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Evaluation of the effects of enzymatic and non-enzymatic treatments on the quality and chemical composition of orange peel essential oils

Catherine Ohunene Olaitan^{1,2}, Sarafadeen Olateju Kareem², Olufunke Bolatito Shittu², Oluseye Adeboye Akinloye³ Mercy Ovayoza Aliu⁴ and Zakari Adeiza David^{1,2}

¹Department of Microbiology, Prince Abubakar Audu University PMB 1008, Anyigba, Kogi State, Nigeria. E-mail: olaitan.co@ksu.edu.ng

²Department of Microbiology, Federal University of Agriculture, PMB 2240, Abeokuta Ogun State, Nizeria.

³Department of Biochemistry, Federal University of Agriculture, PMB 2240, Abeokuta Ogun State, Nizeria.

⁴Department of Microbiology, Confluence University of Science and Technology, Osara, Kogi State, Nigeria.

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Abstract

The extraction and utilization of essential oils from citrus peels, particularly orange peels, have garnered considerable attention due to their rich bioactive composition and industrial applications. This study evaluated the impact of enzymatic and non-enzymatic extraction methods on the quality and chemical composition of orange peel essential oils. Orange peels (*Citrus sinensis*) were subjected to hydrodistillation with and without cellulase enzymes (CAPD from *Bacillus megaterium* and POME from *Bacillus pumilus*) at different enzyme-to-peel ratios (1:2 and 1:3). The extracted oils were analyzed for physicochemical properties including acid value, iodine value, saponification value, peroxide value, density, specific gravity, and refractive index. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to determine chemical composition. Results showed that enzymatic treatments significantly influenced oil properties, with reduced acid values (1.009-1.369 mg KOH/g vs 1.122 mg KOH/g in control), altered saponification values (19.6-33.7 vs 121.67 in control), and modified chemical profiles. The control yielded the highest oil content (5.08%), while enzymatic treatments produced lower yields (1.2-2.54%) but enhanced concentrations of bioactive compounds such as α -terpineol and β -bisabolene. GC-MS analysis revealed that enzymatic extraction altered the dominance of d-limonene and increased oxygenated terpenes. Despite lower yields, enzymatic treatments improved oil quality through selective extraction of bioactive compounds, offering advantages for applications prioritizing quality over quantity in pharmaceutical, cosmetic, and food industries., *Non-Coding DNA, Gene Regulation, Long Non-Coding RNAs, Genome Evolution, Transcriptomics*

Key words: Orange peel essential oil, Enzymatic extraction, Cellulase, d-limonene, α -terpineol, GC-MS analysis, Bioactive compounds

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1. Introduction

The extraction and utilization of essential oils from citrus peels, particularly orange peels, have garnered considerable attention due to their rich bioactive composition and applications in diverse industries, including cosmetics, pharmaceuticals, and food. Orange peel essential oil is a prominent byproduct of citrus fruit

* Corresponding author: Catherine Ohunene Olaitan, Department of Microbiology, Prince Abubakar Audu University PMB 1008, Anyigba, Kogi State. E-mail: olaitan.co@ksu.edu.ng.

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processing, valued for its characteristic aroma, antioxidant activity, and potential health benefits. Its primary constituents include terpenes, alcohols, aldehydes, and esters, with d-limonene being the most abundant compound, known for its antimicrobial, anti-inflammatory, and anticancer properties (Bakkali *et al.*, 2008; Guimarães *et al.*, 2019).

Traditional methods of essential oil extraction, such as hydrodistillation and steam distillation, often fail to achieve optimal yields due to the structural integrity of the peel matrix. Recent advancements in enzymatic extraction techniques have shown promise in overcoming these limitations. Enzymes like cellulase facilitate the breakdown of cellulose and other polysaccharides in the cell wall, enhancing the release of bound oil and improving extraction efficiency (Kowalska *et al.*, 2021). This enzymatic approach is particularly beneficial in maximizing the recovery of bioactive compounds while preserving their functional integrity.

The quality of essential oils is determined by a range of physicochemical and sensory properties, such as acid value, iodine value, peroxide value, density, and refractive index. These attributes reflect the oil's chemical composition, stability, and purity, which are critical for its industrial applicability (Marongiu *et al.*, 2004). Gas Chromatography-Mass Spectrometry (GC-MS) analysis further provides a detailed chemical profile, identifying key compounds and their relative abundances. Such analyses are essential for assessing the impact of different extraction methods on the quality of the oil.

In developing countries, the adoption of advanced extraction techniques is often constrained by cost and resource limitations, leading to a reliance on traditional methods. This study aims to evaluate the impact of enzymatic and non-enzymatic extraction methods on the quality and chemical composition of orange peel essential oils. By comparing physicochemical characteristics and GC-MS profiles, the research seeks to provide insights into the effectiveness of enzymatic treatments in enhancing oil quality and yield. Additionally, the study explores the potential of enzyme-assisted extraction as a cost-effective and sustainable approach for improving essential oil production in resource-limited settings.

2. Materials and methods

2.1. Materials

2.1.1. Sample collection

Fresh orange peels (*Citrus sinensis*) were collected from local markets. The peels were thoroughly washed to remove dirt and impurities, then air-dried under shade to prevent thermal degradation of volatile components. Once dried, the peels were ground into fine particles using a mechanical grinder (model: XYZ). The processed samples were stored in airtight containers at room temperature until further analysis.

2.1.2. Enzymes

Two cellulase enzyme preparations were utilized for enzymatic treatments:

- **CAPD:** Derived from *Bacillus megaterium*.
- **POME:** Derived from *Bacillus pumilus*. The enzymes were obtained from a certified commercial supplier and prepared according to the manufacturer's instructions.

2.1.3. Chemicals and reagents

Analytical-grade reagents were used for all analyses, including ethanol, potassium hydroxide, iodine, and chloroform. All chemicals were procured and met the specifications outlined by the American Chemical Society (ACS).

2.2. Essential oil extraction

Essential oil extraction was performed using hydrodistillation, employing a modified Clevenger apparatus. The process was carried out under two conditions: a control (without enzymes) and enzymatic treatments.

2.2.1. Control (W)

For the control treatment, 100 g of orange peel powder was mixed with 1 L of distilled water (1:10 w/v) and subjected to hydrodistillation. The extraction process was carried out for 3 hours at a controlled temperature of 100°C.

2.2.2. Enzymatic treatments

For enzymatic treatments, two cellulase enzymes (CAPD and POME) were applied at different enzyme-to-peel ratios. The treatments were as follows:

- Treatment A: CAPD A 1:2 and A 1:3

CAPD cellulase was added to the peel powder at ratios of 1:2 and 1:3 (enzyme:peel, w/w).

- Treatment B: POME B 1:2 and B 1:3

POME cellulase was applied at similar ratios (1:2 and 1:3).

In each enzymatic treatment, the enzyme mixtures were incubated with orange peel powder in 100 mL of distilled water at 45°C for 2 hours with gentle stirring. After incubation, the mixtures underwent hydrodistillation for 3 hours under the same conditions as the control.

The essential oil yield was calculated as:

$$\text{Yield (\%)} = \frac{\text{Weight of essential oil extracted (g)}}{\text{Dry weight of orange peel powder (g)}} \times 100$$

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This method was adapted from the works of Singh *et al.* (2020) and modified to optimize enzyme activity and oil recovery.

2.3. Physicochemical characterization

The physicochemical properties of the extracted oils were analyzed using standard methods.

1. **Acid, iodine, saponification, peroxide, and free acid value:** These were determined using protocols outlined in the *Official Methods of Analysis* by the Association of Official Analytical Chemists (AOAC, 2005).
2. **Density and Specific Gravity:** Density was measured using a calibrated pycnometer, and specific gravity was calculated relative to the density of water at 25°C.
3. **Refractive Index:** The refractive index was measured at 25°C using an Abbe refractometer (model: ABBE-3L, Atago Co., Japan).
4. **Sensory Evaluation:** Color, odor, and tactile properties were assessed by a panel of five trained sensory analysts following ISO 5492:2008 guidelines for sensory evaluation.

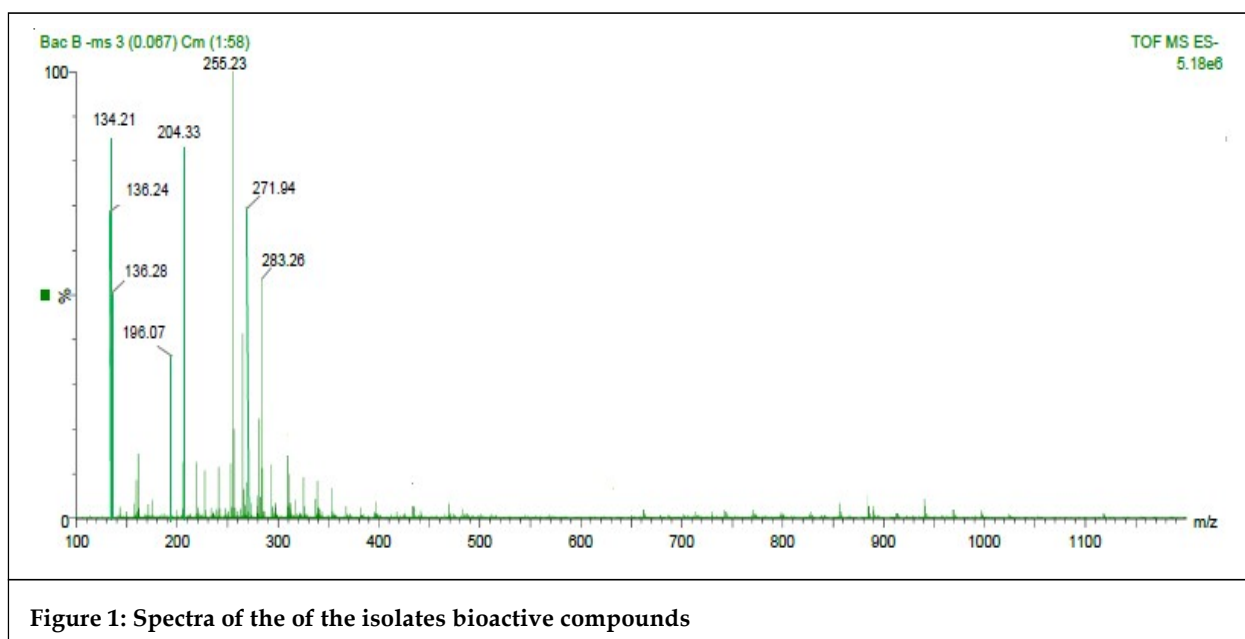
2.4. Gas Chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was conducted using an Agilent 7890A GC system equipped with a 5975C mass-selective detector and an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). Helium served as the carrier gas at a constant flow rate of 1 mL/min.

Instrumental Conditions:

- Injection Temperature: 250°C.
- Split Ratio: 1:20.
- **Oven Temperature Program:**
 - Initial temperature: 50°C (held for 2 minutes).
 - Ramp: 10°C/min to 250°C (held for 5 minutes).

Mass spectra were acquired in electron ionization mode at 70 eV. Peaks were identified by comparing retention times and mass fragmentation patterns with the National Institute of Standards and Technology (NIST) spectral library (Figure 1). This approach was based on the method described by Wang *et al.* (2018).



2.5. Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using SPSS (version 26.0). Results were expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was performed to evaluate differences between treatments. Post-hoc Tukey's multiple comparison test was used to identify significant differences ($p < 0.05$) among means.

3. Results and discussion

3.1. Physicochemical properties of orange peel essential oils

This study investigated the impact of enzymatic treatments on the physicochemical properties and chemical composition of orange peel essential oils. Key parameters analyzed included acid value, iodine value, saponification value, peroxide value, and free acid value, alongside physical attributes like yield, density, specific gravity, refractive index, and sensory characteristics. The results highlight significant differences between the control and enzymatically treated samples, underscoring the influence of enzymatic hydrolysis on oil quality and composition.

3.1.1. Acid value and free acid value

Acid value, which reflects free fatty acid content, varied across the samples as seen in Table 1, with the lowest value (1.009 mg KOH/g) observed in the B 1:3 treatment and the highest (1.369 mg KOH/g) in B 1:2. Free acid values mirrored this trend, ranging from 0.500 in A 1:2 to 0.677 in the control. The reduction in acid and free acid values in enzymatic treatments suggests that cellulase enzymes may break down lipid precursors, mitigating free fatty acid accumulation. These findings align with Guimarães *et al.* (2019), who reported that enzymatic extraction reduces oxidative degradation and free fatty acid content compared to conventional methods.

Table 1: Effect of treatments on quality of oil

Extract	Acid value(mgKOH/g)	Iodine value	Saponification value	Peroxide value	Free acid value
Control (W)	1.122	37.258	121.67	0.012	0.677
A 1:3	1.178	41.705	22.4	0.016	0.607
A 1:2	1.178	36.594	19.6	0.013	0.500
B 1:2	1.369	46.791	22.4	0.016	0.501
B 1:3	1.009	43.750	33.7	0.016	0.550

3.1.2. Iodine value

The iodine value, indicative of unsaturation levels observed in Table 1, was highest in B 1:2 (46.791) and lowest in A 1:2 (36.594). Higher iodine values in POME-treated samples suggest enhanced release of unsaturated compounds, consistent with Kowalska *et al.* (2021), who demonstrated that enzymatic treatments increase the recovery of unsaturated terpenes like d-limonene. Conversely, the lower iodine value in the control (37.258) reflects the limitations of non-enzymatic extraction in liberating unsaturated compounds from the peel matrix.

3.1.3. Saponification and peroxide values

Saponification values, representing molecular weight as seen in Table 1, were markedly lower in enzymatically treated samples (e.g., 22.4 in A 1:3 and 19.6 in A 1:2) compared to the control (121.67). This reduction suggests enzymatic hydrolysis disrupted ester bonds, producing smaller molecules, as similarly noted by Bakkali *et al.* (2008). Peroxide values, indicative of oxidation, remained uniformly low across all samples, with enzymatic treatments exhibiting the highest value of 0.016. These results reinforce findings from Marongiu *et al.* (2004), who reported that enzymatic extraction minimizes oxidative stress on essential oils.

3.2. Physical characteristics of orange peel essential oils

3.2.1. Yield

The control yielded the highest essential oil content ($5.08 \pm 0.19\%$) (Table 2), while enzymatic treatments produced lower yields, with B 1:3 achieving the highest among enzymatic methods (2.54%). Reduced yields in enzymatic treatments may result from selective extraction processes, where enzymes target specific compounds while leaving others intact. These findings are consistent with Marongiu *et al.* (2004), who observed selective compound recovery in enzymatic extraction.

3.2.2. Density, specific gravity, and refractive index

Density values ranged from 0.75 g/cm^3 (control) to 0.84 g/cm^3 (A 1:2) as seen in Table 2, and specific gravity remained consistent across enzymatic treatments (0.843). The refractive index, highest in B 1:3 (0.96), reflects a concentration of aromatic compounds. These physical property enhancements corroborate the observations of Guimarães *et al.* (2019), who noted improved bioactive molecule concentration with enzymatic treatments.

3.2.3. Sensory characteristics

The sensory attributes (Table 2) of all samples, including color, odor, and touch, remained uniform, indicating that enzymatic extraction does not compromise the organoleptic properties of the oils. This finding supports the potential of enzymatic methods for preserving sensory integrity, as highlighted in studies by Bakkali *et al.* (2008).

Table 2: Physical characteristics of orange peel essential oils

Solvent	Control (W)	A 1:3	A 1:2	B 1:2	B 1:3
Yield (%)	5.08 ± 0.19	2	1.33	1.2	2.54
Color	Yellow to orange	Yellow to orange	Yellow to orange	Yellow to orange	Yellow to orange
Odor	Fresh	Fresh	Fresh	Fresh	Fresh
Touch	Oily	Oily	Oily	Oily	Oily
Solubility	Insoluble in water	Insoluble in water	Insoluble in water	Insoluble in water	Insoluble in water
Density(g/cm^3)	0.75 ± 0.02	0.80	0.84	0.76	0.82
Specific gravity	0.74 ± 0.01	0.843	0.843	0.843	0.843
Refractive index	1.56 ± 0.03	0.92	0.84	0.88	0.96

3.3. Chemical composition of orange peel essential oils

The chemical composition of the essential oils, analyzed using GC-MS, revealed significant variations influenced by enzymatic treatments as shown in Tables 3 to 5.

3.3.1. GC-MS analysis of control samples

In the control sample (Table 3), major compounds included 3-chloro-2-nitrophenyl methanol (33.50%), d-limonene (7.23%), and α -terpineol (21.25%). These findings align with Costa *et al.* (2021), who identified d-limonene as a dominant hydrocarbon monoterpene in citrus oils, contributing to their characteristic aroma. α -Terpineol, known for its antimicrobial properties, was also prominent (de Moraes *et al.*, 2022).

Table 3: GC-MS components of orange peel oil extracted with water (Control)

S. No.	Compounds	RT	Peak area (%)	Formula	MW
1	3-chloro-2-nitrophenyl methanol	8.34	33.50	CHCINO	187.58
2	D-limonene 1-methyl-4-(prop-1-en-2-yl) cyclohex-1-ene	5.43	7.23	C H	136.24
3	α -Terpineol 2-(4-methylcyclohex-3-en-1-yl) propan-2-ol	16.75	21.25	CHO	154.25
4	1-isopropyl-4-methylcyclohex-3-en-1-ol	5.39	36.02	CHO	154.25
5	2-(4-chlorophenyl)-5,5-dimethyl-1-oxaspiro[2.5]octan-4-one	12.88	0.41	CHCLO	264.75
6	β -bisabolene (S)-1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohex-1-ene10.60	1.60	CH	204.36	
Note: RT = Retention time, MW = Molecular weight.					

3.3.2. GC-MS analysis of enzymatically treated samples

CAPD Treatments (A 1:3 and A 1:2): Increased α -terpineol (2.60%) and altered d-limonene content suggest enzymatic modification of certain terpenes, consistent with findings by Guimarães *et al.* (2019).

Table 4: GC-MS components of orange peel oil extracted with cellulase CAPD (*Bacillus megaterium*)

S. No.	Compounds	RT	Peak area (%)	Formula	MW
1	(E)-3,7-dimethylocta-2,6-dien-1-yl acetate	6.97	2.54	CHO	196.29
2	(1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	10.08	0.43	CH	136.24
3	1-isopropyl-4-methylcyclohexa-1,4-diene	17.62	41.76	C H	136.24
4	[5,5,10,10-tetrachlorotricyclo 7.1.0.0(4,6)]decane	5.44	30.17	CHCl	271.97
5	β -bisabolene (S)-1-methyl-4-(6-methylhepta-1,5-dien-2-yl) cyclohex-1-ene	25.43	7.34	CH	204.36
6	D-limonene 1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	16.32	2.60	CH	136.24
7	o-cymene 1-methyl-2-(propan-2-yl)benzene	20.23	15.09	CH	134.22
Note: RT = Retention time, MW = Molecular weight.					

POME Treatments (B 1:3 and B 1:2): Enhanced concentrations of β -bisabolene (11.48%) and (Z)-3,7-dimethylocta-2,6-dien-1-ol (14.33%) highlight the enzymatic specificity in releasing bioactive compounds. These compounds, valuable in cosmetics and pharmaceuticals, corroborate observations by Kumar *et al.* (2020).

Table 5: GC-MS components of orange peel oil extracted with cellulase POME (*Bacillus pumilus*)

S. No.	Compounds	RT	Peak area (%)	Formula	MW
1	β -bisabolene (S)-1-methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohex-1-ene	17.50	11.48	CH	204.36
2	β -Terpineol 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	12.12	1.27	CHO	154.25
3	D-limonene 1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	11.81	3.57	CH	136.24
4	(Z)-3,7-dimethylocta-2,6-dien-1-ol	3.59	14.33	C HO	154.25
5	[5,5,10,10-tetrachlorotricyclo 7.1.0.0(4,6)]decane	13.27	16.24	C HCl	271.97
6	(1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	26.43	14.08	CH	136.24
7	1-isopropyl-4-methylcyclohex-3-en-1-ol	21.49	0.86	CHO	154.25
8	1-isopropyl-4-methylcyclohexa-1,4-diene	2.53	38.15	CH	136.24

Note: RT = Retention time, MW = Molecular weight.

The reduced dominance of d-limonene in enzymatic treatments compared to the control suggests potential enzymatic transformation, a phenomenon previously noted by Bakkali *et al.* (2008).3.3.3. Comparison with previous studies

The results align with findings by Kowalska *et al.* (2021), who demonstrated the efficacy of cellulase enzymes in enhancing oil quality and selectively extracting bioactive components. However, the reduced yields observed in this study contrast with Marongiu *et al.* (2004), who reported higher yields with enzymatic methods. These discrepancies may arise from differences in enzyme type, substrate composition, and extraction parameters.

3.3.4. Implications and applications

The variation in chemical composition and improved physicochemical properties of enzymatically extracted essential oils underscore their potential applications in food, cosmetics, and pharmaceuticals. Enhanced concentrations of bioactive terpenes and oxygenated compounds suggest therapeutic benefits, as supported by Kumar *et al.* (2020). Further research optimizing enzymatic conditions could expand the utility of this approach in industrial applications.

4. Conclusion

This study investigated the impact of enzymatic treatments using cellulase CAPD and cellulase POME on the extraction and composition of essential oils from orange peels. The results demonstrated that enzymatic extraction significantly influences the physicochemical properties, chemical composition, and yield of the essential oils, offering a tailored approach to enhancing specific desirable attributes of the oils for various industrial applications.

The enzymatic treatments reduced acid, iodine, and saponification values, suggesting a decrease in unsaturated compounds and molecular weights. This indicates that enzymes can selectively hydrolyze complex molecules, leading to oils with potentially improved oxidative stability and specific bioactive properties. The GC-MS analysis revealed distinct compositional shifts, with increased concentrations of bioactive components such as α -terpineol and β -bisabolene, particularly in samples treated with cellulase POME. These findings highlight the potential of enzymatic extraction to enhance the release of oxygenated terpenes and other bioactive compounds, aligning with prior research on enzyme-assisted extraction methods.

Despite the reduced oil yield in enzymatic treatments compared to the control, the qualitative improvements in chemical composition suggest that enzymatic methods are advantageous for applications prioritizing oil quality over quantity. This aligns with previous studies emphasizing the importance of enzymatic specificity in targeting bioactive compounds for use in pharmaceuticals, cosmetics, and food industries.

In conclusion, enzymatic extraction using cellulase CAPD and POME offers a promising alternative to conventional hydrodistillation, allowing for the production of essential oils with enhanced physicochemical properties and tailored chemical profiles. Future research should explore optimization of enzyme concentrations, reaction conditions, and substrate variations to maximize both yield and quality. Additionally, the environmental benefits and scalability of enzymatic methods present opportunities for sustainable and efficient essential oil production.

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Declaration

All sources of information have been duly acknowledged. All authors also declare that we have adhered to the ethical guidelines for research and authorship.

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