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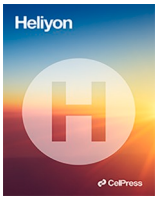

Research article

The investigation of thermal stability and GC-MS analysis of *Acorus tatarinowii* and *Atractylodes lancea* volatile oils treated by β cyclodextrin inclusion and Pickering emulsion technologies

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ABSTRACT

Objective: To investigate the stability of *Acorus tatarinowii* and *Atractylodes lancea* essential oils (ATaAL-EO) under a hot environment at 60 °C, and to analyze the differences in component, quantity, and quality changes, as well as variations in the main components, under different treatment methods of crude oil, β-cyclodextrin inclusion of ATaAL-EO, and Pickering emulsion, to improve the stability and quality of ATaAL-EO.

Methods: The stability of the ATaAL-EO group, the β-cyclodextrin inclusion ATaAL-EO group, and the Pickering emulsion group were investigated under a 60 °C heat environment. Volatile oil retention rate and peroxide value were collected and measured. The volatile oil components of each group were determined by GC-MS, and t-tests were used to screen for differential components. PCA plots for each group were constructed using the OmicShare online platform. Line plots were generated using the Rmisc and reshape2 packages. Upset Venn diagrams under different hot environments were created using the OmicShare online platform to identify quantitative and qualitative changing components and heat map stack plots for newly generated compounds and connected line plots for disappearing compounds were produced for each group. Boxplots for the main component compounds under different hot environments were generated using the reshape2 and ggplot2 packages.

Results: In a hot environment of 60 °C, the β-cyclodextrin inclusion ATaAL-EO and Pickering emulsion group with 1, 3, and 8 h of placement showed higher retention and lower oxidation degree compared to the stability of the ATaAL-EO group. GC-MS analysis results showed that the stability of volatile components in the Pickering emulsion group and β-cyclodextrin inclusion ATaAL-EO group was significantly improved compared to the crude oil group.

Conclusion: β-cyclodextrin inclusion complexes with ATaAL-EO, as well as Pickering emulsions, can significantly enhance the stability and quality of ATaAL-EO. Pickering emulsions have more advantages.

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1. Introduction

The essential oil derived from *Acorus calamus* contains an array of volatile compounds, prominently methyl eugenol, *cis*-methyl isoeugenol, γ-asarone, *β*-asarone and α-asarone [1]. In addition to its pronounced nootropic and neuroprotective properties, the essential oil of *Acorus calamus* exhibits significant cardioprotective effects. Moreover, it helps alleviate asthma, enhance intestinal motility, and mitigate symptoms associated with morphine withdrawal [2]. Modern research has revealed that the volatile oil of *Atractylodes lancea* primarily composed of sesquiterpenes, possesses notable anticancer, antioxidant, anti-inflammatory, antibacterial, and hepatoprotective effects [3]. The chemical composition of the volatile oil of *Atractylodes lancea* is complex, with sesquiterpenes being its main constituents [4]. ATaAL-EO, drawn from these plants, can be leveraged as natural constituents for the formulation of diverse products like herbal harmonies, traditional Chinese medicine prescriptions, fragrances, food additives, and skin care products [5,6]. However, it is important to note that ATaAL-EO is sensitive to high temperatures, as extended exposure can lead to degradation and volatilization of its components. Moreover, ATaAL-EO may potentially induce toxicity, allergic reactions, and have limitations in usage. Additionally, it is important to consider potential interactions of ATaAL-EO with other pharmaceuticals [7].

Pickering emulsion was a unique type of emulsion that relied on solid particles to stabilized the system, while *β*-cyclodextrin was a molecular encapsulant known for its inclusion and self-assembly properties. In the case of Pickering emulsion, the solid particles created a stability structure at the emulsion interface, effectively stabilizing volatile oils. Compared to traditional emulsifiers, the solid particles formed Pickering structures similar to nanoparticles within the emulsion, resulting in stronger stability that prevented oil-water phase separation and colloidal particle precipitation. By providing additional protection, Pickering emulsion reduced the impact of external environmental factors such as light, oxygen, and temperature on volatile oils. The solid particles acted as a protective layer, slowing down the rate of oil volatilization, delaying oxidation and decomposition processes, and maintaining the stability and efficacy of the volatile oils. By harnessing Pickering emulsion to prepare emulsifiers that encapsulated volatile oils, reliance on artificially synthesized surfactants was reduced, resulting in better sustainability [8]. On the other hand, *β*-cyclodextrin formed inclusion complexes with the ATaAL-EO. This inclusion effect enhanced the compatibility and stability between the volatile oils and emulsions. By forming inclusion complexes, *β*-cyclodextrin protected the volatile oils from environmental factors, reducing their rates of volatility and oxidation. Consequently, the stability of the volatile oils in the emulsions was improved [9].

GC-MS analysis was a widely method in chemical analysis that combined gas chromatography and mass spectrometry techniques. It was employed to identify identify compounds, analyze their composition and structure and found applications in various fields such as chemistry, food safety, environmental monitoring, and pharmaceutical analysis. The technique proved particularly useful for identifying unknown substances, determining compound structures, analyzing mixture components, and quantifying compound content. With its high sensitivity, efficient separation capability, and adaptability to complex samples, GC-MS gained wide acceptance as a powerful chemical analysis method [10]. The objective of this study was to employ GC-MS analysis to determine the volatile compositions of crude oil, *β*-cyclodextrin-encapsulated ATaAL-EO, and Pickering emulsion under a thermal environment of 60 °C. Additionally, by utilizing the R-language, a systematic analysis was conducted on the variations in different components, both quantitatively and qualitatively, as well as the main components under different treatment conditions. The aim was to elucidate the technical advantages of incorporating *β*-cyclodextrin encapsulation and Pickering emulsion, with the ultimate goal of enhancing the stability and quality of ATaAL-EO.

2. Materials and methods

2.1. Materials

JY-3002 1/10,000 precision analytical balance (Shanghai Puchun Instrument Co., Ltd.), IKA T18 digital display high-speed disperser (Shanghai Tusen Vision Technology Co., Ltd.), DHG-9140A electric blast drying oven (Shanghai-Heng Scientific Instruments Co., Ltd.), Agilent 7890B/5977B gas chromatograph-mass spectrometer (Agilent Technologies, USA), sodium thiosulfate (Batch No. 20180806); anhydrous sodium carbonate (Batch No. 20210506); soluble starch (Batch No. 20180808); Sodium chloride (batch number 20210302); chloroform (batch number 20210203) were purchased from Tianjin Tianli Chemical Reagent Co., Ltd.; potassium iodide (Tianjin Kamio Chemical Reagent Co., Ltd., batch number 20220422); n-Hexane (Grace chemical technology co. LTD, Lot: 2112091); n-Docosane (Grace chemical technology co. LTD, Lot: G171809, purity: 99.6%); mixed volatile oil extract of *Acorus tatarinowii and Atractylodes lancea* (Shaanxi Momei De Qi Xuehe Pharmaceutical Co., Ltd., batch number S-230204).

2.2. Preparation of *β*-cyclodextrin inclusion of ATaAL-EO and pickering emulsion

The complexes of Beta-Cyclodextrin with ATaAL-EO and Pickering's emulsion were prepared by the previous project team. Precisely weigh 5.00 g of PEG4000 and 1.00 g of Indigo powder. Place the PEG4000 in a 100 mL evaporating dish, transfer it to an electric heating jacket, set the temperature to 228 °C, and preheat the electric heating jacket for 5 min. After melting the PEG4000 by heating, add indigo, stir rapidly with a glass rod for 5 min, remove the evaporating dish, dry at room temperature for 1 h, crush it, and reserve. Weigh 0.50 g of modified indigo separately, add 5 mL of ATaAL-EO and 15 mL of water to a 50 mL centrifuge tube, and shear it at 14000 rpm for 2 min with a high-speed shearing machine, to get the modified Indigo Pickering emulsion.

Precisely weigh 72.00 g of Beta-Cyclodextrin and put it into a ball mill. Add 30 agate grinding balls, and then add one and a half times the amount (108 mL) of purified water to grind for 10 min, to prepare Beta-Cyclodextrin suspension. When the suspension formed, slowly add a diluted solution of equal proportions of volatile oil and ethanol, 12 mL, and slowly add it to the ball mill to mix

with the Beta-Cyclodextrin suspension. Grind for 40 min, vacuum filter, wash 3 times with anhydrous ethanol, about 45 mL each time, and dry at 55 °C for 12–14 h; Coarse crushing of 40 meshes, thus obtained.

Based on the above conditions, the process of β-Cyclodextrin complexation with ATaAL-EO and Pickering emulsion was optimized.

2.3. Characterization of β-cyclodextrin inclusion complex of ATaAL-EO and pickering emulsion

The β-cyclodextrin, β-cyclodextrin and ATaAL-EO physical mixture (6.3:1), and β-cyclodextrin inclusion of ATaAL-EO were separately placed on clean filter paper to compare their differences. A suitable amount of β-cyclodextrin, physical mixture, and inclusion complex were dissolved in water and mixed with the oil-soluble dye Sudan III. The mixture was then spread on glass slides, covered with a coverslip, and observed under an optical microscope (100 × magnification) to examine its morphology. After taking 0.10 g of β-cyclodextrin, inclusion, physical mixture, and 1 μL of ATaAL-EO respectively, 1 mL of methanol was added and the supernatant was taken after shaking. Silica gel G plates were used with petroleum ether: ethyl acetate (9:1) spreading solvent. Spraying with a 5% vanillin sulfuric acid solution, the plates were heated at 105 °C until the spots became clear and visible. The steam distillation method was employed to extract the ATaAL-EO from *Acorus tatarinowii* and *Atractylodes lancea* (AT-AL), as well as from the β-cyclodextrin inclusion complex. GC-MS analysis was conducted following the conditions and methods described in section 2.5 β-cyclodextrin, physical mixture, inclusion complex, and ATaAL-EO were taken in appropriate amounts and subjected to KBr pellet technique for infrared spectrum analysis.

A small amount of Pickering emulsion was dropped and spread on filter paper, followed by dilution with water. The Pickering emulsion samples were coated on glass slides and stained with the oil-soluble dye Sudan III and the water-soluble dye methylene blue. The samples were then observed under a microscope (100 × magnification). The particle size of the Pickering emulsion was measured using an S3500 laser particle size analysis. The zeta potential of the Pickering emulsion was determined using a Zetasizer Nano ZS90 nanoparticle size and zeta potential analyzer. An appropriate amount of ATaAL-EO, as well as indigo suspension and the Pickering emulsion samples, were placed in a rotating sample cup for spectral collection. The collection was performed using an integration sphere with a collection delay of 0 s, resolution of 8 cm^{-1}, and gain of × 2. Air was used as a reference for background subtraction. Each sample was collected three times, and the average near-infrared spectra (NIRS) were obtained.

2.4. The collection of volatile oils in a hot environment and the determination of their retention rate and peroxide content

The mixed volatile oil extract of AT-AL, β-cyclodextrin inclusion of volatile oil extracted from AT-AL, and Pickering emulsion was placed in a 100 mL evaporation dish and placed in an oven at 60 °C for 1,3, and 8 h. The volume of volatile oil extracted from AT-AL in the crude oil group was recorded. The β-cyclodextrin inclusion group and the Pickering emulsion group were extracted by steam distillation to extract the ATaAL-EO, distilled for 5 h, separated the oil phase, and recorded the volume. Three parallel experiments were conducted. Precisely draw 500 μL of the above sample. Take 10.00 mL of a mixture of chloroform-acetic acid (V1/V2 = 4:6) and add it to a conical flask. Add 1.00 mL of saturated potassium iodide solution, seal tightly, shake for 0.5 min, and place in the dark for 3 min. Remove and add 30.00 mL of water. Add 1.00 mL of 1% starch indicator. Titrate with 0.00001 mol L^{-1} sodium thiosulfate standard solution until the blue color of the solution disappears. Record the volume of sodium thiosulfate standard solution consumed and calculate the POV value.

2.5. Determination of volatile oil components under a hot environment by GC-MS

2.5.1. Preparation of internal standard solution

We accurately measured 50.50 mg of docosane standard using a high-precision analytical balance. The docosane standard was then carefully transferred into a 5 mL volumetric flask. To ensure precise measurement, we added n-hexane solution slowly until the solution reached the scale line on the flask. Subsequently, we vigorously shook the flask to thoroughly mix the contents, resulting in a docosane internal standard solution with a concentration of 10 mg/mL.

2.5.2. Preparation of the test solution

100 μL volatile oil samples of each group under item 2.4 were placed in a 10 mL volumetric flask, 100 μL internal standard solution of docosane was added, hexane was added to the scale, appropriate amount of anhydrous sodium sulfate was added to remove water, shaken well, and 0.22 μm organic filter membrane was passed to obtain different groups of volatile oil test solution.

2.5.3. Analytical conditions for GC-MS

According to the method of liquid injection, different GC conditions, MS conditions, etc. were examined to investigate the impact on the chromatographic information of the test sample. The chromatographic information abundance was used as the evaluation index to select the best chromatographic conditions that can provide the most component information. The optimal conditions were found to be: Using an HP-5 MS quartz capillary column (30 m × 0.25 mm × 0.25 μm), the carrier gas is helium (purity 99.999%), with a flow rate of 1 mL min^{-1}. The injection volume is 1 μL, and the split ratio is 10:1. The temperature of the vaporization chamber is 230 °C. The chromatographic column adopts a programmed temperature increase method, starting at 50 °C and increasing at a rate of 15 °C·min^{-1} until reaching 140 °C. Then, it is further increased at a rate of 0.4 °C·min^{-1} until reaching 144 °C and maintained for 5 min. After that, the temperature is increased at a rate of 10 °C·min^{-1} until reaching 250 °C and maintained for 2 min. Finally, the temperature is increased at a rate of 4 °C·min^{-1} until reaching 280 °C and maintained for 2 min. The ionization mode is EI, with an electron energy of

70 eV. The ion source temperature is 230 °C, and the quadrupole temperature is 150 °C. The scan range is 35~500 *m/z*, with a solvent delay of 3 min.

2.5.4. GC-MS operation and analysis

After transferring the prepared test solution into the injection vial through a syringe and a 0.22 μm microporous filter membrane, the samples' ions were generated through electron bombardment (EI) using the optimal method described above. This process was conducted on an Agilent 7890B/5977B gas chromatograph-mass spectrometer equipped with a Single Quadrupole mass spectrometer. The volatile oil sample was initially vaporized within the GC, and its individual components were subsequently separated using an HP-5 MS quartz capillary column with dimensions of 30 m × 0.25 mm × 0.25 μm. The fractions obtained from the chromatographic separation then entered the ion source of the Single Quadrupole mass spectrometer, aided by a gas stream. Within the ion source, high-energy electrons emitted by a hot cathode were accelerated to high speeds. The volatile oil sample molecules collided with these energetic electrons. Upon capture, the sample molecules experienced ionization, losing one electron and resulting in the formation of positively charged ions (cations). Subsequently, the cations were separated based on their mass-to-charge ratio (*m/z*) using a quadrupole mass filter. Only ions with specific *m/z* values were able to pass through the quadrupole and reach the detector. The ions captured by the detector were converted into electrical signals, which were then processed and exported through the data system to generate a mass spectrogram. After the completion of GC-MS data acquisition, the constituent components were analyzed and identified using the Agilent database analysis software, Data Analysis. Specifically, the NIST 14.0 database was employed for this purpose. The software compares the acquired mass spectra of the sample components with the reference spectra from the NIST 14.0 database to identify and match the compounds present in the sample. By utilizing the vast library of reference spectra contained within the NIST database, the software provides reliable and accurate identification of the constituent components based on their mass spectral patterns. This approach allows for comprehensive analysis and characterization of the sample, providing valuable information about its composition.

2.6. Volatile oil quantitative change difference compounds under a hot environment

2.6.1. Selection of volatile oil quantitative change differential compounds under a hot environment

The *t*-test was used to select the quantitative change differential compounds of volatile oil extracted from the mixture of AT-AL at 60 °C. By using the heatmap package [11], a heatmap of differential components was plotted. Using the OmicShare online platform (https://www.omicshare. com/tools), PCA plots were generated for the crude oil group, *β*-cyclodextrin inclusion group, and Pickering emulsion group.

2.6.2. Analysis of volatile oil quantitative change difference compounds under thermal environment

The Rmisc [12] and reshape2 [13] package was used to draw the linear diagram of the volatile oil qualitative change difference compounds.

2.7. Volatile oil qualitative change difference compounds under thermal environment

2.7.1. Selection of volatile oil qualitative change differential compounds under thermal environment

Creating Upset Venn diagrams for the Crude Oil group, *β*-Cyclodextrin Inclusion complex group, and Pickering emulsion group in a thermal environment using the OmicShare online platform. The horizontal bars on the left indicate the number of compounds in each set. The individual dots in the matrix below represent the unique compounds in a specific set. The lines between the dots represent the intersection between the connected sets. The vertical bars above represent the number of unique or intersected compounds in the statistics.

2.7.2. Analysis of newly generated qualitative change differential compounds in volatile oil under thermal environment

Utilizing the OmicShare online platform, we generated a stacked heatmap of newly formed compounds in the thermal environment of the crude oil group, *β*-cyclodextrin inclusion complex group, and Pickering emulsion group. The heatmap is used as a sample and grouping horizontally, with compounds listed vertically. Each colored grid in the heatmap represents the abundance of a compound in a sample. The stacked bar chart represents the abundance of compounds in different groups horizontally, with compounds listed vertically. The color of each bar represents the different groups, and the height of the bar represents the abundance of the compound in each group.

2.7.3. Analysis of disappearing qualitative change differential compounds in volatile oil under thermal environment

Using the OmicShare online platform to draw stack plots of disappearing compounds in the heat environment for the crude oil group, *β*-cyclodextrin encapsulation group, and Pickering emulsion group.

2.8. Analysis of the main compounds of volatile oils in high-temperature environments

The main components of ATaAL-EO were obtained by reviewing the relevant literature. We plotted box plots of the main component compounds in the thermal environments of the crude oil group, the *β*-cyclodextrin inclusion group, and the Pickering emulsion group using the reshape2 and ggplot2 packages [14].

2.9. Statistical analysis

SPSS 26 software was used to analyze the experimental data, and the results were expressed as mean ± standard deviation ($\bar{x} \pm s$). We use a two-sample equal variance *t*-test with a condition of a p-value less than 0.05 to screen for differential components, and the statistical results were expressed as $P < 0.05$ to indicate that the differences were statistically significant. Plotting using GraphPad Prism 8.0, RStudio 4.2.1, Data Analysis, the OmicShare online platform (https://www.omicshare. com/tools) etc.

3. Results

3.1. Preparation of β-cyclodextrin inclusion of ATaAL-EO and pickering emulsion

The optimal process for the inclusion of ATaAL-EO is as follows: grinding time of 33 min, β-cyclodextrin to essential oil ratio of 6.3:1, and water to β-cyclodextrin ratio of 2.6:1. Optimal process for Pickering emulsion: PEG type: PEG4000, PEG4000 to natural indigo ratio: 5:1, melting temperature: 228 °C, melting time: 5 min, oil-water ratio: 13:7, modified natural indigo addition: 0.5 g, stirring speed: 10000 rpm, stirring time: 2 min is the optimal preparation method.

3.2. Characterization of β-cyclodextrin inclusion complex of ATaAL-EO and pickering emulsion

β-cyclodextrin and the inclusion complex appeared as white powdered substances, while the physical mixture appeared as a pale yellow powder, as shown in Fig. 1A β-cyclodextrin exhibited cylindrical crystal shapes with no volatile oil, while the inclusion complex displayed granular crystal structures without volatile oil. The physical mixture demonstrated characteristics of both morphologies and contained volatile oil, indicating significant changes in phase morphology before and after inclusion. These results suggest the formation of the inclusion complex, as depicted in Fig. 1B. The spots of the inclusion complex and physical mixture were consistent with ATaAL-EO l, while β-cyclodextrin did not exhibit any corresponding spots, indicating no significant differences in the main components of ATaAL-EO after inclusion in β-cyclodextrin, as shown in Fig. 1C. Information on the ATaAL-EO and the volatile constituents in β-cyclodextrin inclusion complexes can be found in Supplementary Table 1, and the total ion chromatogram is shown in Fig. 1D. The characteristic peaks of the inclusion complex exhibited similarity and similar peak shapes to β-cyclodextrin. The overall infrared absorption characteristics of the physical mixture exhibited features that combined both β-cyclodextrin and the volatile oil. The absorption peaks of the volatile oil at 2930, 1606, 1500, and 1233 cm^{-1} had disappeared or significantly weakened in the inclusion complex, while they persisted in the physical mixture, indicating that the entry of the volatile oil into the β-cyclodextrin cavity limited its infrared vibration. All these observations indicated the successful formation of the β-cyclodextrin inclusion complex of ATaAL-EO using the optimized preparation process, as demonstrated in Fig. 1E.

Pickering emulsion was prepared and diluted using Pickering filter paper, as shown in Fig. 2A. Microscopic observation (× 100) was conducted, and the results are shown in Fig. 2B. The particle size of the Pickering emulsion was determined to be 23.29 ± 0.26 μm, indicating a normal emulsion, as shown in Fig. 2C. The zeta potential of the Pickering emulsion was measured to be 32.00 ± 0.69 mV, indicating moderate stability, as shown in Fig. 2D. By observing the NIRS spectra, it was found that the characteristic absorption peaks

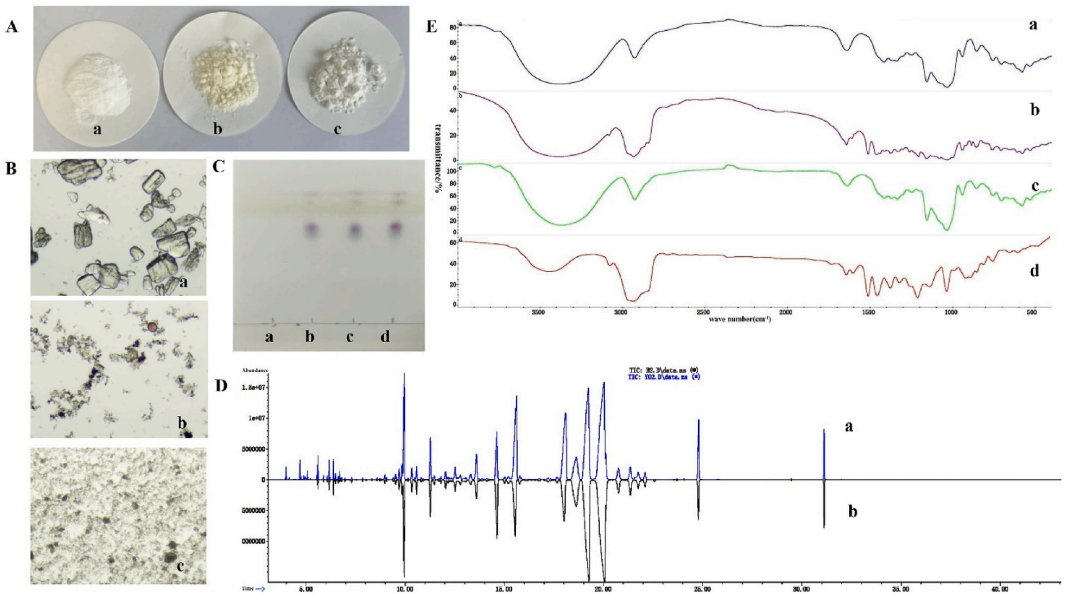

Fig. 1. Characterization of β-cyclodextrin inclusion of ATaAL-EO.

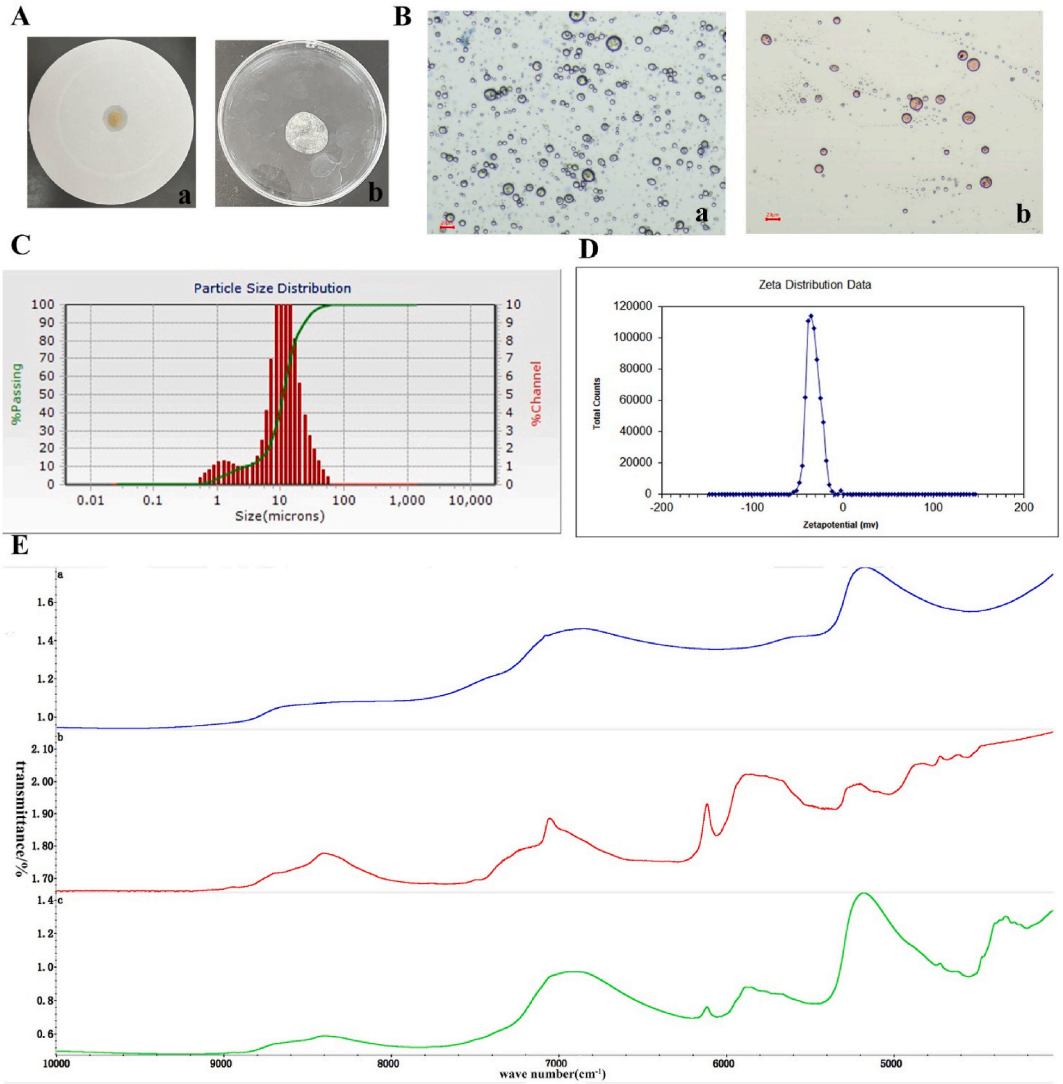

Fig. 2. Characterization of Pickering emulsion. Note: **(A)** Dilution of Pickering Emulsion **a.** Pickering emulsion filter paper method **b.** Pickering emulsion dilution method; **(B)** Microscopic Observation of Pickering Emulsion (100x magnification) **a.** Staining with the water-soluble dye methylene blue **b.** Staining with the oil-soluble dye Sudan III; **(C)** Particle Size of Pickering Emulsion **(D)** Zeta Potential of Pickering Emulsion; **(E) a.** NIRS of indigo **b.** NIRS of ATaAL-EO **c.** NIRS of Pickering emulsion.. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

of ATaAL-EO around the wavenumber of 6000 cm^{-1} were weakened in the Pickering emulsion. The absorption peaks of the Pickering emulsion exhibited similar fluctuations to those of the indigo suspension, and no significant new absorption peaks were generated in the Pickering emulsion. The results are presented in Fig. 2E, indicating that no new chemical bonds were formed in the Pickering emulsion. All of the above observations indicate that the Pickering emulsion prepared using the optimized process is of the oil-in-water (O/W) type Pickering emulsion.

Note: **(A)** Appearance **a.** *β*-cyclodextrin **b.** A physical mixture of ATaAL-EO l with *β*-cyclodextrin **c.** Inclusion complex of ATaAL-EO with *β*-cyclodextrin; **(B)** Microscopic identification (100x magnification) **a.** *β*-cyclodextrin **b.** A physical mixture of ATaAL-EO with *β*-cyclodextrin **c.** Inclusion complex of ATaAL-EO with *β*-cyclodextrin; **(C)** TLC analysis **a.** *β*-cyclodextrin **b.** Inclusion complex of ATaAL-EO with *β*-cyclodextrin **c.** ATaAL-EO **d.** A physical mixture of ATaAL-EO with *β*-cyclodextrin; **(D)** GC-MS analysis **a.** Inclusion complex of ATaAL-EO with *β*-cyclodextrin **b.** ATaAL-EO; **(E)** Infrared spectroscopy **a.** *β*-cyclodextrin **b.** A physical mixture of ATaAL-EO with *β*-cyclodextrin **c.** Inclusion complex of ATaAL-EO with *β*-cyclodextrin **d.** ATaAL-EO.

3.3. The collection of volatile oils in a hot environment and the determination of their retention rate

It can be seen that under the conditions of placing at 60 °C for 1, 3, and 8 h, both the *β*-cyclodextrin group and the Pickering

emulsion group significantly improved the retention rate of the volatile oil compared to the crude oil group ($P < 0.001$). At 1 h, there was no statistical difference in retention rate between the Pickering emulsion group and the β-cyclodextrin group ($P > 0.05$). At 3 h, the retention rate of the Pickering emulsion group was significantly lower compared to the β-cyclodextrin group ($P < 0.05$). At 8 h, the retention rate of the Pickering emulsion group was significantly lower compared to the β-cyclodextrin group ($P < 0.01$). β-cyclodextrin and Pickering system can to some extent delay the loss of ATaAL-EO under high-temperature conditions (60 °C), with β-cyclodextrin performing well and the Pickering system as the second best, and the results are shown in Fig. 3A.

It can be seen that under the conditions of 60 °C for 1, 3, and 8 h, both the β-cyclodextrin inclusion group and the Pickering emulsion group have significantly lower levels of volatile oil peroxides compared to the crude oil group ($P < 0.001$). There was no statistically significant difference in the peroxide content between the Pickering emulsion and β-cyclodextrin inclusion complex groups at 1, 3, and 8 h ($P > 0.05$). This suggests that β-cyclodextrin and the Pickering group can to some extent reduce the peroxide content in the ATaAL-EO under high-temperature conditions (60 °C). The results are shown in Fig. 3B.

3.4. Determination of volatile oil components under a hot environment by GC-MS

The results of GC-MS volatile components of ATaAL-EO in each group under the thermal environment are shown in Supplementary Table 2, and the total ion flow diagram is shown in Fig. 4A.

3.5. Volatile oil quantitative change difference compounds under a hot environment

3.5.1. Selection of volatile oil quantitative change differential compounds under a hot environment

There are 20 differentiating components between crude oil and high temperature for 1 h ($P < 0.05$); there are 23 differentiating components between crude oil and high temperature for 3 h ($P < 0.05$); there are 39 differentiating components between crude oil and high temperature for 8 h ($P < 0.05$), after removing duplicates, there are a total of 39 differentiating components. The information of volatile components in 39 differential components under high temperature environment was shown in Table 1. The clustering results of different thermal environments indicate that the changes in volatile oil components of AT-AL in the crude oil group, β-cyclodextrin inclusion complex group, and Pickering emulsion group are more pronounced under the condition of 60 °C stable for 8 h compared to 60 °C stable for 1 and 3 h. Among them, the β-cyclodextrin inclusion complex group is the most intense for 60 °C stable for 1 and 3 h, while the Pickering emulsion group is more moderate, indicating that the β-cyclodextrin inclusion complex group can effectively delay the loss of volatile oil components of AT-AL under the condition of 60 °C stable for 1 and 3 h. The effect of the Pickering emulsion group is secondary. Under the condition of 60 °C stable for 8 h, the delay effect of the β-cyclodextrin inclusion complex group and Pickering emulsion group is weakened. From the clustering results of volatile components, it can be seen that the characteristic components can

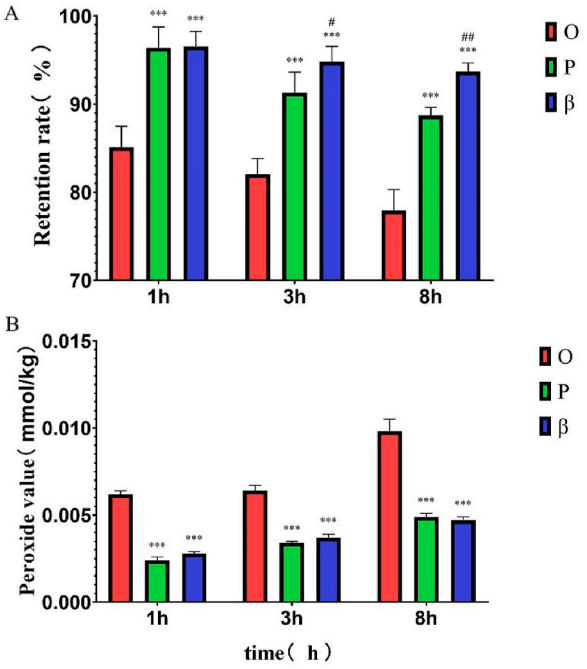

Fig. 3. Change in volatile oil retention and peroxide content determined by extraction from AT-AL by different groups under a hot environment. Notes: Compared to the crude oil group***$P < 0.001$; Compared to the Pickering emulsion system, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. **(A)** The retention rate of volatile oil extracted from the mixture of AT-AL in different thermal environments; **(B)** Quantities of peroxides in different groups under different thermal environments.

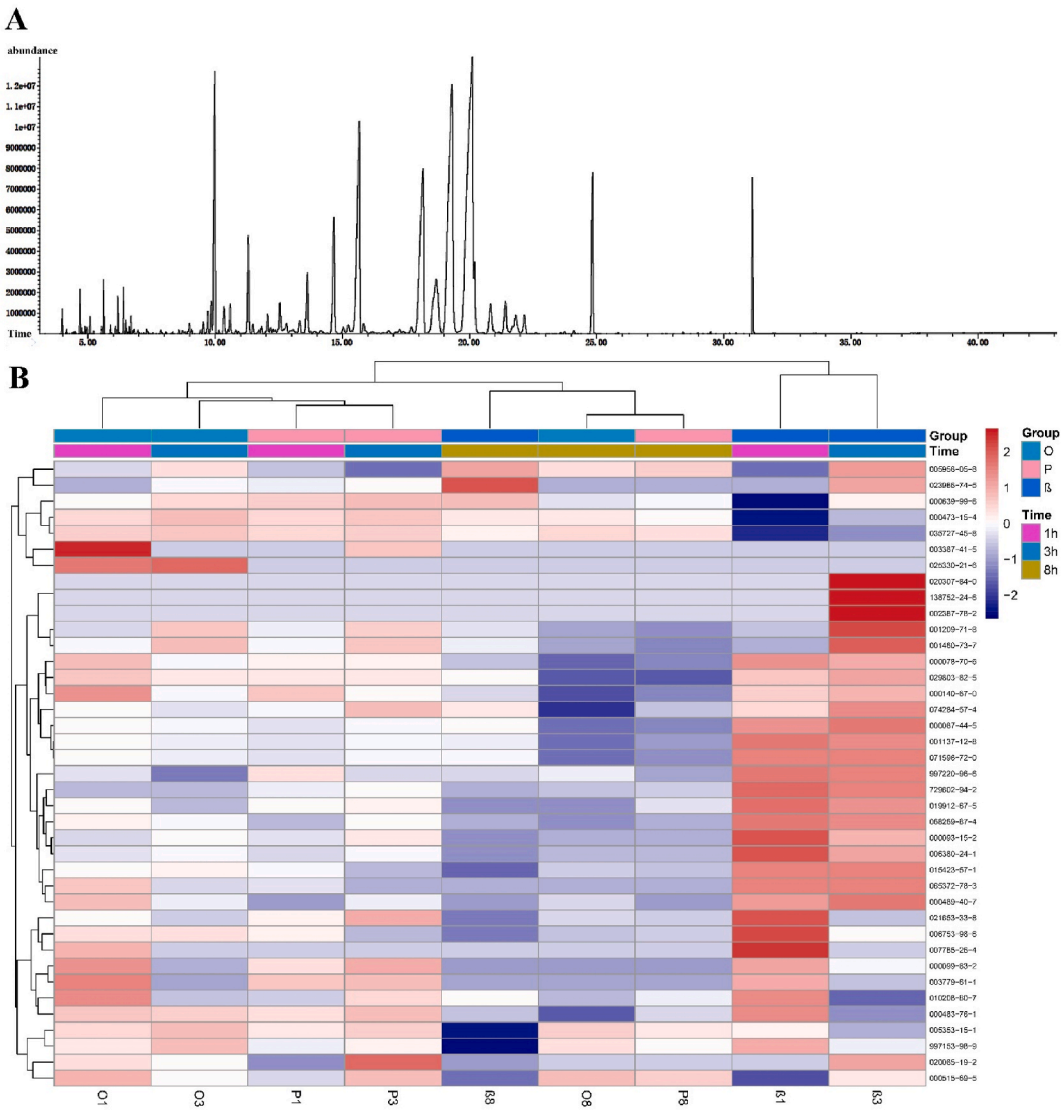

Fig. 4. Selection of differential components under thermal environment. Notes: **(A)** Total ion current diagram of ATaAL-EO; **(B)** 39 differential components Heatmap.

be divided into 5 categories: alpha-Bisabolol; (+)-alpha-amorphene; gamma-Asarone, etc. (E)-delta-Cadinene; (E)-3,7-dimethylocta-1,3,6-triene; (1S)-(−)-alpha-Pinene, etc. (−)-alpha-Gurjunene; 4-Allylanisole; 2-(6,10-dimethyl-2-spiro[4.5]dec-9-enyl)propan-2-ol, etc. Isocalamendiol; Sabinene; Gamma-Decalactone, etc. D Germacrene; Acorenone as shown in Fig. 4B.

After being stabilized at 60 °C for 1, 3, and 8 h, the separation degree between the β-cyclodextrin inclusion complex and both the crude oil and the Pickering emulsion groups increased over time, indicating clear and significant differences, which further illustrates the notable protective effect of the inclusion complex. The reasons for the increasing dispersity might be the gradual increase in different components over time and the significant trends of changes, resulting in a reduced protective effect of the inclusion complex. Under the same conditions of stability at 60 °C for 1, 3, and 8 h, the Pickering emulsion group gradually separated from the crude oil group, suggesting that their differences became increasingly discernible, thus inferring a progressive significance in protective effect. The stability of the crude oil group and consistent trends in unapparent different components exhibited by the Pickering emulsions might have led to the inability to separate some of the differing components. However, as the stabilization time increased, the number of different components also increased and the trend of changes became significant, leading to a gradual separation of the Pickering emulsion group from the crude oil group. The PCA overall graph results showed overlap between the three groups of compounds, for which we displayed the PCA results in 3D, revealing that the β-cyclodextrin inclusion complex occupies a different plane from both the crude oil and Pickering emulsion groups, even though the latter two still overlap considerably. The cause for this overlap could be due to the large time span, the excessive overall sample size, and that some differing components in the crude oil and Pickering emulsion groups exhibit similar properties. Additionally, the low values of PC1 and PC2 may not accurately reflect the differences between each

Table 1
The information of volatile components in 39 differential components under high temperature environment.

CAS	Name of compound	Relative content (mg/mL)								
		1O	1P	1β	3O	3P	3β	8O	8P	8β
000099-83-2	alpha-PHELLANDRENE	1.09 ±0.26	0.67 ±0.28	0.99 ±0.66	0.10 ±0.18	0.98 ±0.42	0.47 ±0.05	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
003387-41-5	Sabinen	0.57 ±0.12	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.23 ±0.40	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
003779-61-1	(E)-beta-ocimene	0.58 ±0.12	0.38 ±0.17	0.44 ±0.46	0.00 ±0.00	0.40 ±0.37	0.06 ±0.11	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
000078-70-6	Linalool	3.25 ±0.41	2.43 ±0.81	3.93 ±0.58	2.18 ±0.29	2.48 ±0.29	3.55 ±0.59	0.41 ±0.05	0.87 ±0.17	1.62 ±0.12
029803-82-5	cis-4-(Isopropyl)-1-methylcyclohex-2-en-1-ol	0.44 ±0.11	0.36 ±0.07	0.44 ±0.05	0.35 ±0.04	0.36 ±0.05	0.51 ±0.03	0.00 ±0.00	0.00 ±0.00	0.32 ±0.02
000140-67-0	Estragole	2.49 ±0.27	2.00 ±0.75	1.94 ±0.22	1.44 ±0.06	1.57 ±0.19	2.17 ±0.11	0.13 ±0.22	0.49 ±0.03	1.17 ±0.04
074284-57-4	Silphinene	0.99 ±0.51	0.97 ±0.21	1.16 ±0.13	0.84 ±0.41	1.32 ±0.14	1.61 ±0.46	0.08 ±0.14	0.72 ±0.05	1.12 ±0.09
001137-12-8	(+)-Longicyclene	1.01 ±0.13	0.89 ±0.15	1.64 ±0.09	0.91 ±0.07	0.97 ±0.11	1.58 ±0.35	0.43 ±0.03	0.57 ±0.04	0.91 ±0.10
068269-87-4	Modephene	1.97 ±0.25	1.24 ±1.09	3.32 ±0.13	1.80 ±0.14	1.88 ±0.21	3.15 ±0.86	0.85 ±0.05	1.11 ±0.08	1.15 ±1.00
065372-78-3	(−)-Isocomene	3.00 ±0.39	1.03 ±1.79	4.71 ±0.28	0.88 ±1.52	0.00 ±0.00	4.70 ±0.98	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
000093-15-2	Methyleugenol	34.09 ±4.03	34.62 ±2.28	50.44 ±3.12	36.57 ±3.05	38.05 ±4.02	42.87 ±19.59	31.49 ±3.22	31.49 ±3.72	28.82 ±3.87
071596-72-0	beta-Isocomene	3.17 ±0.40	2.84 ±0.42	4.86 ±0.20	2.99 ±0.20	3.07 ±0.34	4.77 ±1.03	1.64 ±0.11	1.96 ±0.18	3.07 ±0.36
000087-44-5	Caryophyllene	3.92 ±0.51	3.54 ±0.47	5.55 ±0.10	3.77 ±0.23	3.81 ±0.44	5.86 ±1.15	2.15 ±0.16	2.45 ±0.23	3.94 ±0.51
006380-24-1	(Z)-Methyl isoeugenol	11.61 ±1.41	11.35 ±1.21	17.42 ±1.00	12.04 ±0.46	12.11 ±1.31	15.07 ±6.44	10.47 ±1.23	10.32 ±1.23	9.27 ±1.23
006753-98-6	Humulene	1.15 ±0.15	1.06 ±0.12	1.87 ±0.05	1.13 ±0.06	0.72 ±0.63	1.01 ±0.88	0.74 ±0.07	0.78 ±0.09	0.45 ±0.79
729602-94-2	(1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl)spiro[4.5]dec-7-ene	0.55 ±0.48	0.75 ±0.09	1.42 ±0.03	0.54 ±0.47	0.80 ±0.10	1.33 ±0.52	0.59 ±0.06	0.62 ±0.07	0.51 ±0.44
997220-96-6	gamma.-Curcumene	1.84 ±1.62	2.51 ±0.27	3.74 ±0.11	0.86 ±1.49	1.72 ±1.50	3.61 ±0.90	1.94 ±0.20	1.22 ±1.06	1.75 ±1.53
020085-19-2	Zizanene	0.57 ±0.50	0.31 ±0.54	0.44 ±0.76	0.53 ±0.46	0.85 ±0.11	0.72 ±0.62	0.43 ±0.38	0.43 ±0.38	0.33 ±0.58
010208-80-7	alpha-Muurolene	2.44 ±0.30	0.89 ±1.55	2.46 ±2.13	0.76 ±1.31	1.68 ±1.49	0.00 ±0.00	0.69 ±1.20	1.16 ±1.01	1.30 ±1.13
000483-76-1	(+)-delta-Cadinene	9.82 ±1.27	9.35 ±1.22	11.39 ±0.31	9.61 ±0.87	10.08 ±1.10	6.14 ±6.93	4.91 ±4.25	7.79 ±0.92	7.16 ±0.95
000639-99-6	Elemol	23.16 ±2.84	24.70 ±2.73	14.13 ±1.74	24.53 ±0.78	25.63 ±3.06	23.28 ±8.06	21.80 ±2.87	22.58 ±2.82	25.47 ±3.04
015423-57-1	Germacrene B	1.35 ±0.16	1.29 ±0.12	1.90 ±0.13	1.40 ±0.07	1.07 ±0.49	1.91 ±0.40	1.16 ±0.14	1.10 ±0.14	0.76 ±0.43
000489-40-7	(−)-alpha-Gurjunene	1.36 ±1.24	0.00 ±0.00	1.70 ±1.48	0.64 ±1.11	0.65 ±1.12	1.98 ±1.73	0.47 ±0.82	0.00 ±0.00	0.00 ±0.00
005353-15-1	gamma-Asarone	54.62 ±6.87	53.03 ±5.25	51.39 ±3.74	57.76 ±2.27	55.69 ±6.06	41.52 ±19.88	55.83 ±7.52	53.08 ±6.29	24.42 ±3.27
019912-67-5	Epicubenol	1.22 ±0.17	1.21 ±0.13	2.03 ±0.12	0.86 ±0.75	1.27 ±0.13	1.81 ±0.77	0.68 ±0.59	1.08 ±0.14	0.70 ±0.61
001209-71-8	gamma-EUDESMOL	27.13 ±3.62	27.48 ±2.79	26.92 ±2.44	28.85 ±1.00	28.69 ±3.41	31.30 ±2.04	26.32 ±3.68	26.01 ±3.26	27.44 ±3.49
001460-73-7	Agarospirol	129.09 ±17.66	129.08 ±13.05	124.29 ±12.18	135.40 ±5.54	134.33 ±15.50	143.26 ±7.02	123.21 ±17.13	121.03 ±15.28	128.56 ±16.43
000473-15-4	beta-EUDESMOL	206.94 ±29.08	205.63 ±21.21	137.06 ±15.96	215.52 ±8.91	212.48 ±24.99	178.47 ±22.59	200.62 ±27.98	197.05 ±24.49	201.00 ±22.59
005956-05-8	Acorenone	2.57 ±4.45	2.14 ±3.70	0.00 ±0.00	4.57 ±3.96	0.00 ±0.00	6.88 ±6.44	4.72 ±4.09	5.10 ±4.51	6.62 ±0.82
000515-69-5	alpha-Bisabolol	6.82 ±0.90	4.24 ±3.71	1.26 ±2.18	4.94 ±4.28	6.64 ±0.79	5.58 ±1.03	6.67 ±0.95	6.15 ±0.79	2.01 ±3.48
035727-45-8	(1R,2S,3S,6S)-3-Ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)cyclohexanol	4.15 ±0.61	4.03 ±0.39	0.00 ±0.00	4.40 ±0.15	4.24 ±0.50	1.65 ±1.43	4.00 ±0.58	3.87 ±0.47	3.53 ±0.31
025330-21-6	Isocalamenediol	0.55 ±0.07	0.00 ±0.00	0.00 ±0.00	0.61 ±0.03	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

(*continued on next page*)

Table 1 (*continued*)

CAS	Name of compound	Relative content (mg/mL)								
		1O	1P	1β	3O	3P	3β	8O	8P	8β
997153-98-9	2-[(1E,7E)-Nona-1,7-dien-3,5-diynyl]furan	16.89 ±2.29	15.97 ±1.34	18.64 ±1.28	18.19 ±0.63	16.72 ±1.99	15.91 ±6.62	17.24 ±2.50	16.35 ±2.11	10.31 ±1.44
007785-26-4	(−)-alpha-Pinene	0.23 ±0.20	0.00 ±0.00	0.46 ±0.48	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
021653-33-8	Acorenone B	4.05 ±3.51	4.47 ±3.93	10.07 ±0.82	2.40 ±4.17	6.89 ±0.83	2.35 ±4.07	3.00 ±5.19	2.47 ±4.27	0.00 ±0.00
023986-74-5	(−)-Germacrene D	0.00 ±0.00	0.15 ±0.27	0.00 ±0.00	0.17 ±0.29	0.21 ±0.36	0.45 ±0.39	0.00 ±0.00	0.00 ±0.00	0.69 ±0.27
138752-24-6	Silphiperfol-5-ene	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.21 ±0.19	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
020307-84-0	delta-Elemene	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.45 ±0.40	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
002387-78-2	(−)-Cyperene	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.15 ±0.25	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

Note: O, P, and β represent the crude oil group, Pickering emulsion group, and β-cyclodextrin inclusion complex group, respectively. 1, 3, and 8 represent the stability time in hours of each group at a temperature of 60 °C.

group. Therefore, we compared different time points within each group, and the results showed that the crude oil group exhibited clear differences at high temperatures over different time periods, whereas the Pickering emulsion and the β-cyclodextrin inclusion complex groups showed little difference at various time intervals as shown in Fig. 5.

3.5.2. *Analysis of volatile oil quantitative change difference compounds under thermal environment*

At a temperature of 60 °C, the relative content of the differential component Linalool in the crude oil group exhibited a decrease from 2.84 mg/mL at 1 h to 8 h, whereas the relative content in the Pickering emulsion group decreased from 2.43 mg/mL at 1 h to 0.87 mg/mL at 8 h, indicating a decline of 1.56 mg/mL. The decreasing trend in relative content was partially alleviated. Regarding the differential component β-Caryophyllene, the relative content in the crude oil group decreased by 1.77 mg/mL from 1 h to 8 h, while in the Pickering emulsion group, it decreased by 1.09 mg/mL. Similarly, for the differential component 4-Allylanisole, the relative content in the crude oil group registered a decrease of 2.36 mg/mL from 1 h to 8 h, with the corresponding value in the Pickering emulsion group exhibiting a decline of 1.51 mg/mL. Additionally, the differential components (+)-DELTA-CADINENE, (+)-LONGICYCLENE, α-muurolene, (+)-Epicubenol, Modhephene, (+-)-beta-Isocomene, and1-Silphinene displayed similar trends, with their decreasing tendencies being mitigated in the Pickering emulsion group. At a temperature of 60 °C, the relative content of the differential component Linalool in the β-cyclodextrin inclusion group remained stable at 3.93, 3.55, and 1.61 mg/mL at 1, 3, and 8 h respectively. In contrast, the Pickering emulsion group exhibited stable relative contents of 2.43, 2.48, and 0.87 mg/mL at 1, 3, and 8 h

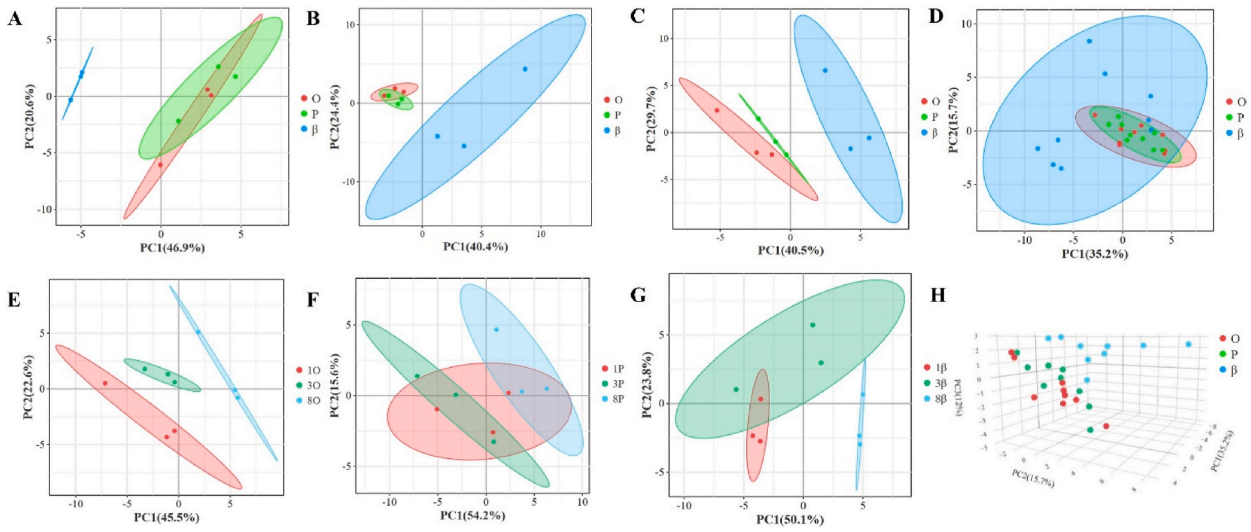

Fig. 5. PCA plot of differential components under thermal environment. Note: **(A)** PCA plot of differential components among groups at 1 h; **(B)** PCA plot of variance components among groups at 3 h; **(C)** PCA plot of variance components among groups at 8 h; **(D)** PCA summary plot of differential components by group; **(E)** PCA plots of 1, 3, and 8 h differential components in the crude oil group; **(F)** PCA plots of 1, 3, and 8h differential components in the Pickering emulsion group; **(G)** PCA plots of 1, 3, and 8 h differential components in the cyclodextrin inclusion group; **(H)** Aggregated 3D plots of PCA of differential components in each group.

respectively. Similarly, in the crude oil group, the relative contents remained stable at 3.25, 2.18, and 0.41 mg/mL respectively at 1, 3, and 8 h. Notably, the relative contents at 1 h, 3 h, and 8 h in the *β*-cyclodextrin inclusion group at 60 °C were consistently higher compared to those in the Pickering emulsion group and crude oil group. The same trend was observed for components Caryophyllene, (+)-Longicyclene, gamma-EUDESMOL, *cis*-2-Menthenol, (−)-alpha-Isocomene, (−)-Modhephene, beta-Isocomene, 1-Silphinene, Spiro [4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, (1R,4R,5S)-, and gamma-Curcumene, as depicted in Fig. 6.

3.6. Volatile oil qualitative change difference compounds under thermal environment

3.6.1. Selection of volatile oil qualitative change differential compounds under thermal environment

Compared to crude oil, after undergoing high-temperature stabilization for 1 h, the crude oil group yielded 22 new compounds, while the Pickering emulsion group produced 18 new compounds and the *β*-cyclodextrin inclusion group generated 15 new compounds. This indicates that both the *β*-cyclodextrin inclusion group and the Pickering emulsion group can partially alleviate the rise in newly formed compounds from the volatile oil caused by changes in the hot environment. In contrast to crude oil, after 1 h of stability in a hot environment, the crude oil group experienced a loss of 34 compounds, the Pickering emulsion group lost 34 compounds, and the *β*-cyclodextrin inclusion group lost 33 compounds, as shown in Fig. 7A. Following 3 h of stability in a hot environment, the crude oil group produced 19 new compounds, while the Pickering emulsion group and the *β*-cyclodextrin inclusion group generated 17 and 21 new compounds, respectively. These results further support the notion that the *β*-cyclodextrin inclusion group and the Pickering emulsion group can to some extent delay the increase in new compounds due to thermal environment changes. Notably, the *β*-cyclodextrin inclusion group exhibited a higher number of new compounds compared to the crude oil group, possibly attributed to a diminished delaying effect with prolonged stability time. Additionally, the stability of newly formed compounds during the initial hour further reinforces the increase in new compounds. The analysis suggests that new compounds gradually accumulate with an extended stability period. In the case of 3 h of stability in a hot environment compared to crude oil, the crude oil group lost 32 compounds, while the Pickering emulsion group and the *β*-cyclodextrin complex group lost 33 and 29 compounds, as depicted in Fig. 7B. After 8 h of stability in a hot environment, the crude oil group generated 14 new compounds, while the Pickering emulsion group and the *β*-cyclodextrin inclusion group generated 14 and 20 new compounds, respectively. As the stabilization time increased, the generation of new compounds gradually diminished, aligning with the hypothesis of the *β*-cyclodextrin inclusion group and further indicating

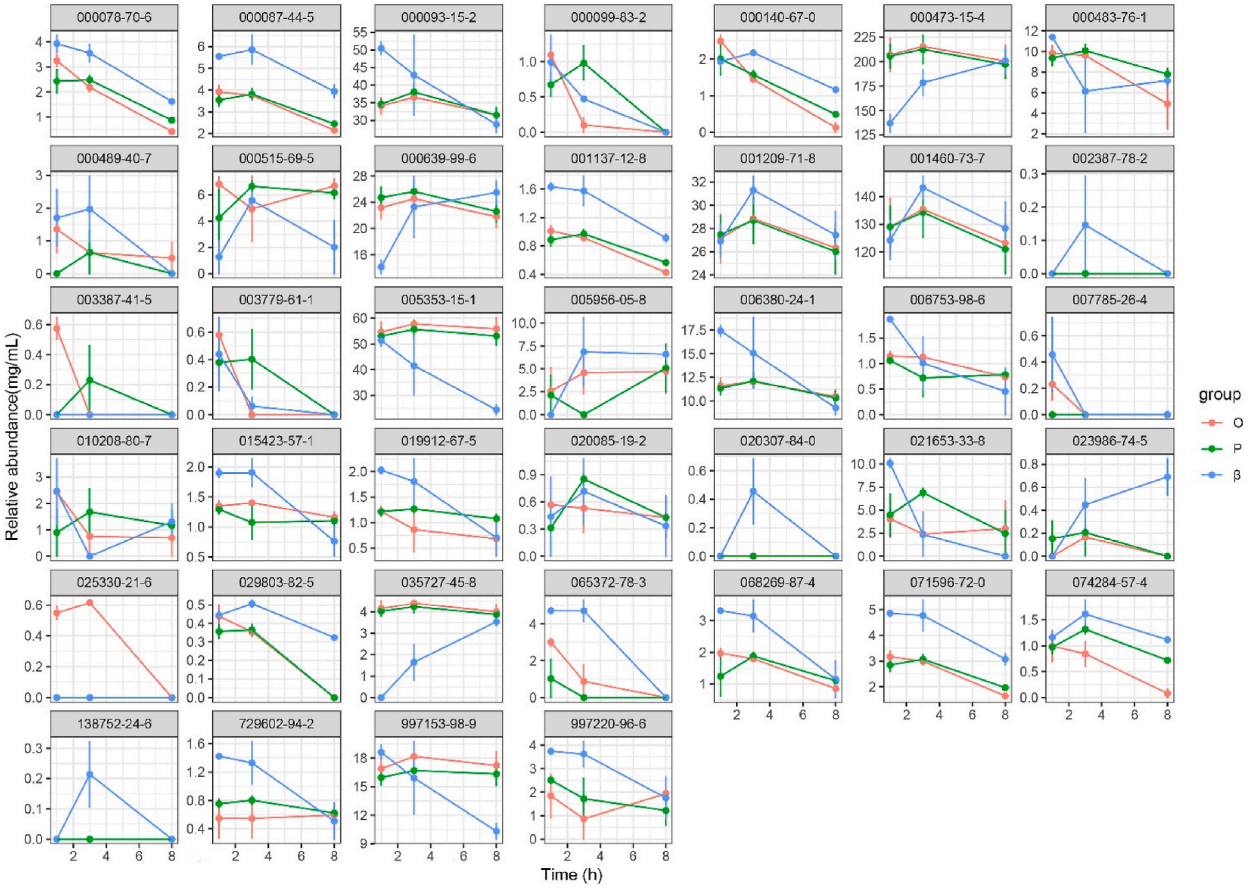

Fig. 6. Difference composition lineshape plots among groups in a hot environment.

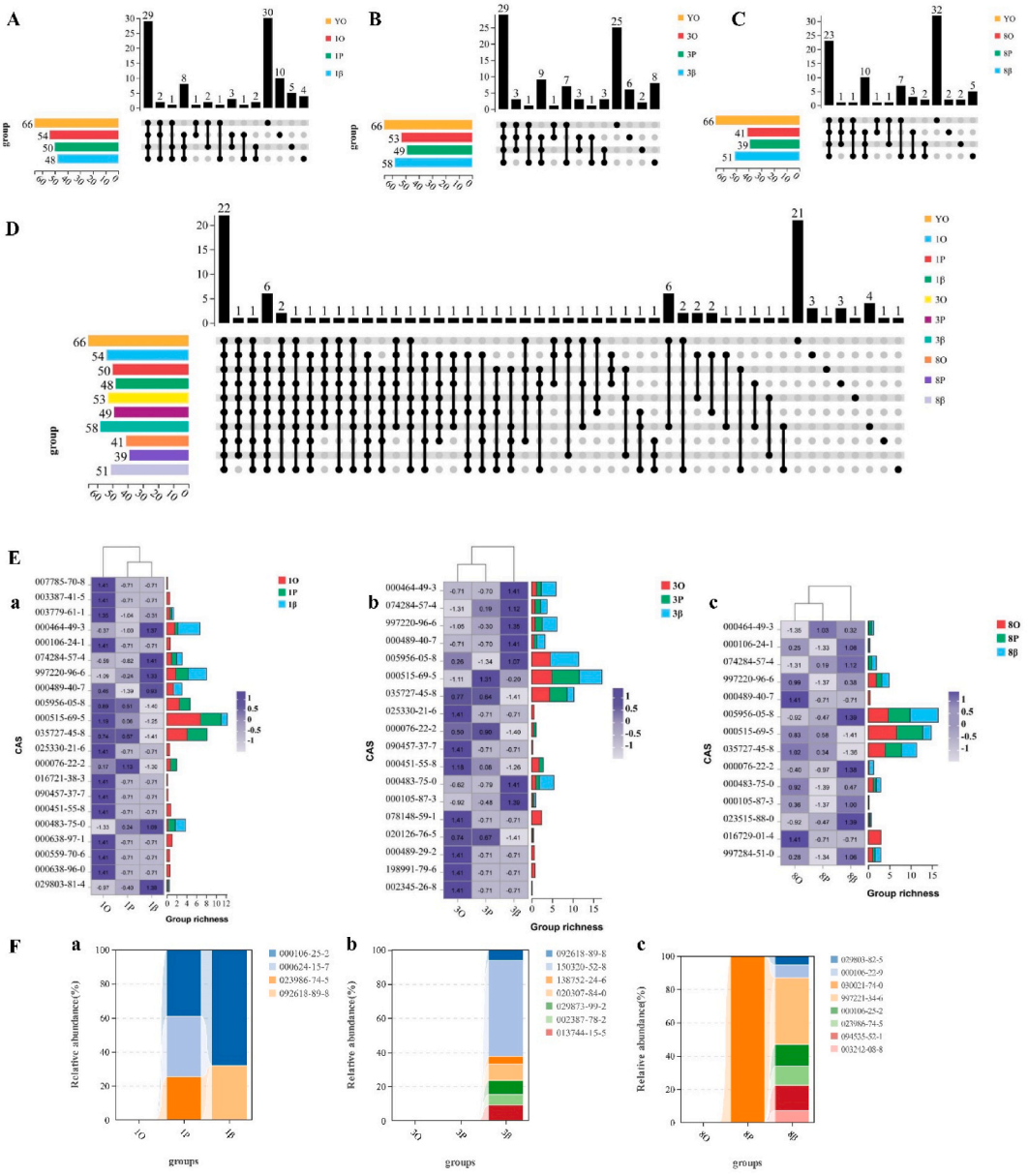

Fig. 7. Selection and analysis of Volatile oil qualitative change difference compounds under thermal environment. Note: **(A)** 1 h upset plot of each group; **(B)** 3 h upset plot of each group; **(C)** Upset plot of each group at 8 h; **(D)** Overall diagram of sets; **(E) a.** The heat map stacking diagram of newly formed compounds from ATaAL-EO in each group after 1 h; **b.** The heat map stacking diagram of newly formed compounds from ATaAL-EO in each group after 3 h; **c.** The heat map stacking diagram of newly formed compounds from ATaAL-EO in each group after 8 h; **(F) a.** The stacked line graph of disappeared compounds from ATaAL-EO after 1 h at 60 °C; **b.** The stacked line graph of disappeared compounds from ATaAL-EO after 3 h at 60 °C; **c.** The stacked line graph of disappeared compounds from ATaAL-EO after 8 h at 60 °C.

their potential in partially mitigating the upward trend of newly formed compounds in volatile oils due to changes in thermal environmental conditions. Comparatively, when stabilized for 8 h in a hot environment, the crude oil group experienced a disappeared of 39 compounds, the Pickering emulsion group lost 41 compounds, and the *β*-cyclodextrin inclusion complex group disappeared 35 compounds, as illustrated in Fig. 7C. With the extension of the stabilization period, the number of disappearing compounds progressively increased. In Fig. 7D, it can be observed that there are still 22 common compounds in the stable groups of crude oil and the hot environment after 1, 3, and 8 h. Additionally, there are still 6 common compounds in the stable groups of the hot environment after 1, 3, and 8 h. Furthermore, there are 21 unique common compounds in the crude oil. In the stable group after 1 h, the crude oil group has 3 unique compounds, while the Pickering emulsion group has 1 unique compound. The *β*-cyclodextrin inclusion group has 3 unique compounds. In the stable group after 3 h, the crude oil group has 3 unique compounds, while the *β*-cyclodextrin inclusion group

has 4 unique compounds. In the stable group after 8 h, the crude oil group has 1 unique compound, and the β-cyclodextrin inclusion group has 1 unique compound.

3.6.2. Analysis of newly generated qualitative change differential compounds in volatile oil under thermal environment

According to Fig. 7E–a, it was observed that under stable conditions of 1 h in a hot environment, new differentiating components were generated in the crude oil group, such as (+)-alpha-Pinene, Sabinen, Geraniol, Isocalamendiol, cis-Piperitol, Spiro[4.5]dec-7-ene,1,8-dimethyl-4-(1-methylethenyl)-,[1S-(1.alpha.,4.beta.,5.alpha.)], delta-bisabolene, Olean-12-en-3-one, Beta-Amyrin, and alpha-Amyrenone. However, these components were not present in the β-cyclodextrin inclusion group and the Pickering emulsion group. The newly formed differential components (E)-beta-ocimene and Bisabolol had higher relative content in the crude oil phase, while they had lower relative content in the β-cyclodextrin inclusion complex and Pickering emulsion groups. Based on the data presented in Fig. 7E–b, it was observed that under stable conditions in a hot environment for 3 h, the newly formed differential components Isocalamendiol, Spiro[4.5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, [1S-(1.alpha.,4.beta.,5.alpha.)]-, Anymol, beta-maaliene, (2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol, and Geranyl isobutyrate were generated in the crude oil group, while they were not present in the β-cyclodextrin complex and Pickering emulsion groups. The newly generated differential component Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]- had a higher relative content in the crude oil phase, while it had lower relative content in the β-cyclodextrin inclusion complex group and the Pickering emulsion group. The relative content of newly generated differential components (−)-Acorenone, 1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,3-diene, and 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- was higher in the crude oil group and lower in the Pickering emulsion group. The newly generated differential component Camphor had higher relative content in the crude oil group, while it has a lower relative content in the β-cyclodextrin inclusion group. Analysis of Fig. 7E–c revealed that under stable conditions at 8 h in a hot environment, the newly synthesized differentiating components 1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]- and 1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene were present in the crude oil group but absent in the β-cyclodextrin inclusion group and Pickering emulsion group. The newly generated differential components gamma-Curcumene, alpha-Bisabolol, Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]-, and Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)- had relatively higher relative content in the crude oil group, while they had relatively lower relative content in the β-cyclodextrin inclusion group and Pickering emulsion group. The newly generated differential components Geraniol, 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-, and 2-(4a,8-Dimethyl-2,3,4,5,6,7-hexahydro-1H-naphthalen-2-yl)propan-2-ol had a relatively high relative content in the crude oil group, while they had a relatively low relative content in the Pickering emulsion group.

3.6.3. Analysis of disappearing qualitative change differential compounds in volatile oil under thermal environment

Based on the data provided in Fig. 7F–a, it was observed that under stable conditions for 1 h in a hot environment, the disappearing compound Nerol could be effectively preserved in the Pickering emulsion and β-cyclodextrin inclusion groups. Additionally, the disappearing compounds 2,6-Octadien-1-ol, 3,7-dimethyl- and (−)-Germacrene D could be effectively preserved in the Pickering emulsion group, whilethe disappearing compound Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester could be effectively preserved in the β-cyclodextrin inclusion group. Analyzing Fig. 7F–b, it can be concluded that when exposed to stable conditions for 3 h in a hot environment, the disappearing compounds Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester, Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-, Silphiperfol-5-ene, Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-, gamma-Elemene, 3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3a.alpha.,4.beta.,7.

Table 2
Volatile component information of the main components of *Acorus tatarinowii* and *Atractylodes lancea* essential oils in high-temperature environment.

CAS	Name of compound	Relative content (mg/mL)								
		1O	1P	1β	3O	3P	3β	8O	8P	8β
000099-83-2	alpha-PHELLANDRENE	1.09 ±0.26	0.67 ±0.28	0.99 ±0.66	0.10 ±0.18	0.98 ±0.42	0.47 ±0.05	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
000078-70-6	Linalool	3.25 ±0.41	2.43 ±0.81	3.93 ±0.58	2.18 ±0.29	2.48 ±0.29	3.55 ±0.59	0.41 ±0.05	0.87 ±0.17	1.62 ±0.12
000093-15-2	Methyl eugenol	34.09 ±4.03	34.62 ±2.28	50.44 ±3.12	36.57 ±3.05	38.05 ±4.02	42.87 ±19.59	31.49 ±3.22	31.49 ±3.72	28.82 ±3.87
000087-44-5	Caryophyllene	3.92 ±0.51	3.54 ±0.47	5.55 ±0.10	3.77 ±0.23	3.81 ±0.44	5.86 ±1.15	2.15 ±0.16	2.45 ±0.23	3.94 ±0.51
006380-24-1	(Z)-Methyl isoeugenol	11.61 ±1.41	11.35 ±1.21	17.42 ±1.00	12.04 ±0.46	12.11 ±1.31	15.07 ±6.44	10.47 ±1.23	10.32 ±1.23	9.27 ±1.23
006753-98-6	alpha-Humulene	1.15 ±0.15	1.06 ±0.12	1.87 ±0.05	1.13 ±0.06	0.72 ±0.63	1.01 ±0.88	0.74 ±0.07	0.78 ±0.09	0.45 ±0.79
000483-76-1	(+)-delta-Cadinene	9.82 ±1.27	9.35 ±1.22	11.39 ±0.31	9.61 ±0.87	10.08 ±1.10	6.14 ±6.93	4.91 ±4.25	7.79 ±0.92	7.16 ±0.95
005273-86-9	beta-Asarone	56.31 ±7.57	51.74 ±4.60	56.19 ±4.39	59.48 ±2.09	53.90 ±5.90	45.51 ±22.92	55.90 ±7.74	50.99 ±7.07	26.76 ±3.43
000473-15-4	beta-Eudesmol	206.94 ±29.08	205.63 ±21.21	137.06 ±15.96	215.52 ±8.91	212.48 ±24.99	178.47 ±22.59	200.62 ±27.98	197.05 ±24.49	201.00 ±22.59

alpha.)]-, 1H-Cyclopenta [1,3]cyclopropa [1,2]benzene, 2,3,3a.alpha.,3b.alpha.,4,5,6,7-octahydro-4.alpha.-isopropyl-7.beta.-methyl-3-methylene- could be effectively preserved by inclusion in β-cyclodextrin. Moreover, as depicted in Fig. 7F–c, it is apparent that in the enduring ambiance of a scorching environment for a span of 3 h, the evanescent entity known as gamma.-Muurolene could be diligently conserved within the confines of the Pickering emulsion system. Additionally, the disappearing compounds 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, *cis*-, Citronellol, (6S,7R)-2,2,6-Trimethyl-10-methylene-bicyclo[5.4.0]undec-1(11)-ene, 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-, (−)-Germacrene D, (1R,3aS,4aS,8aS)-1,4,4,6-Tetramethyl-1,2,3,3a,4,4a,7,8-octahydrocyclopenta [1,4]cyclobuta [1,2]benzene, and Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)- could be effectively preserved in the inclusion complex of β-cyclodextrin.

3.6.4. Analysis of the main compounds of volatile oils in high-temperature environments

Volatile component information of the main components of ATaAL-EO under thermal environment was shown in Table 2. At a temperature of 60 °C, the relative content of the major componentalpha-PHELLANDRENE in the crude oil group decreased to 0.10 mg/mL after 3 h of exposure to high temperature, while the relative content in the Pickering emulsion group decreased to 0.00 mg/mL after 8 h of exposure. The relative content of the major component Linalool in the crude oil group decreased by 1.07 mg/mL from 1 h to 3 h of high temperature exposure, and further decreased by 1.77 mg/mL from 3 h to 8 h of high temperature exposure. In contrast, the relative content in the Pickering emulsion group showed no decrease between 1 h and 3 h of high temperature exposure, and decreased by 1.77 mg/mL from 3 h to 8 h. Similarly, the main constituents Caryophyllene, cis-Methylisoeugenol, and (+)-delta-Cadinene exhibited similar trends, with the decreasing in relative content in the Pickering emulsion group slightly delayed compared to the crude oil group. Under the condition of 60 °C stabilization for 1, 3, and 8 h in the β-cyclodextrin inclusion group, the relative contents of the main components Linalool and Caryophyllene were higher than those in the Pickering emulsion group and crude oil group. Additionally, under the condition of 60 °C stabilization for 3 h in the β-cyclodextrin inclusion group, the relative contents of the main components Methyleugenol, Humulene, and beta-Asarone were higher than those in the crude oil group. All of these relative contents were higher compared to the crude oil group, as shown in Fig. 8.

4. Discussion

The pharmacological activity of volatile oils in traditional Chinese medicine is highly significant. However, their formulations often suffer from poor stability due to their inherent volatility and susceptibility to oxidation [15,16]. In environments with elevated temperatures, the volatile oils are particularly prone to oxidative reactions triggered by oxygen. These reactions can result in alterations to the composition of volatile oils and the generation of oxidation products, ultimately compromising their stability and overall

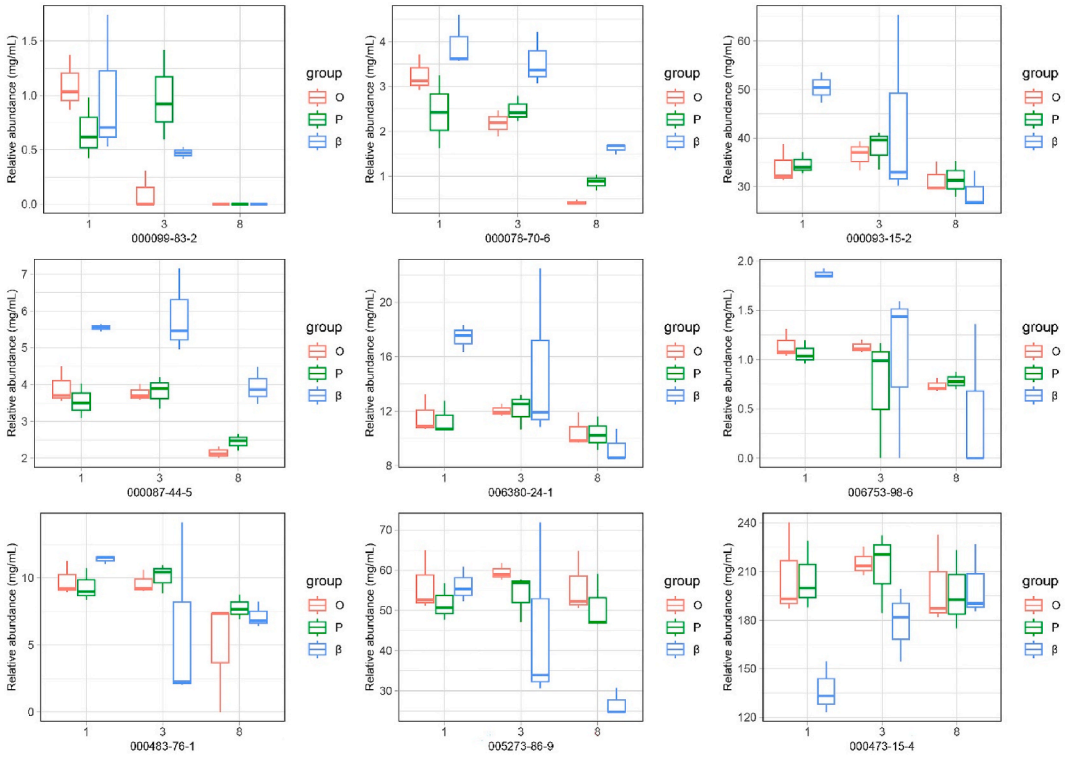

Fig. 8. Boxplots of principal component compounds in each group under a thermal environment.

quality [17]. Additionally, higher temperatures promote the evaporation rate of volatile oils, leading to their loss and a subsequent decrease in concentration [18]. At elevated temperatures, certain volatile oils may even undergo decomposition reactions, leading to changes in their molecular structure and the formation of unstable compounds [19]. Furthermore, temperature exerts a profound influence on the aroma components within essential oils, as high temperatures can induce their decomposition, volatilization, and loss. This, in turn has a direct impact on the aroma quality and longevity of the essential oils [20,21].

Based on this, we introduced the *β*-cyclodextrin inclusion and Pickering emulsion technology. After subjecting the ATaAL-EO to a high-temperature environment of 60 °C for 8 h, it was observed that the retention rate of the crude oil group decreased by 22.05%, while the *β*-cyclodextrin inclusion group only decreased by 6.32% and the Pickering emulsion group decreased by 11.28%. Furthermore, at high temperatures of 1, 3, and 8 h, the peroxide values in the crude oil group were higher compared to those in the Pickering emulsion and *β*-cyclodextrin inclusion groups. This suggests that the introduction of Pickering emulsion and cyclodextrin inclusion technology can effectively delay the loss of volatile oil and reduce its peroxide value under high-temperature conditions.

Through a comprehensive analysis of the volatile components in ATaAL-EO, we found that Pickering emulsion technology can partially prevent the fluctuation of relative content in most of the differential and major components in the crude oil group under a thermal environment of 60 °C, thus demonstrating a certain degree of stability. On the other hand, *β*-cyclodextrin inclusion technology can protect the relative content of major components from being lost. Moreover, after 1 h of high-temperature exposure, Pickering emulsion and *β*-cyclodextrin were able to prevent the formation of 10 newly generated differential compounds in the crude oil group, reduce the relative content of 2 newly generated compounds, and prevent the loss of 3 and 2 disappearing compounds, respectively. At a high temperature of 3 h, Pickering emulsion and *β*-cyclodextrin were able to prevent the formation of 6 newly generated differential components, reduce the relative content of 4 and 2 newly generated compounds, and prevent the loss of 7 vanishing compounds in the crude oil group. Furthermore, after 8 h of high-temperature exposure, Pickering emulsion and *β*-cyclodextrin were able to prevent the formation of 2 newly generated differential components in the crude oil group. The Pickering emulsion group was able to reduce the relative content of 7 newly generated differential components, while *β*-cyclodextrin prevented the loss of 7 vanishing compounds in the crude oil group. Overall, both Pickering emulsion and *β*-cyclodextrin exhibited the ability to prevent the formation of most newly generated differential components in volatile oil under the thermal environment of 60 °C. Pickering emulsion demonstrated a significant advantage, followed by *β*-cyclodextrin.

5. Conclusions

This study compared and analyzed the retention rate, peroxide content, and composition change trends of ATaAL-EO, Pickering emulsions, and *β*-cyclodextrin at a 60 °C thermal environment. In terms of improving the retention rate, the inclusion technique using *β*-cyclodextrin showed greater advantages compared to the Pickering emulsion. In reducing the peroxide content, both *β*-cyclodextrin inclusion and Pickering emulsions demonstrated advantages. Pickering emulsions could to some extent delay the trend of differential and major component content changes in the crude oil group under a 60 °C thermal environment, showing certain stability. *β*-cyclodextrin was able to effectively protect against the loss of relative content in most components. In terms of preventing the formation of new differential components and slowing down the trend of increased relative content of newly formed compounds due to the thermal environment, the effectiveness of Pickering emulsions was more significant, with *β*-cyclodextrin in the secondary position. Additionally, both methods could prevent the disappearance of some compounds in a high-temperature environment to a certain extent, with *β*-cyclodextrin having a better performance. The results emphasized the respective advantages of Pickering emulsion and *β*-cyclodextrin in stabilizing ATaAL-EO, providing valuable insights for improving the quality of ATaAL-EO.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the corresponding author. Researchers interested in accessing the data can contact jun-bo zou email at 2051078@sntcm.edu.cn.

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Declaration of competing interest

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Appendix A. Supplementary data

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