International Food Research Journal 32(4): 1126 - 1138 (August 2025)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of pre-processing conditions on composition and properties of ginger (*Zingiber officinale* Roscoe) essential oil

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Article history

Received:
30 July 2024
Received in revised form:
1 October 2025
Accepted:
3 November 2025

Keywords

antibacterial, antioxidant, essential oil, ginger, pretreatment, volatile compounds

Abstract

Ginger (*Zingiber officinale* Roscoe) is a herbaceous perennial plant, widely used as a spice and in traditional medicine. Essential oil extracted from ginger exhibits various beneficial effects due to its antioxidant and antibacterial properties. The present work evaluated the effects of three pretreatment methods (fresh, frozen, and heat pump-dried) on the quality attributes of ginger essential oils. Gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the volatile compounds in the three essential oils. The yield of ginger essential oil was found to be highest in the heat pump drying method. The essential oil obtained from dried ginger samples contained the greatest number of volatile compounds (19), whereas fresh and frozen ginger essential oils contained 12 and 17 compounds, respectively. Volatile compounds present at levels greater than 2% were detected in all three ginger essential oil samples (fresh, frozen, and heat pump-dried), including camphene, eucalyptol, neral, α-citral, zingiberene, and cedrene. The results of principal component, hierarchical cluster, and heat map analyses confirmed significant changes in the volatile compounds of ginger essential oil obtained from the three different pretreatment methods. The antioxidant and antibacterial properties of heat pump-dried ginger essential oil were significantly higher than those of the other two essential oil samples. Pearson correlation analysis revealed that variations in the volatile compounds of ginger essential oil were significantly associated with its antioxidant and antibacterial properties. These findings may assist growers and producers in selecting appropriate pretreatment methods for the extraction of ginger essential oil.

DOI
https://doi.org/10.47836/ifrj.32.4.17

Introduction

Ginger (*Zingiber officinale* Roscoe) is a perennial herbaceous plant cultivated in countries with temperate, subtropical, and tropical climates (Jiang *et al*., 2006). In Vietnam, it is one of the most popular fresh spices and has considerable export potential (Nguyen *et al*., 2022). Ginger has been known since ancient times and is an important herb and spice in daily life (Abdo *et al*., 2018). Ginger can be used in either dried or fresh form. Due to its distinctive flavour and aroma, ginger is incorporated into various foods, such as ginger tea, bread, cookies, and confectionery products (El-Ghorab *et al*., 2010; Ajayi *et al*., 2013). In addition, due to its excellent pharmacological effects, ginger is also used as a traditional remedy in many cultures (Lua *et al*., 2015). Several studies have demonstrated the medicinal importance of ginger due to its hepatoprotective, hypoglycaemic, anti-tumour, anti-inflammatory, antibacterial, and antioxidant effects (Jiang *et al*., 2006; Garza-Cadena *et al*., 2023). In particular, ginger has been reported to mitigate harmful factors associated with COVID-19, such as oxidative stress, and elevated prostaglandin levels. Moreover, it has been suggested that ginger may have therapeutic potential against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and inflammation (Jafarzadeh *et al*., 2021).

Ginger essential oils are volatile aromatic compounds extracted from fresh or dried ginger rhizomes. Ginger essential oil has been reported to

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possess notable therapeutic properties, including antioxidant, anti-infective, anti-inflammatory, anti-cancer, analgesic, and cough-suppressant activities (Sritoomma *et al*., 2014; Munda *et al*., 2018). The two most important phenolic compounds identified are gingerols and shogaols. Among them, gingerols are responsible for the characteristic pungent aroma of ginger (Bhattarai *et al*., 2018). Shogaols are formed through the dehydration of gingerols during the drying process of fresh ginger, and they contribute to the pungent aroma of dried ginger (Huang *et al*., 2011). In addition, ginger essential oil contains other important compounds such as curcumene, sesquiphellandrene, bisabolene, zingiberene, 6-dehydrogingerdione, galanolactone, ginger sulfonic acid, geraniol, neral, monoacyldigalactosylglycerols, and ginger glycolipids (Kamal *et al*., 2023).

Several conventional methods are commonly used to extract essential oils, including steam distillation, mechanical pressing, and supercritical CO_2 extraction (Shukla *et al*., 2019; Da Silva Moura *et al*., 2020). Pretreatment methods can also be applied to improve the yield and composition of essential oils. Such methods help extend the shelf life of raw materials, prevent the growth of harmful microorganisms, and inhibit enzyme activities (García-Segovia *et al*., 2011). Drying methods prior to essential oil extraction have been reported to reduce nutrient loss, minimise aroma and colour changes, and promote the development of antioxidant compounds (Hossain *et al*., 2010; Ozdemir *et al*., 2018). For example, drying methods have been shown to affect the quality of Thomson navel orange essential oil, with freeze-drying reported to achieve the highest efficiency in essential oil recovery and retention of biological activities (Ozdemir *et al*., 2018).

The concentrations of volatile compounds in essential oils can either decrease or increase depending on the pretreatment method applied (Calín-Sánchez *et al*., 2013). Each pretreatment method has a distinct mechanism for transferring energy into the feedstock at different rates and durations. This results in changes to the structural, physical, and mechanical properties of the material, particularly in terms of irreversible chemical and biological reactions (Xing *et al*., 2017). Osae *et al*. (2021) reported that the freeze-drying method enhanced the number of volatile compounds in Ghanaian ginger essential oil, yielding 24 compounds, a number higher than that obtained using other drying techniques such as relative humidity convective, infrared, microwave, and pulsed vacuum drying. Similarly, Aabha *et al*. (2022) observed that dehydration of ginger rhizomes increased the levels of zingiberene, geraniol, neral, and geranial, while reducing those of geranyl acetate, β-phellandrene, and camphene. Previous studies have primarily focused on the effects of pretreatment methods on the number and concentration of volatile compounds in essential oils, whereas information regarding the influence of ginger pretreatment on its biological activities has rarely been reported. Therefore, the present work was systematically conducted to evaluate the effects of different ginger pretreatment methods (including fresh, frozen, and heat pump-dried) on critical parameters such as essential oil yield, density, and volatile compound composition, with particular emphasis on the oil's biological activities. The findings provide evidence-based guidance for growers and manufacturers in selecting pretreatment methods that extend the storage life of raw ginger and address a substantial gap in optimising the production of ginger essential oil with enhanced bioactivity. Ultimately, these results are expected to contribute to improved ginger essential oil quality for applications in the food, pharmaceutical, and nutraceutical industries.

Materials and methods

Materials

Eight-month-old ginger rhizome samples were purchased from a local market in Ho Chi Minh City, Vietnam, and were confirmed to be fresh, undamaged, and free from rot. The samples were divided into three portions. The first portion was extracted and analysed in the fresh state. The second and third portions were subjected to pretreatment prior to essential oil extraction. The second portion was dried in a heat pump dryer (Dragon Food Equipment. Ltd., Viet Nam) at 45°C with an air velocity of 40 Hz for 16 h. The third portion was frozen (Model XLT C400, Nordic Lab, Denmark) at -30°C for 24 h.

Chemicals

Some chemicals used include DPPH and ABTS reagents originating from Sigma-Aldrich (USA), *n*-hexane (95%; Fisher Chemical), methanol (99.5%; Xiling, China), and dimethyl sulfoxide (99.5%; GHTech, China).

Microbiological culture mediums Muller Hinton (MH) and Muller Hinton Agar (MHA) were provided by HiMedia (India). The microbial strains used included two Gram-positive bacterial strains (*Bacillus cereus* NRRL B-3711 and *Staphylococcus aureus* NRRL B-313) and two Gram-negative bacterial strains (*Campylobacter jejuni* DSM 24114 and *Escherichia coli* NRRL B-409) provided by Institute Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

Extract of essential oils

Fresh, heat pump-dried, and frozen ginger samples were distilled by steam entrainment at a solvent-to-fresh material ratio of 2:1 (L/kg) and a temperature of 130°C for approximately 180 min (measured from the appearance of the first drop) using a Clevenger-type apparatus. The essential oils were dehydrated with anhydrous sodium sulphate and stored at -4°C until analysis.

Yield and density

The yield and density of ginger essential oil were calculated according to Kamal *et al*. (2011), using Eq. 1:

$$Y(\%) = \left(\frac{mass\ of\ essential\ oil\ after\ dehydration}{mass\ of\ raw\ material}\right) \times 100 \quad \text{(Eq. 1)}$$

The density of ginger essential oil at 25°C was determined by weighing 1 mL of the essential oil using a PA214 four-decimal-place analytical balance (Ohaus, USA).

Gas chromatography-mass spectrometry (GC-MS) assay

The volatile components of the three ginger essential oils were determined using a Waters-Agilent-Pal RSI-coupled series GC-MS system. A DB 5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) was used for compound separation. Helium was employed as the carrier gas at a flow rate of 1 mL/min. Each essential oil sample (1 µL) was diluted in *n*-hexane at a ratio of 1:100 (v/v) and injected automatically. The mass spectrometer was operated at an ionisation energy of 70 eV. Compounds in the ginger essential oils were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) library.

DPPH assay

The DPPH free radical scavenging activity of ginger essential oils was determined according to Brand-Williams *et al*. (1995), with minor modifications. Briefly, methanol was used to prepare a stock DPPH solution (0.004%). After 24 h of incubation, the stock solution was diluted with methanol to obtain a working DPPH solution with an absorbance of 1.10 ± 0.02. Subsequently, 10 µL of ginger essential oil was added to 190 µL of the working DPPH solution. After 30 min of incubation at room temperature, the absorbance was measured at 517 nm. The DPPH free radical scavenging activity was calculated using Eq. 2:

$$DPPH\ (\%) = \frac{Abs_{blank} - Abs_S}{Abs_{blank}} \times 100\% \quad \text{(Eq. 2)}$$

where, Abs_{blank} = absorbance of the solvent blank, and Abs_S = absorbance of the working DPPH solution after the addition of essential oil.

ABTS assay

The ABTS free radical scavenging activity of ginger essential oils was determined according to Re *et al*. (1999) with minor modifications. Briefly, ABTS solution (0.007 M) was mixed with potassium persulphate (0.0049 M) at a 1:1 ratio and incubated in the dark for 24 h. The resulting solution was diluted with methanol to obtain a working ABTS solution with an absorbance of 1.10 ± 0.02. Subsequently, 10 µL of ginger essential oil was added to 190 µL of working ABTS solution. After 30 min of incubation at room temperature, the absorbance was measured at 734 nm. The ABTS free radical scavenging activity was calculated using Eq. 3:

$$ABTS\ (\%) = \frac{Abs_{blank} - Abs_S}{Abs_{blank}} \times 100\% \quad \text{(Eq. 3)}$$

where, Abs_{blank} = absorbance of the solvent blank, and Abs_S = absorbance of the working ABTS solution after the addition of essential oil.

Antibacterial activity

The antibacterial activity of ginger essential oils (fresh, frozen, and heat pump-dried) was determined following the agar disk diffusion method described by Abdullahi *et al*. (2020) with minor

modifications. Ginger essential oil samples (400 µL) were diluted in dimethyl sulfoxide (DMSO; 1 mL). All microorganisms were adjusted to a turbidity equivalent to McFarland No. 0.5 in MH medium. Then, 15 mL of MHA medium was poured into each Petri dish. After solidification, 100 µL of bacterial suspension was spread evenly over the agar surface. Sterile filter paper disks (6 mm diameter) impregnated with 10 µL of diluted ginger essential oil were placed onto the inoculated agar. The positive control disks were impregnated with Ciprofloxacin (0.01 mg/mL), and the negative control disks contained only DMSO.

To prevent evaporation of the essential oils, all Petri dishes were sealed with sterile Parafilm. The dishes were kept at room temperature for 30 min to allow the essential oils to diffuse. The diameter of the inhibition zones (mm) was measured after 24 h of incubation at 37°C to assess the antibacterial activity.

Statistical analysis

Results were expressed as mean ± standard deviation of three replicates. SPSS software version 22 (SPSS Inc., Chicago, IL, USA) was used to perform a One-way ANOVA, and the Tukey's test was applied to determine statistically significant differences at the 5% significance level. Origin Pro 21 software (Origin Lab, USA) was used to calculate the principal component analysis (PCA), hierarchical cluster analysis (HCA), Pearson correlation, and construct graphs.

Results and discussion

Yield and density of ginger essential oils

The yield and density of ginger essential oil samples (fresh, frozen, and heat pump-dried) are presented in Table 1. The yield of ginger essential oil was significantly affected by the pretreatment method. In particular, the heat pump-drying method produced a significantly higher yield ($p < 0.05$) than the frozen and fresh methods. Although the yield of frozen ginger essential oil was greater than that of fresh ginger, the difference was not statistically significant. In a study on purple basil landrace essential oil extraction, dried samples also exhibited higher yields than fresh samples (Pirbalouti *et al*., 2013a). For the frozen method, when the temperature is reduced to -30°C, both free water molecules and chemically bound water in ginger are frozen. Upon thawing, these water molecules are released from the material, altering its structure. This structural change facilitates the release of essential oils from the material, thereby increasing the yield.

Table 1. Yield, density, and antioxidant capacity of essential oils.

Treatment	Yield (%)	Density (g/mL)	DPPH (%)	ABTS (%)
Fresh	0.030 ± 0.010^{a}	0.882 ± 0.006^{a}	24.92 ± 1.51^{a}	16.73 ± 1.20^{a}
Frozen	0.077 ± 0.015^{a}	0.882 ± 0.007^{a}	31.84 ± 1.07^{b}	20.39 ± 1.06^{b}
Heat pump - Dried	0.177 ± 0.032^{b}	0.871 ± 0.004^{a}	41.33 ± 1.04^{c}	27.30 ± 1.08^{c}

Means followed by different lowercase superscripts within similar column are significantly different among samples according to Tukey's *post hoc* test ($p < 0.05$).

The density values of the essential oil samples were similar and were not significantly affected by either drying or freezing methods. The densities (g/mL) of fresh, frozen, and heat pump-dried ginger essential oils were 0.882 ± 0.006, 0.882 ± 0.007, and 0.871 ± 0.004, respectively. These values are consistent with previous reports on the densities of Chinese and Thai ginger essential oils (Kamal *et al*., 2023).

Volatile compounds of ginger essential oils

The quality of ginger essential oil is largely determined by its volatile compounds. Therefore, GC-MS analysis was conducted to evaluate changes in the volatile compounds of three types of ginger essential oils (fresh, frozen, and heat pump-dried). A total of 22 compounds were identified (Table 2). The volatile compounds in ginger essential oil (fresh, frozen, and heat pump-dried) comprised five monoterpene hydrocarbons, one ketone, two alcohols, five oxygenated monoterpene hydrocarbons, two esters, one aldehyde, and six sesquiterpene hydrocarbons. The types and quantities of volatile compounds varied among the ginger essential oil samples, suggesting that pretreatment influenced their composition. Sesquiterpenes were the most abundant compounds, followed by aldehydes (α-citral), monoterpenes, and oxygenated

monoterpenes. Similarly, sesquiterpenes and monoterpenes have been reported as the predominant volatile compounds in sweet ginger (*Alpinia coriandriodora* D. Fang) essential oil (Dong *et al*., 2020). Another study also reported that the types and quantities of volatile compounds in essential oils were significantly affected by the material pretreatment method (Pirbalouti *et al*., 2013b).

Volatile compounds belonging to the sesquiterpene, monoterpene classes are known to play important roles in the antioxidant, cytotoxic, anti-inflammatory, antimicrobial, and insecticidal activities of essential oils (Silva *et al*., 2021). Variations in the composition and quantity of volatile compounds in the essential oil samples (fresh, frozen, and heat pump-dried) are illustrated in Figure 1A. The results indicated that the freezing and heat pump-drying processes significantly increased the levels of certain volatile compounds.

Table 2. Categories of volatile compounds.

Code	Compound	Formula	Yield (%)		
			Fresh	Frozen	Heat pump - Dried
Monoterpene hydrocarbon					
1	α-pinene	$C_{10}H_{16}$	4.40 ± 0.01	1.07 ± 0.02	3.10 ± 0.01
2	Camphene	$C_{10}H_{16}$	4.80 ± 0.02	3.08 ± 0.02	10.22 ± 0.03
3	β-pinene	$C_{10}H_{16}$	1.10 ± 0.01	0.75 ± 0.02	1.72 ± 0.01
4	α-phellandrene	$C_{10}H_{16}$	ND	ND	0.58 ± 0.01
5	3-carene	$C_{10}H_{16}$	ND	3.66 ± 0.02	6.80 ± 0.04
Ketone					
6	Sulcatone	$C_8H_{14}O$	ND	ND	1.31 ± 0.02
Alcohol					
7	Eucalyptol	$C_{10}H_{18}O$	2.50 ± 0.02	2.40 ± 0.01	11.00 ± 0.05
8	Linalool	$C_{10}H_{18}O$	1.80 ± 0.01	ND	1.47 ± 0.01
Oxygenated monoterpene hydrocarbon					
9	Borneol	$C_{10}H_{18}O$	ND	1.46 ± 0.02	3.10 ± 0.03
10	α-terpineol	$C_{10}H_{18}O$	ND	1.28 ± 0.01	2.54 ± 0.02
11	Neral	$C_{10}H_{16}O$	8.60 ± 0.02	8.04 ± 0.02	15.05 ± 0.05
12	Geraniol	$C_{10}H_{18}O$	ND	3.18 ± 0.01	2.16 ± 0.01
13	β-terpineol	$C_{10}H_{18}O$	1.30 ± 0.01	ND	ND
Ester					
14	Citronellol acetate	$C_{12}H_{22}O_2$	ND	2.02 ± 0.04	1.81 ± 0.01
15	Geranyl acetate	$C_{12}H_{20}O_2$	1.40 ± 0.02	1.16 ± 0.01	0.87 ± 0.01
Aldehyde					
16	α-citral	$C_{10}H_{16}O$	19.80 ± 0.06	16.32 ± 0.04	20.08 ± 0.05
Sesquiterpene hydrocarbon					
17	Copaene	$C_{15}H_{24}$	ND	0.65 ± 0.01	0.30 ± 0.01
18	Curcumene	$C_{15}H_{22}$	ND	5.73 ± 0.04	3.81 ± 0.03
19	Zingiberene	$C_{15}H_{24}$	34.50 ± 0.07	28.91 ± 0.06	7.22 ± 0.04
20	α-farnesene	$C_{15}H_{24}$	12.50 ± 0.04	ND	ND
21	β-bisabolene	$C_{15}H_{24}$	ND	12.94 ± 0.02	3.91 ± 0.01
22	Cedrene	$C_{15}H_{24}$	6.90 ± 0.01	7.05 ± 0.01	2.67 ± 0.02

ND: not detected. Values represent descriptive GC-MS data (mean ± SD, $n = 3$) without statistical comparison, as the table is intended to provide a compositional profile. Statistical analyses of treatment effects on essential oil yield are presented in Table 1.

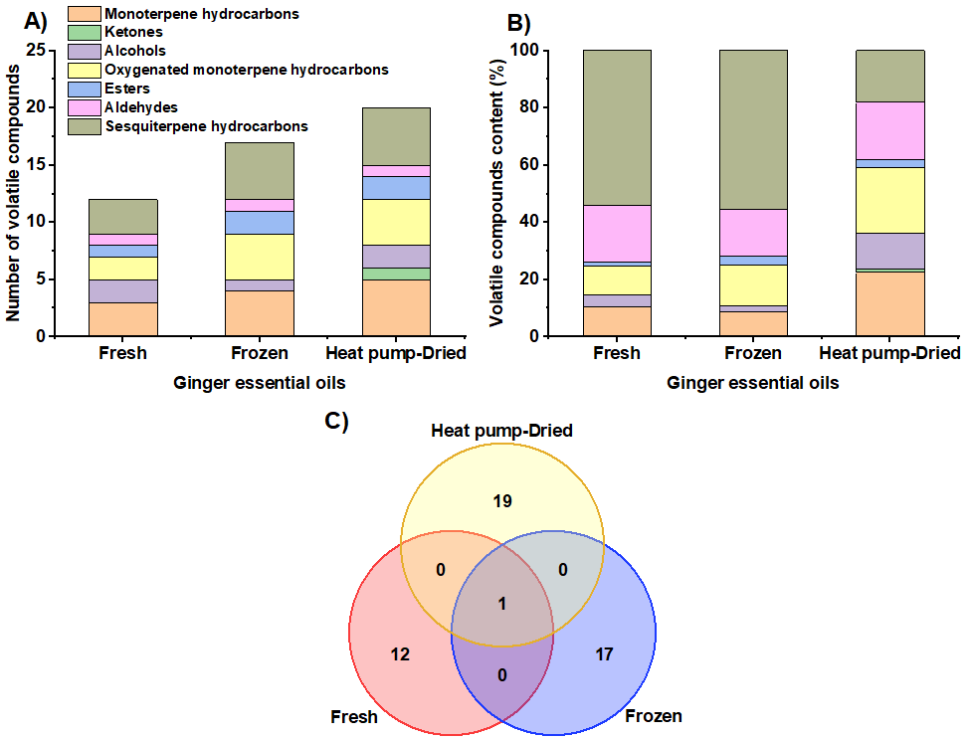

Figure 1. Volatile compounds of three ginger essential oils: **(A):** types of volatile compounds; **(B)** percentage of volatile compounds; and **(C)** percentage of volatile compounds Venn diagram.

In particular, the number of oxygenated monoterpene compounds increased to four; the number of ester compounds increased to two; and the number of sesquiterpene compounds increased to five. For monoterpenes, the number of compounds increased to four in ginger essential oil subjected to freezing and to five in ginger essential oil subjected to heat pump-drying. Notably, in heat pump-dried ginger essential oil, the presence of sulcatone (a ketone) was also detected. The results demonstrated that the number of volatile compounds in ginger essential oil increased the most when subjected to heat pump-drying. This may be attributed to the higher essential oil recovery efficiency of the heat pump-dried ginger sample compared with the fresh and frozen samples.

The proportions of volatile compounds in ginger essential oils (fresh, frozen, and heat pump-dried) are illustrated in Figure 1B. The results showed that sesquiterpenes and aldehydes (α-citral) were the two most dominant compound classes in both fresh and frozen ginger essential oils. In fresh ginger essential oil, the proportions of sesquiterpenes and aldehydes were 53.90 and 19.8%, respectively. In frozen ginger essential oil, the proportions of sesquiterpenes and aldehydes were 55.28 and 16.32%, respectively. In addition, the heat pump-drying process increased the proportions of monoterpenes (22.42%), ketones (1.31%), alcohols (12.42%), oxygenated monoterpenes (22.85%), and aldehydes (α-citral) (20.08%), while the proportion of sesquiterpenes decreased to 17.91%. Kamal *et al.* (2023) also reported that sesquiterpenes are the predominant compounds in Chinese and Thai fresh ginger essential oils; however, sun- and oven-drying processes significantly reduced their proportion.

The Venn diagram of the proportions of volatile compounds in ginger essential oils (fresh, frozen, and heat pump-dried) is illustrated in Figure 1C. The results showed that fresh ginger essential oil contained a total of 12 volatile compounds. Freezing and heat pump-drying increased the number of volatile compounds to 17 and 19, respectively. These findings indicated that both pretreatment processes (freezing and heat pump-drying) significantly increased the number of volatile compounds. In particular, as shown in Figure 1C, one volatile compound was common to all three ginger essential oil samples (fresh, frozen, and heat pump-dried), and the main compound was α-citral.

Principal component analysis

PCA was employed to illustrate the variance distribution among volatile compounds in the three types of ginger essential oils (fresh, frozen, and heat pump-dried). As shown in Figure 2, the two principal components (PC1 and PC2) together explained the total variability of volatile compounds in these oils.

Specifically, PC1 accounted for 70.90% of the total variance, while PC2 explained 29.10%. PC1 was positively associated with α-pinene, β-pinene, linalool, neral, β-terpineol, α-citral, and α-farnesene, but negatively associated with 3-carene, borneol, α-terpineol, geraniol, citronellol acetate, curcumene, and β-bisabolene. PC2 was positively associated with camphene, α-phellandrene, sulcatone, and eucalyptol, but negatively associated with geranyl acetate, copaene, zingiberene, and cedrene.

Figure 2 also shows a distinct distribution of three separate clusters corresponding to the three types of ginger essential oils (fresh, frozen, and heat pump-dried). The fresh ginger essential oil cluster was characterised by contributions from β-terpineol and α-farnesene. This finding was consistent with the results in Table 2, where β-terpineol and α-farnesene were detected only in fresh ginger essential oil. The frozen ginger essential oil cluster was characterised by the contribution of copaene, which was also present at the highest level in frozen ginger essential oil. The heat pump-dried ginger essential oil cluster was clearly characterised by α-phellandrene, sulcatone, and eucalyptol. This observation agreed with the results in Table 2, where α-phellandrene and sulcatone were detected only in heat pump-dried ginger essential oil, while eucalyptol was also found at its highest level in this group. These results indicated that the composition and content of volatile compounds in ginger essential oil are influenced by different pretreatment methods, with heat pump drying causing the most pronounced compositional changes. Moreover, the PCA results provided quantitative evidence of variance distribution, thereby strengthening the interpretation of pretreatment method discrimination based on volatile composition.

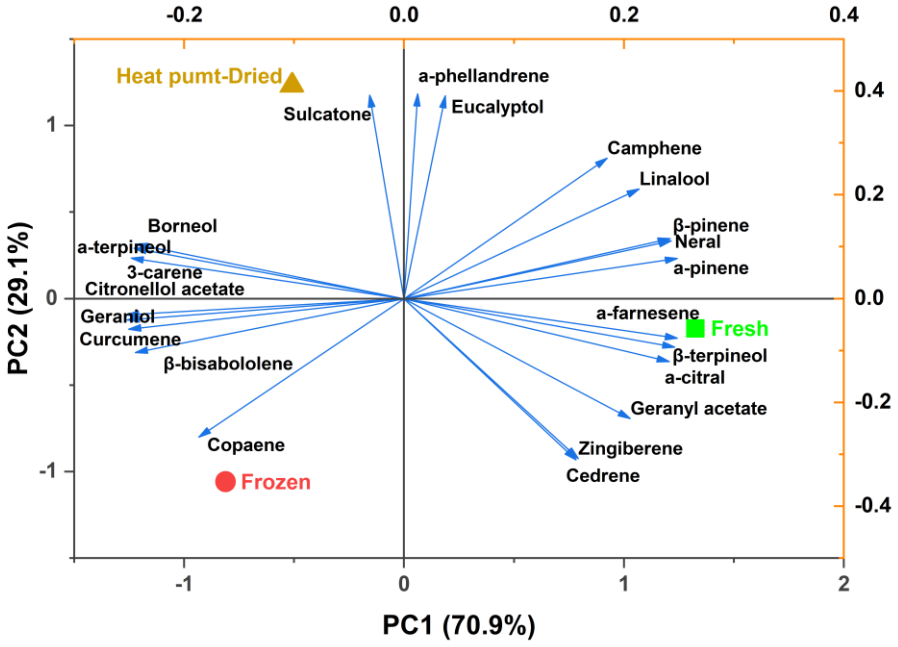

Figure 2. Principal component analysis of major compounds of three ginger essential oils.

Hierarchical cluster analysis of ginger essential oils

HCA is a commonly used statistical tool for classifying samples so that those within the same group are more similar to each other and different from those in other groups. In the present work, HCA was applied to explore changes in the volatile compounds of ginger essential oils (fresh, frozen, and heat pump-dried).

The HCA plot of the three ginger essential oils is shown in Figure 3A. The results indicated three distinct groups in the dendrogram with significant average distances, corresponding to fresh, frozen, and heat pump-dried ginger essential oil. The dendrogram revealed clear cluster formation. Among them, the heat pump-ginger essential oil group formed the first cluster, which had the highest average distance (100) compared with the other two clusters. The second cluster was positioned between the fresh and frozen ginger essential oil groups, with an average distance of 66.33. The average distance between the fresh and frozen ginger essential oil groups was 33.67. These findings suggested that the heat pump drying method

altered the composition of ginger essential oil to a greater extent than the freezing method, consistent with the PCA results.

The volatile compounds responsible for the clustering in Figure 3A were identified using a heat map (Figure 3B). The relative abundances of volatile compounds are expressed with values ranging from low to high, corresponding to a colour change from green to orange-brown. The six most abundant volatile compounds in fresh ginger essential oil are α-pinene, linalool, β-terpineol, geranyl acetate, zingiberene, and α-farnesene. However, α-citral, zingiberene, and α-farnesene were the three predominant compounds in fresh ginger essential oil assessed in the present work. The six most abundant volatile compounds in frozen ginger essential oil were geraniol, citronellol acetate, copaene, curcumene, β-bisabolene, and cedrene. Among these, α-citral, zingiberene, and β-bisabolene were the three predominant compounds. In heat pump-dried ginger essential oil, ten volatile compounds were detected at the highest abundances: camphene, β-pinene, α-phellandrene, 3-carene, sulcatone, eucalyptol, borneol, α-terpineol, neral, and α-citral. Of these, camphene, eucalyptol, neral, and α-citral were the four predominant compounds.

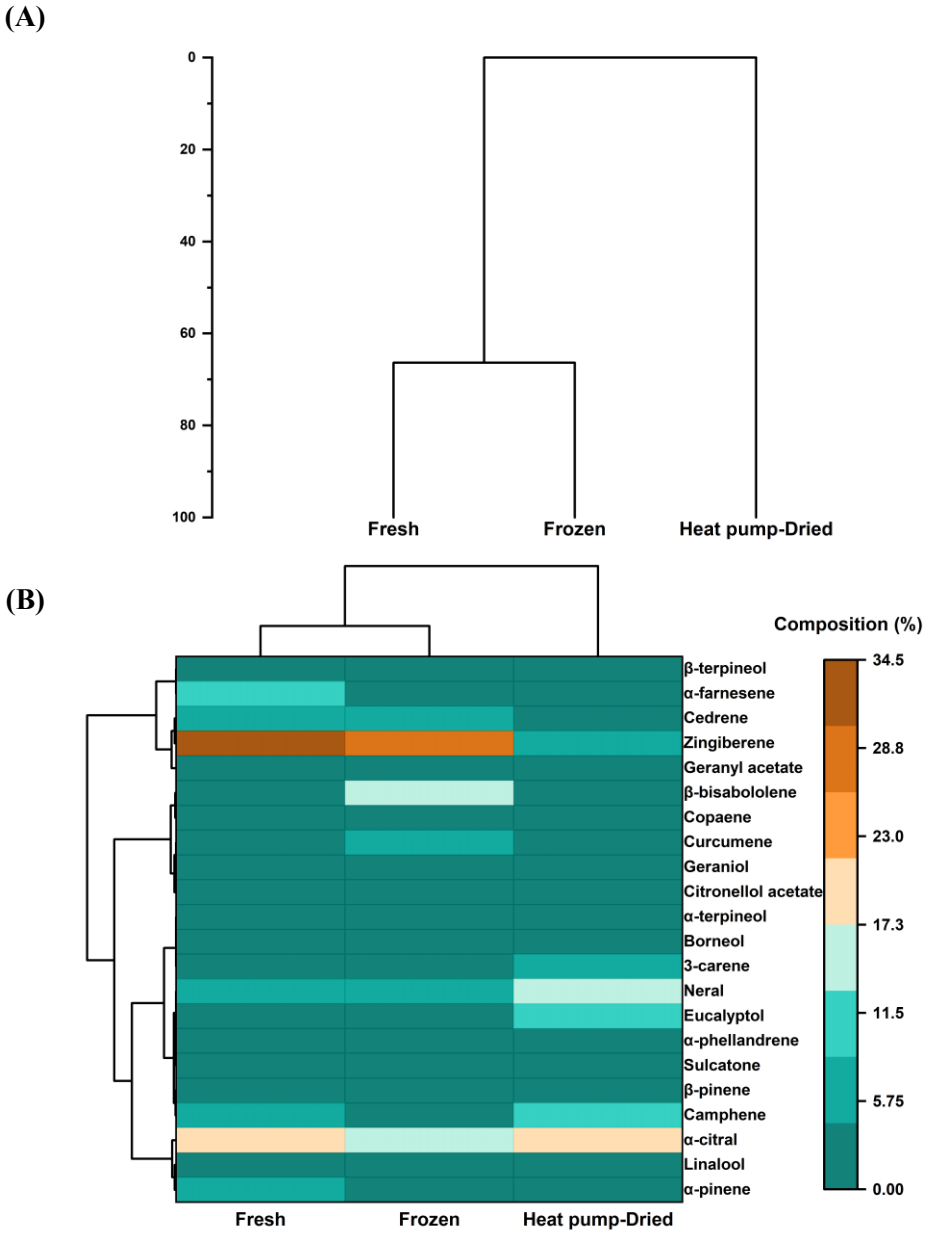

Figure 3. Volatile compounds of three ginger essential oils. **(A)**: hierarchical cluster analysis; and **(B)** heat map analysis of volatile compounds' abundance.

Antioxidant capacity of ginger essential oils

The antioxidant capacity of ginger essential oils (fresh, frozen, and heat pump-dried) was evaluated using their DPPH and ABTS free radical scavenging activities (Table 1). Overall, the antioxidant activity of ginger essential oils was significantly affected by the different pretreatment methods. The results showed that the DPPH and ABTS free radical scavenging activities of heat pump-dried ginger essential oil were the highest ($p < 0.05$), with values of 41.33 ± 1.04 and 27.30 ± 1.08, respectively. In contrast, the DPPH and ABTS free radical scavenging activities of fresh ginger essential oil were the lowest ($p < 0.05$), with values of 24.92 ± 1.51 and 16.73 ± 1.20, respectively. Drying methods have also been reported to significantly increase the free radical scavenging activity of *Origanum vulgare* L. and *O. onites* L. essential oils (Ozdemir *et al*., 2018). The antioxidant capacity of dried ginger essential oil was higher than that of the fresh and frozen samples, possibly due to the higher essential oil recovery efficiency of the drying method. Heat pump-dried ginger essential oil contained increased proportions of several volatile compounds, including camphene, β-pinene, α-phellandrene, sulcatone, eucalyptol, borneol, α-terpineol, neral, and α-citral. This likely contributed to the improved antioxidant capacity of the dried ginger essential oil, as these compounds have been reported to possess antioxidant properties (Badr *et al*., 2021; Carpes *et al*., 2021; Luo *et al*., 2022).

Antibacterial activity of ginger essential oils

The antibacterial activity of ginger essential oils (fresh, frozen, and heat pump-dried) and the antibiotic ciprofloxacin was evaluated using the agar disk diffusion method against Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative bacteria (*Campylobacter jejuni* and *Escherichia coli*). Both ginger essential oils and the antibiotic exhibited antibacterial activity against all tested bacterial strains, although at different levels (Figure 4A). In general, the diameters of the inhibition zones for all bacteria increased significantly in the following order: frozen ginger essential oil, heat pump-dried ginger essential oil, and antibiotics. However, the differences in inhibition zone diameters of the four bacterial strains between fresh and frozen ginger essential oil were not statistically significant. Drying methods have also been reported to enhance the antibacterial activity of *Mentha aquatica* L. essential oil to bacteria (*E. coli* and *S. aureus*) (Djamila *et al*., 2021). Two pretreatment methods (freezing and heat pump-drying) enhanced the antibacterial activity of ginger essential oil. This effect may be attributed to an increase in oxygenated monoterpenes, esters, and sesquiterpenes in these two essential oil samples. However, the heat pump-dried ginger essential oil exhibited a greater increase in monoterpenes and the presence of ketones, which further improved its antibacterial activity. Several other studies have also reported that volatile compounds such as camphene, β-pinene, α-phellandrene, 3-carene, sulcatone, eucalyptol, borneol, α-terpineol, neral, and α-citral are responsible for the antibacterial activity of essential oils (Muilu-Mäkelä *et al*., 2022; Hachlafi *et al*., 2023; Fatimazahra *et al*., 2024). Bacteria are inhibited because ginger essential oil directly affects the cell membrane, disrupting the cell structure, thereby increasing membrane permeability, and leading to the loss of essential structural functions (Wang *et al*., 2020). In addition, the lipophilic regions of isolated cell membranes and mitochondria can be readily penetrated by hydrophobic compounds in ginger essential oil, which compromise membrane integrity and impair key cellular functions, including nucleic acid stability, protein synthesis, enzyme activities, and energy metabolism in bacterial cells (Beristain-Bauza *et al*., 2019).

The three types of ginger essential oils (fresh, frozen, and heat pump-dried) all exhibited larger inhibition zone diameters against Gram-positive bacteria (*B. cereus* and *S. aureus*) than against Gram-negative bacteria (*C. jejuni* and *E. coli*). This difference is attributed to the distinct cell envelope structures of Gram-positive and Gram-negative bacteria. Gram-positive bacteria possess a thick peptidoglycan layer, whereas Gram-negative bacteria have an additional outer membrane containing a thick lipopolysaccharide layer (Hsouna *et al*., 2011). The lipid bilayer structure of Gram-negative bacteria enhances their resistance to antibacterial compounds (Beristain-Bauza *et al*., 2019).

Correlation between volatile compounds with the antioxidant and antibacterial properties of ginger essential oils

Pearson correlation analysis was conducted to evaluate the relationships between antioxidant capacity (DPPH and ABTS), antibacterial capacity (*B. cereus*, *S. aureus*, *C. jejuni*, and *E. coli*), and the

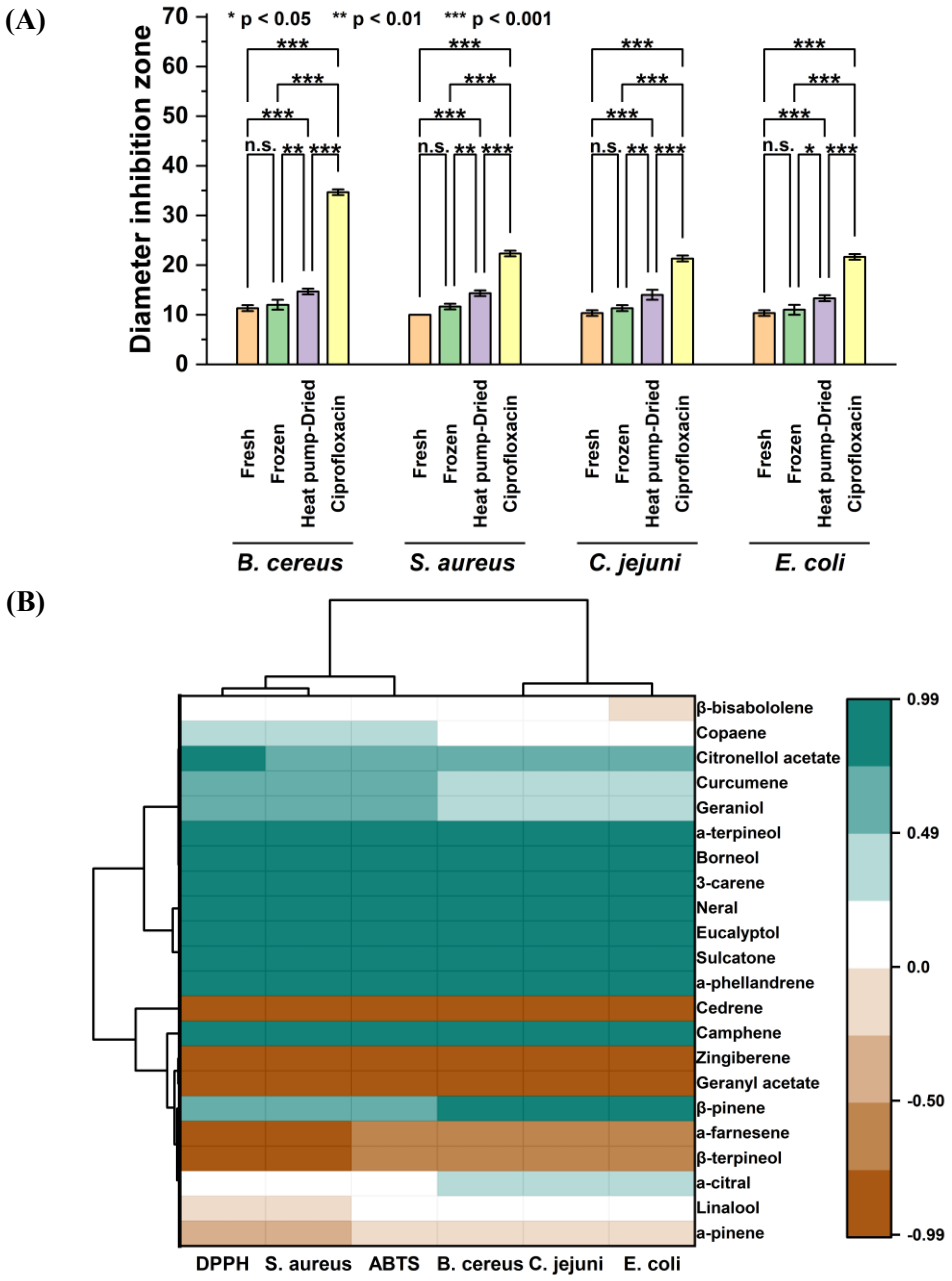

Figure 4. Properties of three ginger essential oils. **(A)** diameter of inhibition zone of four types of bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Campylobacter jejuni*, and *Escherichia coli*) of ginger essential oil (fresh, frozen, and heat pump-dried) (300 µL/mL), and antibiotic ciprofloxacin (0.01 mg/mL). **(B)** correlation analysis of volatile compounds with antioxidant and antibacterial activities.

volatile compounds of ginger essential oils (Figure 4B). In general, most monoterpene compounds (except α-pinene), ketones, alcohols (except eucalyptol), oxygenated monoterpenes (except β-terpineol), esters (citronellol acetate), aldehydes, and sesquiterpenes (copaene and curcumene) were positively correlated with free radical scavenging capacity (DPPH and ABTS) and bacterial inhibition zone diameters (*B. cereus*, *S. aureus*, *C. jejuni*, and *E. coli*). In contrast, cedrene, zingiberene, geranyl acetate, α-farnesene, β-terpineol, and α-pinene were negatively correlated with antioxidant capacity and antibacterial activity.

Conclusion

Pretreatment methods significantly affected the recovery efficiency, volatile compound composition, and biological activity of ginger essential oil. The ginger drying method significantly increased the essential oil recovery efficiency. The densities of ginger essential oil samples (fresh,

frozen, and heat pump-dried) were similar. GC-MS analysis showed that the numbers of volatile compounds in fresh and frozen ginger essential oils were 12 and 17, respectively, with sesquiterpenes and aldehydes being the two dominant groups. In particular, 19 compounds were detected in heat pump-dried ginger essential oil. Among them, the dominant groups were monoterpenes, oxygenated monoterpenes, aldehydes, sesquiterpenes, and ketones-type compounds (1.31%). The compound α-citral was identified as a common volatile constituent in all ginger essential oil samples (fresh, frozen, and heat pump-dried).

Results from PCA, HCA, and heat map analyses showed a clear distinction in the volatile compound profiles of the three types of ginger essential oils (fresh, frozen, and heat pump-dried). Pretreatment enhanced the free radical scavenging (DPPH and ABTS) and antibacterial (*B. cereus*, *S. aureus*, *C. jejuni*, and *E. coli*) activities of ginger essential oil, with the heat pump drying method showing more pronounced advantages. Pearson correlation analysis also revealed that volatile compounds were associated with the biological activity of ginger essential oil. These findings can assist farmers and essential oil producers in selecting appropriate ginger pretreatment methods to minimise material loss and improve essential oil quality. Future work will explore advanced drying and extraction methods, alongside absolute GC quantification of gingerol and shogaol. MIC assays and cytotoxicity tests will also be conducted to better define the extracts' bioactivity and safety.

Acknowledgement

The authors gratefully acknowledge Nguyen Tat Thanh University, Ho Chi Minh City University of Technology (HCMUT), VNU-HCM, and Hutech University for supporting the present work.

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