellow liquid with a bitter and spicy taste and a characteristic odor, and a relative density of 1.047. Our results were close to those found by Boughendjioua and Djeddi (2018), confirming that EO from cinnamon was a clear liquid, yellowish in color, spicy sweet taste, and aromatic flavor, with a relative density of 1.050 and a refractive index of 1.6020.

Color, density, and refractive index are the essential characteristics of EO’s quality and purity. Color signals the presence of natural components or possible impurities; density helps to confirm whether the oil is pure or diluted; and refractive index is a sensible measure to reveal subtle changes in EO’s composition, making it useful for detecting adulteration. The data are especially valuable in deciding whether EO needs further purification or refinement before it could be used safely, particularly in sensitive applications such as food, medicine, and aromatherapy. However, EO undergoes rigorous analysis and complies with safety and purity standards. The results confirm that it is free from adulteration and suitable for use in food applications. The ongoing monitoring of these parameters remains essential for quality assurance; no further purification is required for our EO at this point of time.

Physicochemical and microbiological parameters

The importance of using orange juice for human health depends on maintaining physical, chemical, and microbiological standards to ensure the quality and safety of the juice (Hossain *et al.*, 2012). The results of CCEO impacts on the physicochemical and microbiological parameters of orange juice during 28 days of storage at $4\pm0.5^{\circ}\text{C}$ are presented in Table 3. Pearson’s correlation coefficients between CCEO dose and the physicochemical and microbiological parameters of orange juice during storage are presented in Table 4.

Classification of Pearson’s correlation coefficients (R) is as follows: 0.00 and 0.199: very low; 0.20–0.399: low; 0.40–0.599: medium; 0.60–0.799: high; 0.80–1.00: very high.

pH and TA

The results of pH and TA during storage are shown in Figure 1. All doses of CCEO did not significantly affect the pH and TA of orange juice samples, compared to the

Table 2. Organoleptic and physical characteristics of CCEO.

Characteristics	CCEO obtained	
Appearance	Clear liquid	
Odor	Characteristic, fresh, and pleasant	
Color	Pale yellow	
Taste	Sweet and spicy	
Flavor	Aromatic	
Relative density	1.053 ± 0.004	1.045–1.063 (AFNOR NFT 75-202, 1999)
Refractive index	1.6034 ± 0.003	1.6020–1.6060 (AFNOR, 2000)
Notes: CCEO: <i>Cinnamomum cassia</i> essential oil; AFNOR: Association Française de Normalisation.		

Table 3. Effect of CCEO on the physicochemical and microbiological parameters of orange juice.

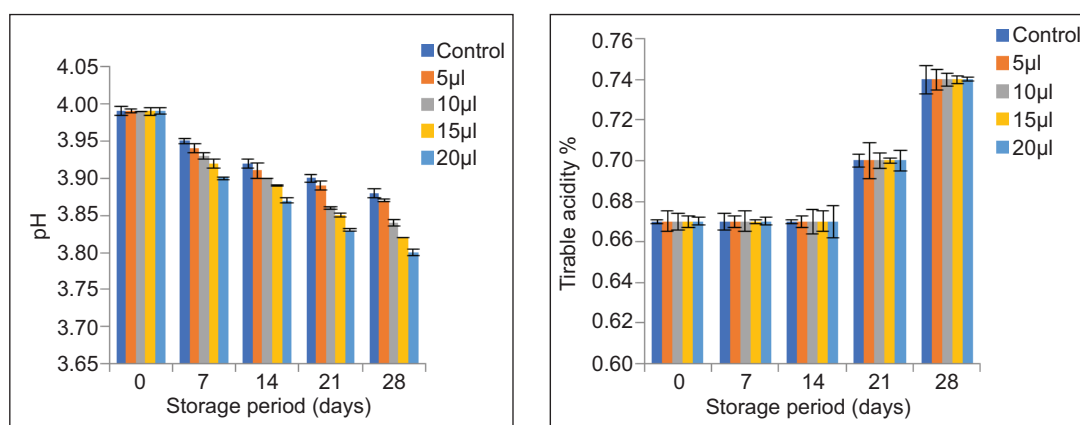
Dose	pH	TA (%)	Vitamin C (mg/100 mL)	TSS °(Brix)	BI (420 nm)	CV (660 nm)	Yeast and mold count (cfu/100 mL)
Control	3.93±0.019 ^a	0.67±0.011 ^a	3.30±0.38 ^e	9.63±0.004 ^{d,e}	0.349±0.06 ^a	0.229±0.03 ^e	2.19×10 ¹ ±0.12 ^a
5 µL/L	3.92±0.020 ^a	0.67±0.013 ^a	4.02±0.27 ^d	9.82±0.005 ^d	0.295±0.04 ^b	0.546±0.04 ^d	0.68×10 ¹ ±0.11 ^b
10 µL/L	3.90±0.026 ^a	0.67±0.012 ^a	4.27±0.23 ^c	10.06±0.004 ^c	0.254±0.03 ^c	0.661±0.05 ^c	0.32×10 ¹ ±0.15 ^c
15 µL/L	3.89±0.029 ^a	0.67±0.010 ^a	4.43±0.22 ^{a,b}	12.08±0.004 ^{a,b}	0.245±0.04 ^d	0.775±0.04 ^b	0.20×10 ¹ ±0.10 ^d
20 µL/L	3.88±0.032 ^a	0.67±0.013 ^a	4.56±0.23 ^a	12.58±0.005 ^a	0.226±0.05 ^e	0.859±0.06 ^a	0.15×10 ¹ ±0.09 ^e

Note: Different superscript letters indicate significant differences ($p < 0.05$).

Table 4. Pearson's correlation coefficients between CCEO dose and orange juice's physicochemical and microbiological parameters.

	pH	TA (%)	Vitamin C (mg/100 mL)	TSS °(Brix)	BI (420 nm)	CV (660 nm)	Yeast and mold count (cfu/100 mL)
Pearson's correlation coefficients (R)	-0.321	0.00	0.581 ^{**}	0.345 [*]	-0.469 [*]	0.891 ^{**}	-0.646 ^{**}

^{**}Significant at the level of 0.01.

**Figure 1.** Effect of CCEO on pH and TA of orange juice.

control ($p > 0.05$). It is commonly observed that beverages with high acidity have a lower pH value. The pH values of orange juice samples ranged from 3.80 to 3.99, which were within the normal range of fresh orange juice, because orange is a natural acidic fruit.

TA analysis showed values ranging from 0.65% to 0.72%, which aligned with the standard range of 0.65–1.85% established by the NMX-F-118-1984 standard (General Directorate of Standards, Mexico, 1984). This showed that the acidity levels of the juice were within the acceptable range set by the standard. After 14 days of storage, all orange juice samples showed a slight decrease in pH and a slight increase in TA, with no significant variation ($p > 0.05$). Pearson's correlation analysis showed a negative correlation between the CCEO dose and pH, but it was not statistically significant ($p > 0.05$). There was no significant correlation between the CCEO dose and TA

($p > 0.05$). This result confirmed that acidity levels are not affected by CCEO doses.

A slight decrease in pH and a slight increase in TA indicated that enzymatic and microbial activity led to the formation of organic acids (Alkuraieef and AlJahani, 2022). Furthermore, increased acidity during storage was caused by lactic acid bacteria converting sugars into organic acid (Natt and Katyal, 2022). Similar findings were reported regarding no significant changes in pH over a 15-day storage period of raw apple juice treated with betel leaf EO (Basak, 2018). Similarly, Kapoor *et al.* (2008) found a slight decrease in pH accompanied by an increase in TA during storage of pineapple juice treated with *Cinnamomum tamala* EO. In contrast, Madhumita *et al.* (2021) reported a gradual decrease in pH and increase in TA in sapota juice treated with cured betel leaf EO. Moreover, Kapoor *et al.* (2011) observed different results

in sweet orange juice with cardamom EO, showing an increase in pH and a decrease in acidity during storage.

Contents of vitamin C

The levels of vitamin C in orange juice samples treated with different doses of CCEO during storage are shown in Figure 2. The results indicate a significant enhancement ($p < 0.05$) in the level of ascorbic acid in the treated orange juice samples, compared to the control. The treated samples showed higher ascorbic acid levels with a range of 4.98–3.89 mg/100 mL for 20 μ L, 4.82–3.73 mg/100 mL for 15 μ L, 4.66–3.52 mg/100 mL for 10 μ L, and 4.51–3.20 mg/100 mL for 5 μ L, compared to the control, with a range of 4.27–2.11 mg/100 mL. However, there was a degradation of ascorbic acid over time because of oxidation and enzymatic activity. Furthermore, the study discovered moderate positive Pearson's correlation coefficients between the dose of CCEO and vitamin C levels in orange juice samples. These statistically significant results indicated that higher doses of CCEO could help to maintain vitamin C content and the nutritional quality of orange juice over time.

Our results were consistent with a research conducted by Kapoor *et al.* (2011), who reported a significant enhancement in ascorbic acid of sweet orange juice treated with cardamom EO during storage. Similar findings were noted in case of fresh orange juice preserved with *Cymbopogon citrate* EO (Adjou *et al.*, 2017). Our findings aligned with the results of a research conducted by Madhumita *et al.* (2021), who reported that cured betel leaf EO significantly improved the ascorbic acid content in sapota fruit juice.

A significant increase in the ascorbic acid content of treated orange juice samples could be due to the ability of

EO to slow down oxidation and the enzyme ascorbinase (ascorbate oxidase) contributing to vitamin C preservation (Kapoor *et al.*, 2011). However, during extended storage, orange juice loses ascorbic acid, changes color, and loses other nutrients. This happens because of dissolved oxygen that starts oxidation. Changes in temperature speed up this process, converting ascorbic acid to dehydroascorbic acid and then to inactive compounds, such as 2,3-diketogulonic acid (Sinchaipanit *et al.*, 2015).

It is scientifically wrong to label cinnamon EO as an “enzyme inhibitor” without further studies supporting this claim. At most, the available data support the hypothesis that cinnamon EO may contribute to slowing the enzymatic activity, possibly through indirect effects (e.g., antioxidant or antimicrobial properties), but not necessarily by direct enzyme inhibition.

Total soluble solids

The results of TSS content ($^{\circ}$ Brix) in orange juice samples treated with various concentrations of CCEO during storage are shown in Figure 2. The results indicate a significant improvement ($p < 0.05$) in the level of TSS in the treated orange juice samples, compared to the control, except for the dose of 5 μ L/L. The treated samples retained higher TSS levels, ranging from 13.60 to 8.50 $^{\circ}$ Brix for 20 μ L, 13.60 to 6.50 $^{\circ}$ Brix for 15 μ L, 13.60 to 5.60 $^{\circ}$ Brix for 10 μ L, and 13.60 to 5.50 $^{\circ}$ Brix for 5 μ L, compared to the control, which ranged from 13.60 to 4.90 $^{\circ}$ Brix, suggesting that CCEO contributed toward enriching the sugar profile of the juice. However, there was a decrease in TSS for all samples over storage. Furthermore, Pearson's coefficient correlation between CCEO dose and TSS was positive and statistically significant. These statistically significant results indicated that higher doses of CCEO could help maintain the sugar content over time.

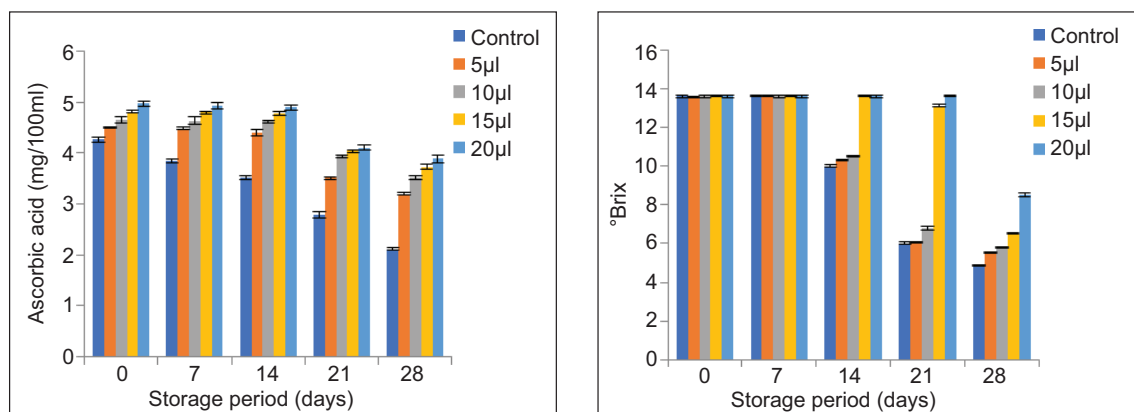


Figure 2. Effect of CCEO on the vitamin C content and total soluble solids of orange juice.

Our findings were in agreement with the research conducted by Charfi *et al.* (2019), who reported a significant enhancement in sugar contents of pomegranate juice treated with *Thymbra capitata* EO. Similarly, Madhumita *et al.* (2021) observed an enhancement in the TSS of sapota juice treated with cured betel leaf EO during storage. This rise in TSS was probably linked to fruit ripening process, where organic acids served as crucial respiratory substrates during postharvest storage.

The addition of EOs improves sugar content in orange juice because of the presence of phenols in EO that preserve sugars and protect these from degradation (Charfi *et al.*, 2019). However, sugar content could decrease during storage because of microorganisms and acids (Sreedevi *et al.*, 2020), creating reactive forms (e.g., HMF and/or 3-deoxyglucosone) that contribute to non-enzymatic browning (NEB; Pham *et al.*, 2019).

Browning index

The results of BI of orange juice samples treated with various concentrations of CCEO during storage are shown in Figure 3. The results indicate a significant decrease ($p < 0.05$) in the BI of orange juice samples treated with CCEO, compared to the control. The treated samples exhibited lower BI values, ranging from 0.160 to 0.283 for 20 μL , 0.160 to 0.295 for 15 μL , 0.160 to 0.308 for 10 μL , and 0.160 to 0.401 for 5 μL , compared to the control, which ranged from 0.169 to 0.517. However, there was an increase in BI for all samples over storage. Pearson's correlation coefficient between CCEO dose and BI was negative and statically significant. This correlation suggested that higher concentrations of CCEO effectively reduced browning reactions in the juice, further supporting the role of EOs in preserving visual quality and extending the shelf life of fruit juices.

This decrease in BI aligned with the findings of Kapoor *et al.* (2014), who found that EO of black pepper

significantly reduced BI of orange juice. The significant decrease in BI could be due to the presence of aldehydes and volatile compounds that slowed down browning and microbial growth (Eissa *et al.*, 2008). However, increase in BI over storage could be linked to NEB and accumulation of brown compounds with pigment degradation (Pham *et al.*, 2019).

Cloud value (CV)

The results of CV of orange juice samples treated with various concentrations of CCEO during storage are shown in Figure 3. The results indicated a significant enhancement ($p < 0.05$) in the CV of orange juice samples treated with CCEO, compared to the control. The treated samples exhibited higher CV values, ranging from 0.951 to 0.751 for 20 μL , 0.882 to 0.652 for 15 μL , 0.791 to 0.501 for 10 μL , and 0.663 to 0.401 for 5 μL , compared to the control, which ranged from 0.343 to 0.157. However, there was a decrease in CV for all samples over storage. Pearson's correlation coefficient between CCEO dose and CV was positive and statistically significant. This proved that higher concentrations of CCEO enhanced the juice's cloudiness and its physical stability.

Cloudiness in fruit juices, as indicated by CV, is mainly attributed to colloidal suspended particles maintained by pectin molecules (Vukić *et al.*, 2017). The presence of cloudiness influences the color and organoleptic characteristics of juice. Decrease in CV or clarification observed during storage is due to the enzymatic activity of PME, also known as pectinase or pectinesterase, which is an enzyme responsible for the de-esterification of methoxylated pectin and formation of insoluble calcium pectate, which reduces juice viscosity (Aghajanzadeh and Ziaifar, 2018). Cloudy juice is prone to sedimentation because of the presence of suspended particles composed of proteins, polysaccharides, and phenols (Yi *et al.*, 2018). After centrifugation, the separation of components in orange juice occurs based on their densities, with the heavier

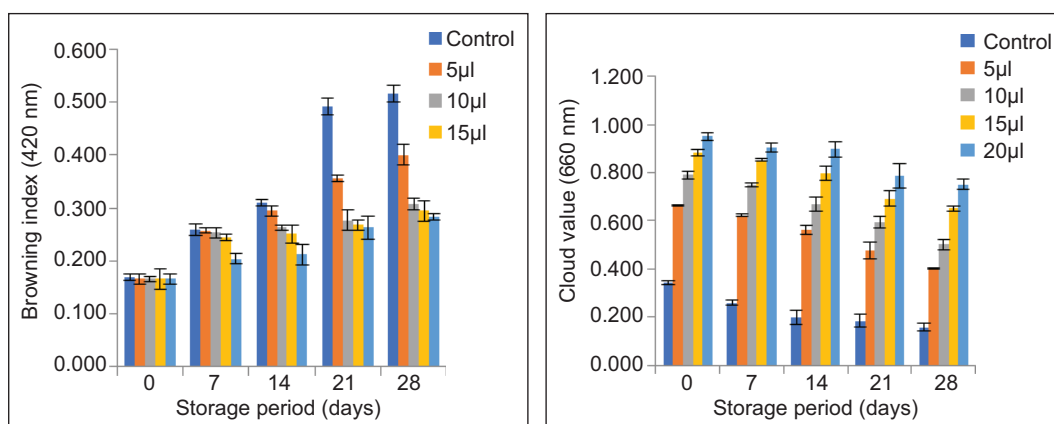


Figure 3. Effect of CCEO on the browning index and cloud value of orange juice.

phase settling at the bottom of the container. This process allows light to pass through a clear aqueous phase, indicating the removal of particulate matter and sedimentation (Wei *et al.*, 2020). Instability index, which reflects the tendency of particles to settle or disperse, increases as the transmission change during centrifugation time increases. A higher instability index signifies decreased stability of dispersion, indicating a greater likelihood of sedimentation occurring over time. The stability of orange juice improved after treatment with EO. As a result, EO treatment may prevent the sedimentation of irregularly shaped particles in the juice, thereby improving its physical stability (Xu *et al.*, 2020). The improved stability could be due to the interaction between EO components and the juice matrix, which could prevent particles from settling and maintaining a more homogeneous dispersion. Furthermore, it is suggested that EO could interfere with the enzymatic activity of PME in orange juice (Xu *et al.*, 2020). Cloud value is an indirect parameter of PME activity because changes in pectin structure, caused by PME, influence particle suspension and juice turbidity. However, it is scientifically inaccurate to refer to CCEO as a PME inhibitor. At most, the available data support the hypothesis that CCEO may contribute to slowing PME activity, possibly through indirect effects, but not necessarily by direct inhibition.

Yeast and mold count

The chemical and physical properties of fruit juices, including low pH, high water activity, and the presence of various nutrients, favor the growth of molds and yeasts, rendering them susceptible to spoilage by fungi while inhibiting bacterial proliferation (Obire *et al.*, 2008; Okigbo and Obire, 2009). This was the main reason for limiting microbiological analysis of fungi (mold and yeast) because they are the most incriminated microbes in orange juice. The results of microbiological analysis are shown in Figure 4. The results indicate that microbial counts (ranging from 0.019×10^1 to 1.19×10^1 colony-forming units (CFU) per 100 mL) conform to the

acceptable range specified by the standard for fruit juices (5.0×10^1 CFU per 100 mL) (International Classification for Standards (ICS) & Standards Organization of Nigeria (SON), 2008). This confirmation of compliance with microbial limits indicates that the product is microbiologically stable and suitable for consumption without posing a risk to consumer health. A statistically significant improvement ($p < 0.05$) in the microbiological quality was observed in orange juice samples treated with CCEO, compared to the control. However, microbial colony counts increased over time in all samples. Pearson's correlation coefficients between the CCEO dose and yeast and mold counts were negative and statistically significant ($p < 0.01$). As a result, the microbiological quality of the orange juice samples improved with increasing CCEO concentration.

Our findings aligned with previous studies that demonstrated the efficacy of various EOs in improving the microbiological quality of fruit juices. Kapoor *et al.* (2008) reported that *Cinnamomum tamala* EO improved the microbiological quality of pineapple juice. In a subsequent study, the same authors (Kapoor *et al.*, 2011) demonstrated the efficacy of cardamom EO in inhibiting the growth of yeasts and molds in sweet orange juice. Similarly, Ogueke *et al.* (2018) found that EOs extracted from *Piper guineense*, *Xylopi aethiopica*, and *Tetrapleura tetraptera* effectively suppressed yeast and mold growth in mixed fruit juice. Charfi *et al.* (2019) reported a significant effect of *Thymbra capitata* EO against yeasts and molds in pomegranate juice. Boukhatem *et al.* (2020) also observed that *Eucalyptus globulus* EO successfully inhibited food spoilage fungi in *Orangina* juice. Additionally, Madhumita *et al.* (2021) found a marked improvement in the microbiological quality of sapota juice treated with cured betel leaf EO.

This improvement could be attributed to the synergistic action of CCEO compounds, which inhibit microbial growth (Kapoor *et al.*, 2014). Our previous study

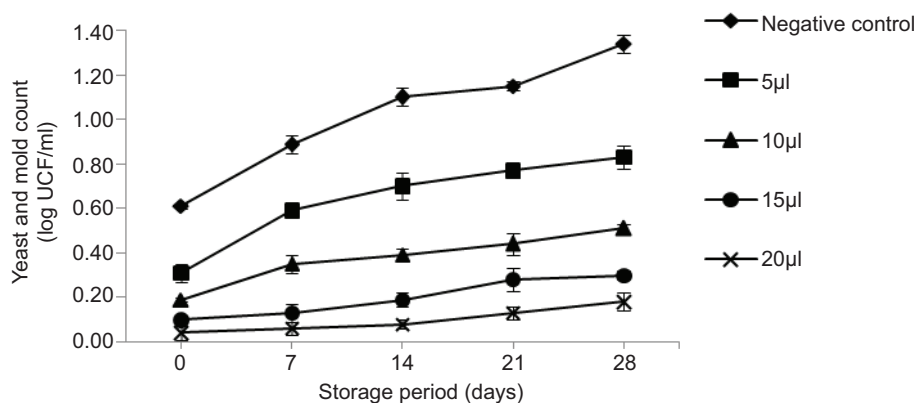


Figure 4. Effect of CCEO on the yeast and mold count of orange juice.

identified 25 compounds, accounting for 99.61% of total composition (Boubrik *et al.*, 2025). The major compounds were (E)-cinnamaldehyde (37.72%), δ -cadinene (5.02%), α -copaene (4.23%), α -murolene (3.90%), and γ -cadinene (3.43%). We demonstrated that CCEO exhibited strong fungicidal activity against *Saccharomyces cerevisiae* and *Acremonium* spp. isolated from orange juice, which could be attributed to the synergistic action of various compounds and/or the high concentration of (E)-cinnamaldehyde (Boubrik *et al.*, 2025). Furthermore, cinnamaldehyde found in cinnamon bark oil as major compound increases cell membrane permeability in microbes, leading to loss of essential nutrients and halting their growth (Bakkali *et al.*, 2008; Lambert *et al.*, 2001; Madhumita *et al.*, 2021). However, the increase in microbial colony counts over time could be due to the survival

and proliferation of certain microorganisms during storage, leading to spoilage, off-flavors, odors, elevated CO₂ levels, and a loss of juice color (Castell *et al.*, 2022). In addition, the activity of EOs, such as CCEO, is linked to oxygenated terpenes, which disrupt key processes in microorganisms, causing their inhibition and elimination (Boukhatem *et al.*, 2020).

Sensory analysis

The effect of CCEO on the sensory characteristics of orange juice is presented in Table 5 and Figure 5. Pearson's correlation coefficients between CCEO dose and the sensory analysis of orange juice during storage are presented in Table 6. The results show a statistically

Table 5. Sensory scores of orange juice samples treated with CCEO during storage.

Dose (μ L/L)	Storage time (days)	Sensory parameters			
		Color	Taste	Flavor	Overall acceptability
0	0	8.01 $\pm$ 0.10 ^a	5.21 $\pm$ 0.08 ^a	5.69 $\pm$ 0.14 ^a	5.65 $\pm$ 0.07 ^a
	7	6.21 $\pm$ 0.15 ^b	4.61 $\pm$ 0.10 ^b	4.45 $\pm$ 0.09 ^b	4.40 $\pm$ 0.18 ^b
	14	4.33 $\pm$ 0.20 ^c	3.72 $\pm$ 0.11 ^c	3.79 $\pm$ 0.18 ^c	3.63 $\pm$ 0.10 ^c
	21	3.20 $\pm$ 0.11 ^d	3.50 $\pm$ 0.22 ^{c,d}	3.50 $\pm$ 0.10 ^{c,d}	3.41 $\pm$ 0.12 ^{c,d}
	28	3.09 $\pm$ 0.10 ^{d,e}	2.30 $\pm$ 0.17 ^e	2.51 $\pm$ 0.13 ^e	2.81 $\pm$ 0.20 ^e
5	0	8.24 $\pm$ 0.14 ^a	5.77 $\pm$ 0.11 ^a	5.86 $\pm$ 0.09 ^a	5.86 $\pm$ 0.10 ^a
	7	7.46 $\pm$ 0.10 ^b	5.68 $\pm$ 0.19 ^{a,b}	5.70 $\pm$ 0.11 ^{a,b}	5.81 $\pm$ 0.15 ^a
	14	6.29 $\pm$ 0.09 ^c	5.42 $\pm$ 0.08 ^b	5.53 $\pm$ 0.05 ^{a,b}	5.68 $\pm$ 0.14 ^{a,b}
	21	4.15 $\pm$ 0.18 ^d	4.85 $\pm$ 0.07 ^c	4.77 $\pm$ 0.08 ^c	4.90 $\pm$ 0.12 ^c
	28	3.11 $\pm$ 0.15 ^e	3.69 $\pm$ 0.05 ^d	4.40 $\pm$ 0.10 ^{c,d}	4.69 $\pm$ 0.16 ^{c,d}
10	0	8.33 $\pm$ 0.21 ^a	6.85 $\pm$ 0.10 ^a	6.77 $\pm$ 0.11 ^a	6.52 $\pm$ 0.10 ^a
	7	7.58 $\pm$ 0.08 ^b	6.57 $\pm$ 0.17 ^{a,b}	5.80 $\pm$ 0.13 ^b	6.40 $\pm$ 0.11 ^a
	14	6.21 $\pm$ 0.12 ^c	5.84 $\pm$ 0.16 ^c	5.58 $\pm$ 0.17 ^{b,c}	6.27 $\pm$ 0.13 ^{a,b}
	21	5.44 $\pm$ 0.10 ^d	5.55 $\pm$ 0.11 ^{c,d}	6.55 $\pm$ 0.20 ^c	5.85 $\pm$ 0.17 ^c
	28	4.05 $\pm$ 0.09 ^e	5.20 $\pm$ 0.15 ^{d,e}	5.36 $\pm$ 0.15 ^d	5.41 $\pm$ 0.11 ^{c,d}
15	0	8.33 $\pm$ 0.21 ^a	7.99 $\pm$ 0.07 ^a	7.89 $\pm$ 0.10 ^a	7.80 $\pm$ 0.09 ^a
	7	8.27 $\pm$ 0.17 ^{a,b}	7.77 $\pm$ 0.10 ^{a,b}	7.79 $\pm$ 0.11 ^a	7.55 $\pm$ 0.07 ^{a,b}
	14	7.65 $\pm$ 0.06 ^c	7.19 $\pm$ 0.17 ^b	7.77 $\pm$ 0.07 ^a	7.34 $\pm$ 0.10 ^{a,b}
	21	7.11 $\pm$ 0.09 ^{c,d}	5.88 $\pm$ 0.09 ^c	5.99 $\pm$ 0.06 ^b	6.61 $\pm$ 0.08 ^c
	28	6.50 $\pm$ 0.10 ^e	5.73 $\pm$ 0.11 ^{c,d}	5.63 $\pm$ 0.17 ^{b,c}	5.68 $\pm$ 0.11 ^d
20	0	8.43 $\pm$ 0.15 ^a	7.96 $\pm$ 0.08 ^a	7.93 $\pm$ 0.10 ^a	7.78 $\pm$ 0.22 ^a
	7	8.36 $\pm$ 0.13 ^a	7.85 $\pm$ 0.11 ^{a,b}	7.78 $\pm$ 0.22 ^{a,b}	7.67 $\pm$ 0.15 ^a
	14	7.81 $\pm$ 0.19 ^b	7.80 $\pm$ 0.22 ^{a,b}	7.71 $\pm$ 0.11 ^b	7.59 $\pm$ 0.16 ^{a,b}
	21	7.29 $\pm$ 0.10 ^{b,c}	6.94 $\pm$ 0.15 ^c	6.60 $\pm$ 0.16 ^c	6.70 $\pm$ 0.08 ^c
	28	6.80 $\pm$ 0.07 ^d	5.99 $\pm$ 0.12 ^d	6.44 $\pm$ 0.09 ^{c,d}	5.53 $\pm$ 0.11 ^d

Note: Different superscript letters represent significant variations ($p < 0.05$).

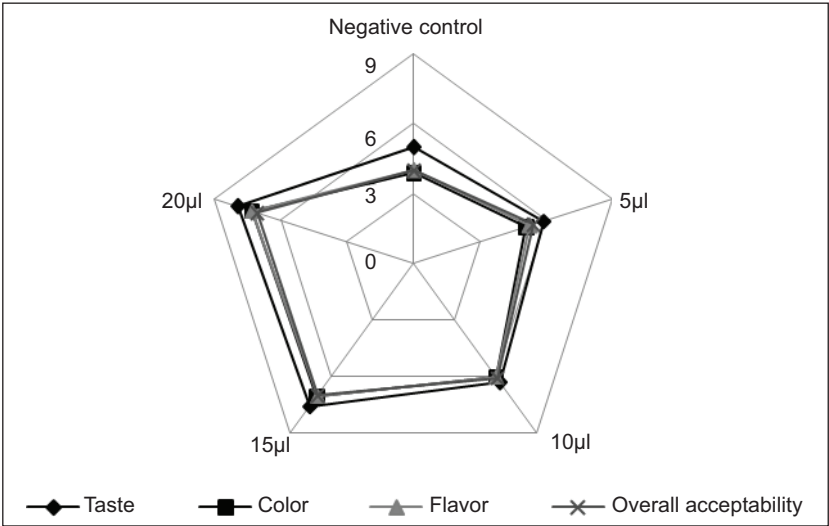


Figure 5. Effect of CCEO doses on the sensory scores of orange juice.

Table 6. Pearson's correlations between CCEO dose and orange juice's sensory parameters.

Sensory parameters	Color	Taste	Flavor	Overall acceptability
Pearson's correlation coefficients (R)	0.573**	0.810**	0.821**	0.809**

Notes: **Significant at the level of 0.01.
Classification of Pearson's correlation coefficients (R) are as follows: 0.00 and 0.199: very low; 0.20–0.399: low; 0.40–0.599: medium; 0.60–0.799: high; 0.80–1.0: very high.

significant improvement ($p < 0.05$) in the sensory attributes of color, taste, flavor, and the overall acceptability of juice samples treated with varying concentrations of CCEO, compared to the control. Color scores ranged from 8.43 to 6.80 for 20 µL, 8.33 to 6.50 for 15 µL, 8.33 to 4.50 for 10 µL, and 8.24 to 3.11 for 5 µL. Taste scores ranged from 7.96 to 5.99 for 20 µL, 7.99 to 5.73 for 15 µL, 6.85 to 5.20 for 10 µL, and 5.77 to 3.69 for 5 µL. Flavor scores ranged from 7.93 to 6.44 for 20 µL, 7.89 to 5.63 for 15 µL, 6.77 to 5.36 for 10 µL, and 5.86 to 4.40 for 5 µL. The overall acceptability scores ranged from 7.78 to 5.53 for 20 µL, 7.80 to 5.68 for 15 µL, 6.52 to 5.41 for 10 µL, and 5.86 to 4.69 for 5 µL. In contrast, the control samples recorded lower scores, ranging from 8.01 to 3.09 for color, 5.21 to 2.30 for taste, 5.69 to 2.51 for flavor, and 5.65 to 2.81 for overall acceptability. No significant difference ($p > 0.05$) was found between 15 µL/L and 20 µL/L doses during storage. The results indicated that samples treated with CCEO at concentrations of 20 µL/L and 15 µL/L achieved the highest sensory evaluation scores, with progressively lower ratings observed in samples treated with 10 µL/L and 5 µL/L CCEO concentrations. Higher CCEO concentrations were generally more acceptable to the sensory panel, as these levels help to reduce harmful microorganisms and enhance flavor, thereby improving the overall juice quality and consumer appeal. However,

there was a decrease in the sensory scores of all samples over storage.

The results of Pearson's correlations, as shown in Table 6, indicate moderate positive correlations between CCEO dose and color, taste, flavor, and the overall acceptability as well as statistically significant results. These findings suggested that the addition of CCEO significantly improved the sensory quality of orange juice, leading to enhancement in color, taste, flavor, and overall acceptability. These positive correlations underscored the potential of CCEO as a natural preservative and flavoring agent in fruit juice applications.

Cloudiness and turbidity are critical parameters influencing the sensory and visual appeal of juice that are important for its sensory and visual qualities, thus affecting the consumer perception. PME promotes juice clearance over time by deesterification of pectin, lowering the viscosity, and influencing clarity and stability (Aghajanzadeh and Ziaifar, 2018). Previous study showed that oxygen caused degradation of ascorbic acid in orange juice, resulting in loss in both color and quality during storage (Sinchaipanit *et al.*, 2015). In addition, natural pigments and other reactions contributed to this degradation. EOs, such as *Cinnamomum cassia* EO, can improve orange

juice's color stability and physical characteristics by stabilizing the colloid system (Xu *et al.*, 2020), and preventing oxidation (Kapoor *et al.*, 2011) and microbial growth (Eissa *et al.*, 2008).

A significant enhancement in taste could be related to preserving EO key compounds such as sugars and organic acids, which are vital for consumer acceptance. The EO preserves key flavor compounds, thereby enhancing the sensory profile of the juice. However, decrease in the taste score over time may be linked to lipid oxidation, which produces off-odors that harm taste characteristic (Madhumita *et al.*, 2021). These off-odors are often associated with rancidity or spoilage, and negatively impact the taste characteristic, resulting in poor taste scores during sensory evaluation.

A significant enhancement in flavor could be related to the specific flavor components present in CCEO, such as coumarin, cinnamaldehyde (the main component responsible for the typical cinnamon flavor found in the oil), cinnamic acid, and cinnamyl alcohol, which create pleasant aroma and taste (Woehrlin *et al.*, 2010). However, decrease in the flavor score over time is due to chemical reactions, such as enzymatic and NEB processes that turn juice brown and develop off-flavors (Paravisini and Peterson, 2018).

A significant improvement in the overall acceptability could be related to the improvement in color, taste, and flavor. However, decrease in the overall acceptability is due to browning, oxidation, and microbial activity that negatively impact sensory qualities of the juice, ultimately influencing consumer acceptance.

In contrast to our findings, Espina *et al.* (2014) found that increasing mint and rosemary EOs reduced acceptance in tomato juice and vegetable soup. In addition, Dwijatmoko *et al.* (2016) noted that high levels of CCEO in dark chocolate decreased consumer acceptance, highlighting the need for careful use of EOs. Similarly, a high concentration of CCEO used for *Thomson navel* orange fruit caused skin burns and the fruit was unacceptable by panel (Khorram and Ramezani, 2021). However, even low concentrations of CCEO in an edible coating of apples harmed their flavor and aroma (Santos *et al.*, 2018).

Researchers and food companies are focusing on creating cinnamon-based functional foods because of their potential health benefits. However, it is challenging to develop such foods because cinnamon has strong aromatic components that could potentially impact consumer acceptance (Dwijatmoko *et al.*, 2016). To our knowledge, there is a lack of sensory evaluations specifically focusing on the incorporation of CCEO in orange juice. Furthermore,

the existing literature on sensory analyses involving CCEO in food products often lacks comprehensive panel information or relies on smaller panel sizes. It is a critical consideration, as the demographic characteristics of panelists, such as age, food preferences, and gender, significantly influence the outcomes and interpretations of sensory studies.

Conclusion

The current study showed that CCEO has a positive impact on orange juice, improving its physicochemical, microbiological, and sensory qualities. First, monitoring of juice quality during storage revealed that CCEO has the ability to stabilize physicochemical (ascorbic acid, TSS, BI, and CV) and microbiological qualities, compared to the negative control. Second, CCEO has shown high efficacy as a flavoring agent by improving sensorial parameters, such as color, taste/flavor, and the overall acceptability with a positive and significant correlation ($p < 0.01$) with CCEO dose. Therefore, the treated orange juice confirmed safety standards and could be consumed safely. These results are very interesting for the food industry, because besides these properties, CCEO is nontoxic and secure for human consumption. It can be an appropriate alternative to chemical preservatives and heat treatment for improving food safety. This study is limited by its short storage period, small sensory panel, and focus on a single fruit juice. Future research should explore longer storage durations to better understand long-term effects, test EOs on various juices to evaluate its broader applicability across different juice matrices and involve a more diverse panel to improve the reliability and consumer relevance of sensory evaluation results.

Data Availability Statement

All the data in the article are available from the corresponding author upon reasonable request.

Author Contributions

Concept and design: Fairouz Boubrik and Nabil Benyoucef. Analysis and interpretation: Fairouz Boubrik and Tahar Boubellouta. Data collection: Fairouz Boubrik. Writing of the article: Fairouz Boubrik. Critical revision of the article: Yuva Bellik, Abderrahmane Ait-Kaddour, and Sureerat Makmuang. Statistical analysis: Fairouz Boubrik and Tahar Boubellouta. Project administration: Fehmi Boufahja and Hamdi Bendif. Supervision, review, and editing: Hamdi Bendif. Final approval of the article was provided by all the authors.

Conflicts of Interest

The authors declared no conflict of interest.

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