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Research on Pickering emulsification technology based on the concept of "combination of medicine and adjuvant" to improve the pH stability of volatile oil in solid preparations—taking Lingzhu Pulvis as an Example

Lei Peng†, Mei Wang†, Xiao-Fei Zhang, Dong-Yan Guo, Bing-Tao Zhai, Jun-Bo Zou* and Ya-Jun Shi*

Abstract

Lingzhu Pulvis are widely used in clinical practice because of their therapeutic effects, but their stability and efficacy are affected by the easy oxidation and volatility of *Acorus calamus*. In this study, we combined the idea of "combination of medicine and adjuvant" and introduced Pickering emulsion technology to use the ingredients of Lingzhu Pulvis as the stabilizer of Pickering emulsion. Pearl powder was selected as the stabilizer by the type of emulsion formation and the state of emulsion layer, and the optimal preparation method was 0.065 $g \cdot ml^{-1}$ of pearl powder and 45% oil, prepared using high-pressure homogenization method (500 bar, three times). The malondialdehyde and peroxide contents in the crude oil and Pickering emulsion groups were analyzed under different pH environments, and it was clear that the Pickering emulsion group possessed higher antioxidant properties. The volatile oil of *Acorus calamus* in different groups was analyzed using GC-MS, and seven differential components were screened by processing the analytical data with R language, which were further determined that the differential components changed more slowly and had significantly improved stability in the Pickering emulsion group. This study provides a reference for the improvement of stability of other solid formulations containing oil in Chinese medicine.

Keywords Stability, Lingzhu Pulvis, Combination of medicine and adjuvant, The volatile oil of *Acorus calamus*, Pickering Emulsion

Pediatric febrile convulsions are common in pediatrics, with a reoccurrence rate of 30–40% after the first episode, mainly manifesting as sudden local or generalized myoclonic, compulsive convulsions (Fang-Yuan et al. 2020; Symonds et al. 2020). In addition to the possibility of causing epilepsy, recurrent convulsive re-seizures can cause irreversible brain damage and mental retardation in the child (Skotte et al. 2022; Watila et al. 2021). Lingzhu Pulvis, a centuries-old scripture formula produced by Lei Yunshang, has been applied as a national intangible cultural heritage and has a high clinical usage rate in the

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treatment of febrile convulsions and external wind chills in pediatric patients (Xiao et al. 2021; Nan et al. 2009). However, the oil of*Acorus calamus*in the remedy has the problem of easy oxidation, volatilization and escape (Hong-Xia 2008; Qi et al. 2022), which can directly affect the stability of the antelope bead preparation and thus the efficacy of the preparation. Therefore, improving the stability of oil is a real problem that needs to be solved urgently for the quality improvement of Lingzhu Pulvis. Pickering emulsion refers to the replacement of traditional chemical molecular emulsifiers by solid particles with suitable surface wettability, which can irreversibly adsorb at the oil/water interface and form a solid spatial shell layer at the interface to protect the oil phase of emulsion droplets and thus improve the stability, which has been rapidly developed at home and abroad in recent years because of its simple preparation process and easy industrialization (Bing-Long et al. 2022; Shi-Xuan et al. 2020) "combination of medicine and adjuvant" is an important principle for the use of excipients in Chinese medicine preparations, and it is also a distinctive feature that distinguishes Chinese medicine preparations from chemical preparations and is universal in Chinese medicine preparations.

In this theoretical hypothesis, from the formulation point of view, the use of the prescription powder, acting as a stabilizer to prepare volatile oil solid particle emulsion, to assist in the formation and stabilization of the formulation, is the embodiment of the "medicine used as adjuvant"; from the therapeutic point of view, the powder acts as a stabilizer to improve the stability of volatile oil, which in turn helps to ensure the clinical efficacy, reflecting the characteristics of "adjuvant used as medicine." The prescription of Lingzhu Pulvis contains different types of powder including animal medicine (Cornu Antelopis, artificial bezoar), plant medicine (calamus), mineral medicine (pearl powder, cinnabar), extracts (*Bombyx batryticatus*, Dan Nanxing extract), and resin (amber), which basically cover the types of stabilizers such as protein, cellulose, mineral, starch, and polysaccharide used in the preparation of solid particulate emulsions. Therefore, in this study, we used Pickering emulsion technology to improve the stability of the oil of *Acorus calamus*in Lingzhu Pulvis and thus improve its efficacy based on the idea of "combination of medicine and adjuvant" (Jun-Hong et al. 2022). It also provides more references for future studies on improving the stability of volatile oils in dispersants. The main pathways for improving volatile oils include inclusion compounds, particulate delivery systems, and macroscopic delivery systems. Among them, the particulate systems can be divided into (1) lipid-based delivery systems, including emulsions, nano-lipid carriers, solid nanoparticles; (2) self-assembled delivery systems, including liposomes, phosphate complexes, ethanolic liposomes, and non-ionic surfactant vesicles, which have the property of self-assembling into bilayer membrane-like vesicles; and (3) polymer-based delivery systems, including nanoparticles, nanocapsules, and nanofibers. The above delivery systems have been used to improve the storage stability, mask the bad taste, protect the volatile components, and reduce the oxidation level, but their core design ideas are all achieved by adding a large amount of excipients, which have obvious effects on the in vitro release and in vivo absorption of the active ingredients of volatile oils, and even bring new drug safety hazards. Therefore, to find a stabilization strategy that does not introduce or rarely introduce exogenous excipients, does not interfere with the in vitro and in vivo behavior of volatile oils, and has high safety and stability is the ideal choice to improve the stability of volatile oils and is also the core technology of this paper to focus on and implement. Compared with the traditional methods to improve the stability of volatile oil, the method adopted in this study takes its original composition as the stabilizer of Pickering emulsion, without adding exogenous excipients or changing the basis of drug action, which is safer and more reliable. Moreover, the preparation is simple and easy to achieve large-scale industrial production. At the same time, the basic research on improving the stability of volatile oil without the use of exogenous excipients was expanded, which provided the basis for improving the stability and efficacy of the whole solid preparation in the next step. It is of great significance to improve the stability of solid preparation of oil-containing traditional Chinese medicine.

Material

The materials are as follows: MH-3000 temperature-regulating electric heating jacket (Beijing Kewei Yongxing Instrument Co., Ltd.), Electric Heating Blast Dryer (Shanghai-Heng Scientific Instruments Co., Ltd.), HG-3 Magnetic Heating Stirrer (Changzhou Guohua Electric Co., Ltd.), JY-3002 one hundred thousandth analytical balance (Shanghai Puchun Instrument Co., Ltd.), IKA T18 digital digital high-speed disperser (Shanghai Tucson Vision Technology Co., Ltd.), AH-BASIC High Pressure Homogenizer (Antos Nano Technology Co., Ltd.), DM3000 Biological Microscope (Leica Microsystems Trading Co., Ltd.), TENSOR-27 Fourier Transform Infrared Spectrometer (Bruker, Germany), ANTIRIS II Fourier Transform Near Infrared Spectrometer (Thermo Fisher Scientific, USA), K100C-KRUSS Automatic surface tension and contact angle tester (KRUSS, Germany), UV spectrophotometer (Shanghai Youke Instrument Co., Ltd.), acid burette, and Kjeldahl flask.

Materials also include the following: volatile oil of *Acorus calamus*, pearl powder, artificial bezoar, amber, cinnabar, bilberry and Dan Nanxing extract from Lei Yun Shang Pharmaceutical Group Co., Ltd, chloroform (Lot No. 20,200,203, Tianjin Tianli Chemical Reagent Co., Ltd.), 2-thiobarbituric acid (TBA, Shanghai Kefeng Industrial Co., Ltd., Lot No. 20,170,921), trichloroacetic acid, and 1,1,3,3-tetraethoxypropane (Shanghai Maclean Biochemical Technology Co., Ltd., lot C10050555, purity ≥ 97%). The water was distilled, and the rest of the reagents were analytically pure.

Methods and results

Characterization, screening, and process optimization of stabilizer powder properties

Determination of solubility, particle size, and contact angle

Solubility Weigh 0.01 g of the mixture of pearl powder, amber, cinnabar, artificial bezoar, Cornu Antelopis, *Bombyx batryticatus*, and Arisaema cum Bile in 100 mL of water, respectively, and shaken strongly for 30 s every 5 min; the dissolution state was observed within 30 min.

Particle size The particle size of samples are determined with the aid of the dry measurement method of the Malvern laser particle sizer. Sample measurement time was set to 12 s, the background measurement time was 10 s, the dispersion air pressure was 200 kPa, the shading range was 0.8~3.0%, the injection speed was 50%, the appropriate amount of sample particles was added, the measurement was started, and the characteristic value of particle size distribution d90 was recorded for each sample.

Contact angle The 0.2 g sample particles were pressed into tablets using an infrared press with a diameter of 10 mm (set pressure of 6 N). The test solution is water. Determination of the wetting properties of pearl powder particles using KRUSS.

Table 1 Results of the study

Samples	Solubility	d_{90}/μm	Contact angle /°
Pearl powder	Insoluble	58.57	73.63 ± 2.58
Amber	Insoluble	61.46	87.27 ± 1.33
Cinnabar	Insoluble	28.82	74.23 ± 2.33
Artificial bezoar	Insoluble	89.01	30.3 ± 1.30
Cornu Antelopis	Insoluble	147.44	88.87 ± 2.66
Bombyx batryticatus and Arisaema cum Bile	Insoluble	260.33	64.07 ± 0.75

The results are shown in Table1. All the powder of Lingzhu Pulvis composition remedy are insoluble substances, and the consumption of dissolved in water when forming emulsion is negligible. The particle size of the mixture of Cornu Antelopis, *Bombyx batryticatus*, and Arisaema cum Bile was too large (> 100 μm) for the preparation of Pickering emulsion. The contact angle of artificial bezoar was too small to be used to form Pickering emulsion. Preliminary screening of pearl powder, amber and cinnabar as stabilizers for Pickering emulsion.

Identification of emulsion types and preparation methods

Preparation of emulsion Measure the appropriate amount of calamus oil and water in a beaker, a total of 3 parts, respectively; add the 0.3 g of pearl powder, amber, and cinnabar into an emulsion using a high-speed disperser; separate the emulsion phase; and observe the color of the emulsion layer.

Filter paper method Aspirate an appropriate amount of emulsion drops onto the filter paper and observe the unfolding phenomenon.

Staining method Sudan III which has a strong affinity for calamus oil can be quickly dissolved in the oil phase; the oil phase discoloration is obvious. And it is difficult to dissolve in water. Methylene blue is easily soluble in water but insoluble in calamus oil, and the color change is obvious in the water phase. The emulsion samples were applied on the slides and stained with Sudan III and methylene blue once each, and the staining results were observed.

Preferred stabilizer concentration Measure 14 mL of water and 6 mL of the oil of *Acorus calamus*, a total of 16 parts, and then add 0.1 g, 0.2 g, 0.3 g, 0.4 g, 0.5 g, 0.6 g, 0.7 g, 0.8 g, 0.9 g, 1.0 g, 1.1 g, 1.2 g, 1.3 g, 1.4 g, 1.5 g, and 1.6 g of pearl powder, respectively, and emulsify using a high-speed disperser. The optimal Pickering emulsion concentration was determined by observing the amount of oil encapsulation and emulsion formation.

Preferred oil-water ratio Take five 45 mL centrifuge tubes; add 6 mL of the oil of *Acorus calamus*; add 14 mL, 11.14 mL, 9 mL, 7.33 mL, and 6 mL of distilled water; and then add 1.30 g, 1.11 g, 0.96 g, 0.87 g, and 0.78 g of pearl powder in turn, and use a high-speed disperser to form an emulsion; observe the amount of emulsion and the amount of oil coating, and determine the optimal oil-water ratio.

Preparation method Take three 45 mL centrifuge tubes, add 6 mL the oil of *Acorus calamus*, 14 mL distilled water and 1.3 g pearl powder, and prepare them using magnetic stirrer, high-speed disperser, and high-pressure homogenizer, respectively. Pickering emulsion morphology was observed using a DM3000 biomicroscope to determine the optimal preparation process.

From Fig. 1A, it can be seen that cinnabar distributed on the wall or bottom of the tube cannot form Pickering emulsion. By the filter paper method and staining method, it was concluded that the Pickering emulsion formed by pearl powder was O/W type and the Pickering emulsion formed by amber was W/O type, as shown in Fig. 1B, C. It was finally decided to use pearl powder as the stabilizer of the Pickering emulsion. With the increment of pearl powder concentration, the emulsion layer formed gradually increased, as shown in Fig. 1D. After the concentration exceeded 0.065 $g{\cdot}mL^{-1}$, the emulsion volume stabilized and the formed emulsion layer stratified with the increase of time, so the maximum emulsion volume without stratification was 0.065 $g{\cdot}mL^{-1}$. The emulsion state under different oil–water ratio were observed, from which we can tell that when under the oil ratio of 30%, 35%, 40%, and 50%, the emulsion layer and water layer stratified, while under the oil ratio 45% without stratification, as in Fig. 1E. It is known that the Pickering emulsion formed at 45% oil to water ratio is the most stable. Subsequently, three different emulsion-forming preparation methods were compared; the solution with an appropriate oil percentage of 45% and a pearl powder concentration of 0.065 $g{\cdot}mL^{-1}$ was prepared using a magnetic stirrer, a high-speed disperser, and a high-pressure homogenizer, respectively. It can be seen that the magnetic stirring method could not form emulsion and then using a DM3000 microscope to observe the emulsion-forming state prepared by high-speed disperser (13,000 rpm, 3 min) and high-pressure homogenization method (500 bar, 3 times), as shown in Fig. 1F, G. Smaller and more uniform particle size of Pickering emulsions prepared by high pressure homogenization method can be observed. Finalized Pickering emulsion using high pressure homogenization method. The optimal preparation method was determined to be Pickering emulsion prepared by high-pressure homogenization (500 bar, 20 min) at room temperature using 0.0064 g/ml pearl powder with an oil-water ratio of 45%.

Fourier transform (FT) NIR spectral characterization

Appropriate amounts of the oil of *Acorus calamus*, pearl powder suspension and Pickering emulsion samples were placed in a 2 mm quartz cuvette for spectral acquisition

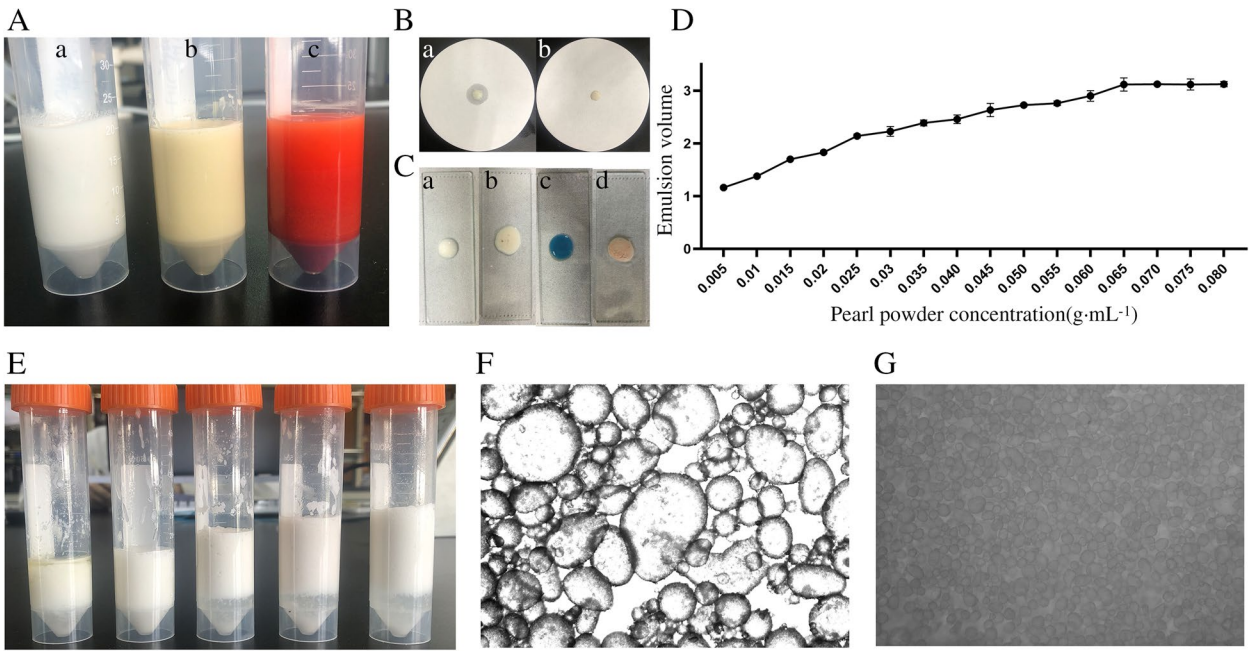

Fig. 1 Identification and preparation method of various medicinal materials into emulsion types. **A** State of forming emulsions ((a) pearl powder; (b) cinnabar; (c) amber). **B** Filter paper method to identify each herb into emulsion type ((a) pearl powder; (b) amber). **C** Staining method to identify the type of each herb into emulsion (before staining: (a) pearl powder, (b) amber; after staining: (c) pearl powder, (d) amber). **D** Emulsion volume of different pearl powder concentration. **E** Emulsion state of different oil-water ratio. **F** Preparation by high-speed dispersion method (× 100). **G** Preparation by high-pressure homogenization method (× 100)

in the range of 4000 cm−1 to 1000 cm−1 with an acquisition temperature of room temperature, a scan number of 32, and a resolution of 8 cm−1 and a gain of 1×. The spectra were collected with air as the reference minus the background, and each sample was repeated three times to obtain the average NIR spectra. The characteristic absorption peaks of the oil of *Acorus calamus* at wave numbers 4413 cm^{-1}, 5888 cm^{-1}, and 7065 cm^{-1} were attenuated in Pickering emulsion by observing the NIR spectra. The absorption peak at 7065 cm^{-1} in the NIR absorption spectrum is the primary vibrational octave band of water and hydroxyl group (−OH), the absorption peak at 5888 cm^{-1} is the combined band of telescoping-bending vibration of water, and the absorption peak at 4413 cm^{-1} is the combined band of telescoping-bending vibration of hydroxyl group (−OH). The absorption peaks of Pickering emulsion and the pearl powder suspension have basically the same amplitude of absorption peak fluctuations, and it can be judged that the Pickering emulsion formed is the outer layer wrapped by pearl powder. Meanwhile, no obvious new absorption peaks were generated in Pickering emulsion, as shown in Fig. 2, indicating that Pickering emulsion could encapsulate the calamine oil and no new chemical bonds were formed.

Stability experiments

Collection of volatile oils in acid-base stability experiments

Measure 6 ml of calamus oil, 13.33 ml of oil–water mixture, and Pickering emulsion in a 100-ml evaporating dish. The pH value was adjusted to 6, 7, and 8 using dilute hydrochloric acid and dilute sodium hydroxide, and then the oil–water mixture and Pickering emulsion were distilled using water distillation for 8 h. The oil phase was separated. Parallel three sets of experiments.

Statistical processing

All experimental data were analyzed using the SPASS25. 0 software for analysis, *t*-test was used to compare data between two groups, and one-way analysis of variance (ANOVA) was used for comparison between groups. Statistical results were presented at $P<0.05$ indicates that the difference is statistically significant.

Determination of malondialdehyde substances in volatile oil

Malondialdehyde is an organic substance produced by peroxidation of the membrane lipids of plant tissues or organs as a result of aging or damage suffered by plant organs under adverse conditions. Its size can be a good reflection of the degree of oxidation of the measured components. Determination of standard curve is as follows: accurately pipette 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 mL of malondialdehyde standard solution (0.315 g of 1,1,3,3-tetraethoxypropane dissolved in water and diluted to 1000 mL) into a 10-mL volumetric flask, and fix the volume with distilled water to 10.00 mL to obtain the concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 μg·mL^{-1} of malondialdehyde standard solution. Add 5 mL of thiobarbituric acid (TBA) solution (0.288 g thiobarbituric acid diluted to 100 mL with water, 0.02 mol·L^{-1}), mix well, heat in a water bath at 90 °C for 40 min, remove, add 5.00 mL of chloroform after cooling, shake well, and let stand for 1 h. The supernatant was measured at 532 nm. The absorbance was measured at 532 nm, and the standard curve was plotted

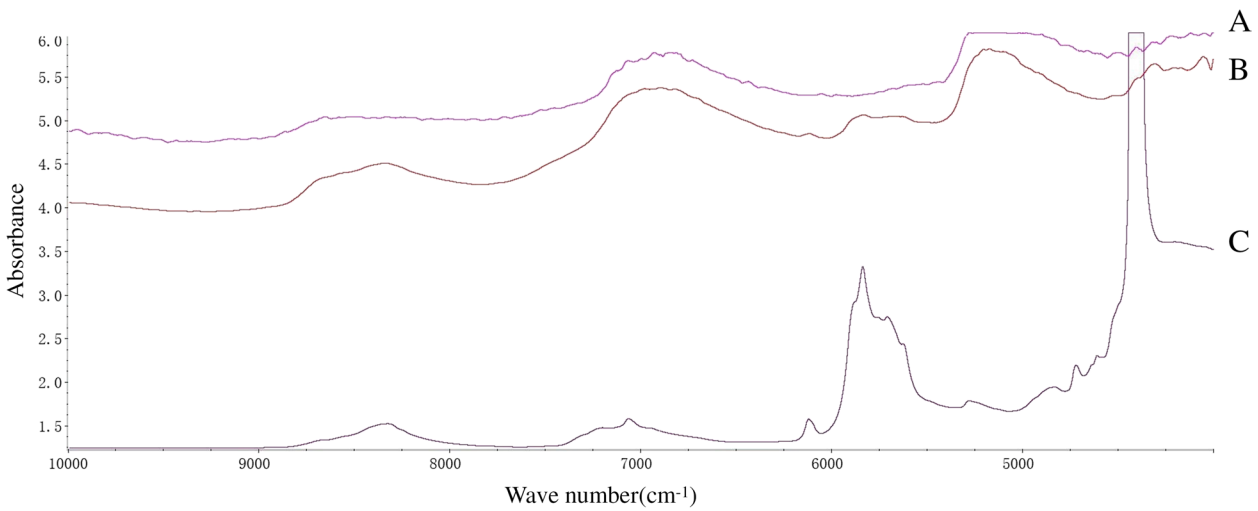

Fig. 2 Near-infrared spectrogram. **A** Pearl powder suspension. **B** Pickering emulsion. **C** Calamus oil

with the mass concentration of the standard series solution as the horizontal coordinate and the absorbance as the vertical coordinate.

Sample preparation and determination: 500 μL of the above volatile oil samples were taken in a 10-mL volumetric flask, mixed solution of trichloroacetic acid (37.5 g trichloroacetic acid and 0.50 g sodium ethylenediaminetetraacetate, dissolved in water and diluted to 500 mL) was fixed to the scale, sonicated for 10 min, and filtered, and 5.00 mL of the filtrate was taken in a conical flask, 5.00 mL of TBA solution was added, heated at 90 ° C for 4 h, removed, cooled away from light for 1 h, added 5.00 mL of chloroform, shaken thoroughly, and left for 1 h. Add 5.00 mL of TBA solution, heat at 90 °C for 4 h, remove, cool for 1 h, add 5.00 mL of chloroform, shake thoroughly, and leave for 1 h. The absorbance of the supernatant was measured at 532 nm, and the malondialdehyde content was calculated. The standard curve of malondialdehyde concentration/absorbance was plotted using 1,1,3,3-tetraethoxypropane with a linear regression equation of $Y = 0.088\ 9x - 0.000\ 7$, $r = 0.998\ 9$, as in Fig. 3A. Based on the standard curve to calculate the malondialdehyde concentration in different samples, it was found that at pH 6, 7, and 8, the malondialdehyde content was significantly lower in the Pickering emulsion group compared to the crude oil group ($P < 0.01$, $P < 0.001$), as shown in Fig. 3B.

Determination of peroxide value in volatile oil

The peroxide value of edible oil is one of the most commonly used physicochemical indicators to evaluate whether the edible oil meets the national health standards. The higher the peroxide value, the higher the degree of oxidation of fats and fatty acids, the more serious the deterioration of edible oil and the greater the danger to human body. Separately, 500 μL of the above volatile oil samples were taken in centrifuge tubes. Take 10 mL of trichloromethane-glacial acetic acid mixture ($V_1/V_2 = 4{:}6$) 10 mL and wash it into a corked conical flask. Accurately add 1 mL saturated potassium iodide solution, tightly plugged, shaken for 0.5 min, placed in the dark for 3 min, removed, added 30 mL of water, added 1 mL saturated potassium iodide solution, tightly plugged, shaken

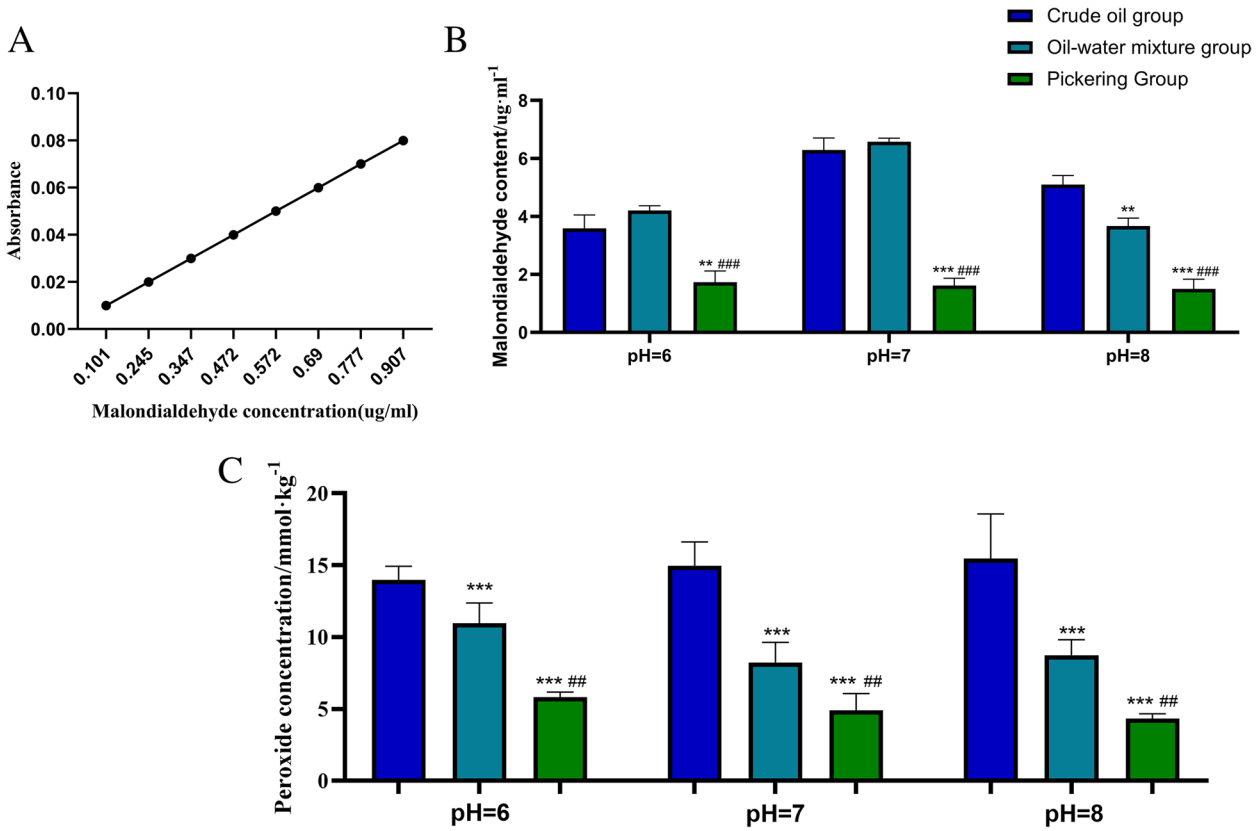

Fig. 3 Standard curve of malondialdehyde and absorbance and comparison of index changes among groups. **A** Standard curves of malondialdehyde concentration and absorbance. **B** Content of malondialdehyde at different time periods. **C** Content of peroxide at different time periods. Compared with the blank group *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with the oil-water mixture group #$P < 0.05$, ##$P < 0.01$, ##$P < 0.001$

for 0.5 min, placed in the dark for 3 min, removed, added 30 mL of water, added 1 mL of 1% starch indicator, titrate with 0.001 mol-L^{-1} sodium thiosulfate standard solution (26 g sodium thiosulfate and 0.20 g anhydrous sodium carbonate dissolved in 1 000 mL water, boil slowly for 10 min, dilute after cooling) until the solution disappears as the end point, record the volume of consumed sodium thiosulfate standard solution and calculate the Peroxide value value.

The Pickering emulsion group had significantly lower peroxide content compared to the oil-water mixture and crude oil groups ($P < 0.01$, $P < 0.001$), as shown in Fig. 3C.

Analysis of volatile oil components by gas chromatography-mass spectrometry

Determination of volatile oil composition by GC-MS after intervention under different conditions

GC-MS is widely used for the separation and identification of complex components, with the high resolution of GC and the high sensitivity of MS. The different components in the sample are separated out by programmed temperature rise, and the spectrum is derived by using mass spectrometry under high vacuum after a series of reactions to make it form charged particles, and the mass-to-charge ratio and relative intensity of each ion are determined by the detector. GC-MS is very suitable for the analysis of the oil of *Acorus calamus*, which has a complex chemical composition and is volatile. GC-MS conditions are as follows: an HP-5MS quartz capillary column (30 m·0.25 mm, 0.25 μm) with a carrier gas of helium (purity of 99.999%) at a flow rate of 1 mL·min^{-1}; injection volume 1 μL; shunt ratio 10:1; inlet temperature 230 °C; heating up procedure; 50 °C for 2 min, to 110 °C at 5 °C·min^{-1} for 2 min, to 120 °C at 2 °C·min^{-1} for 5 min, to 125 °C at 0. 5 °C·min^{-1} to 125 °C for 10 min, 4 °C·min^{-1} to 200 °C for 2 min, 10 °C·min^{-1} to 250 °C for 2 min. Mass spectrometry conditions are as follows: ionization mode EI, electron energy of 70 eV, quadrupole temperature 150 °C, ion source temperature 230 °C, scan range m/z 35–500, solvent delay 3 min.

Sample solution preparation is as follows: Take the upper 2.2. 1 sample 100 μL in a 10 mL brown volumetric flask, fix the volume with ether, add 0.2 g of anhydrous sodium sulfate, weigh 2 mL of sample precisely, filter the membrane in a liquid phase vial, and use GC-MS to determine.

Screening of differential components

After the GC-MS data acquisition was completed, the components were characterized by Agilent database analysis software Data Analysis calling the NIST 14.0 database. Twenty-seven sets of data were summarized and screened for differential components at different pH conditions using the R language limma package, and the differential components were de-weighted and used to create heat maps, PCA maps, time variation maps, and box plots.

The total ion flow diagram of the GC-MS analysis of the oil of *Acorus calamus* is shown in Fig. 4A, and the summary information of the components is shown in Supplementary Table 1. Based on the volcano maps of each group 6, 1 and 6 difference components were obtained, respectively, as shown in Fig. 4B, and a total of 7 difference components were obtained after de-weighting. Based on the heat map information we can get two types of clustering information. The clusters can be divided into two groups according to the groups as (1) pH = 6-Pickering group, pH = 6-oil-water mixture group, pH = 6-crude oil group, pH = 7-Pickering group, pH = 7-oil-water mixture group, pH = 7-Pickering group, pH = 7-oil-water mixture group, pH = 7-crude oil group, and (2) pH = 7-crude oil group. The analysis by chemical composition can be classified as (1) bicyclo[2.2.1]heptane, 2,2,3-trimethyl(000473-19-8), citral(005392-40-5), and (2) 2,6-octadien-1-ol,3,7-dimethyl(000624-15-7), gamma-Elemene(029873-99-2), alpha-calacorene(021391-99-1), (2E)-3, 7-dimethyl-2,6 octadienal(000141-27-5), and alpha-Cubebene(017699-14-8), as in Fig. 4C. According to the PCA diagram, it can be seen that the crude oil group and Pickering emulsion group can be completely separated at pH = 6, 7 ,and 8, which indicates that there is a difference between these two groups not simply mixing. Also, the points in the Pickering emulsion group were more aggregated compared to the crude oil group, indicating that the crude oil group had better stability than the Pickering emulsion group, as shown in Fig. 5A–C. However, according to the PCA plots between the different groups, it can be seen that there is overlap between the crude oil group and the Pickering emulsion group, which is not consistent with the conclusions drawn between the different pH groups, considering that it may be because the mapping data account for a relatively low percentage of data that cannot reflect all the data, as in Fig. 5D. Comparing the line and box plots for each group, it was concluded that the trend of slowing down in the Pickering emulsion group was slowed down for all seven compounds compared to the crude oil group, as shown in Figs. 6 and 7.

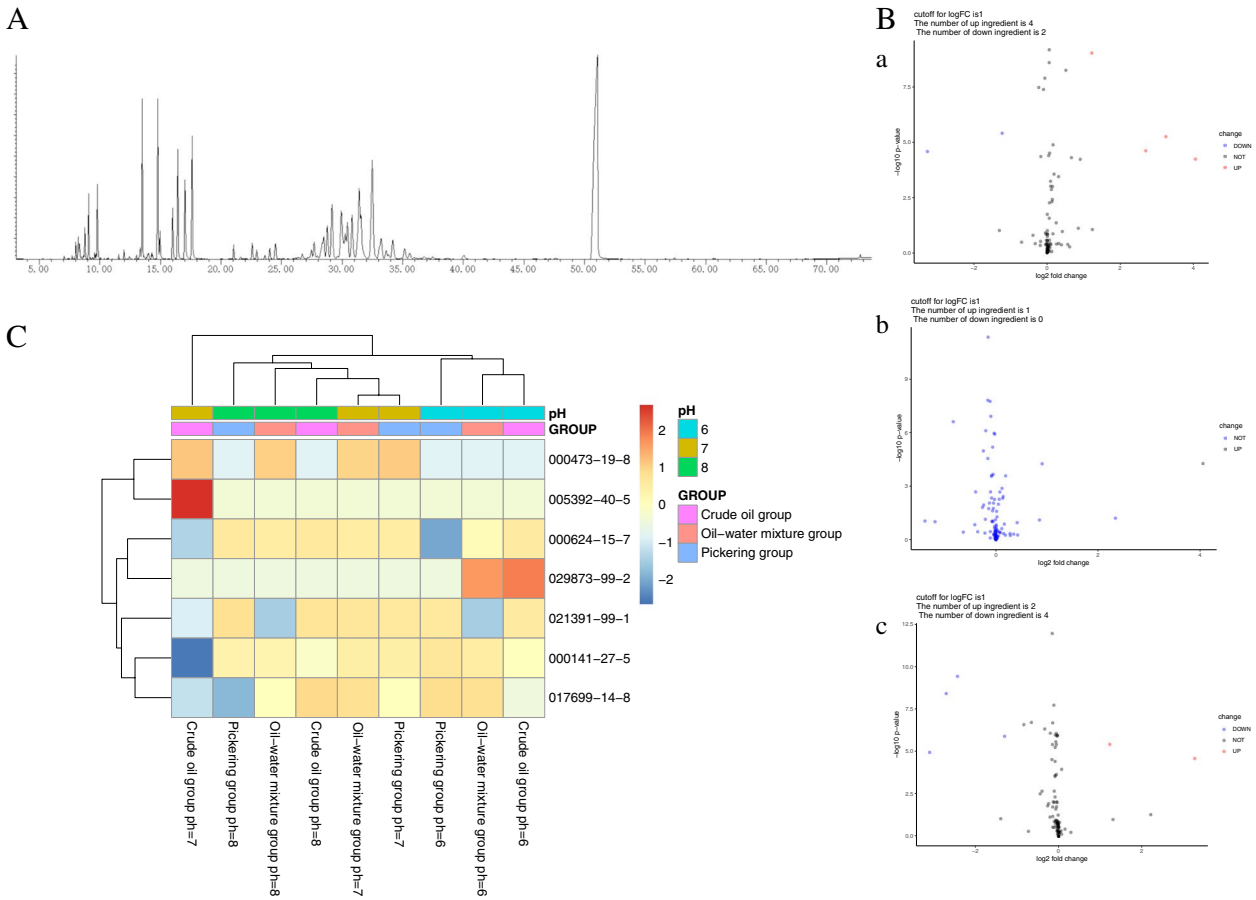

Fig. 4 PCA plot of differential components. **A** PCA plots of the differential components of each group at pH = 6. **B** PCA plots of the differential components of each group at pH = 7. **C** PCA plots of the differential components of each group at pH = 8. **D** PCA plots of the differential components of each group

Discussion and conclusions

Febrile convulsions are a common clinical emergency in pediatrics, as well as one of the most common emergency diseases and emergency hospitalizations in contemporary pediatrics (Xiao-Hui et al. 2009). It occurs mostly in infants and children from 6 months to 3 years of age, and its main clinical manifestations are high fever, convulsions, and delirium (Hui and Shou-Chuan 2021). It has a great impact on the quality of life of the affected children and their families (Ya-Jing 2018). Lingzhu Pulvis is commonly used as an adult formulation for the treatment of pediatric febrile convulsions, and improving the stability of its administration has been a high priority for this formulation (Xiao 2021). In this study, we introduced Pickering emulsion technology based on the idea of "combination of medicine and adjuvant" to solve the defect of unstable the oil of*Acorus calamus*in Lingzhu Pulvis and then improve the stability and efficacy of Lingzhu Pulvis in the future (Jia et al. 2020). The final decision to use pearl powder as a stabilizer for Pickering emulsion was made by comparing particle size, contact angle, and emulsion formation type. It is preferably prepared by a pearl powder concentration of 0.065 g·mL−1, oil phase/water phase 9:11 (V_1/V_2), and using a high-pressure homogenization method. The particle size of Pickering emulsions obtained in this study was micron-sized, and there was a large difference between the particle size of most of the reported nano-sized Pickering emulsions. The analysis of the reason for this may be due to the fact that the stabilizers used in conventional Pickering

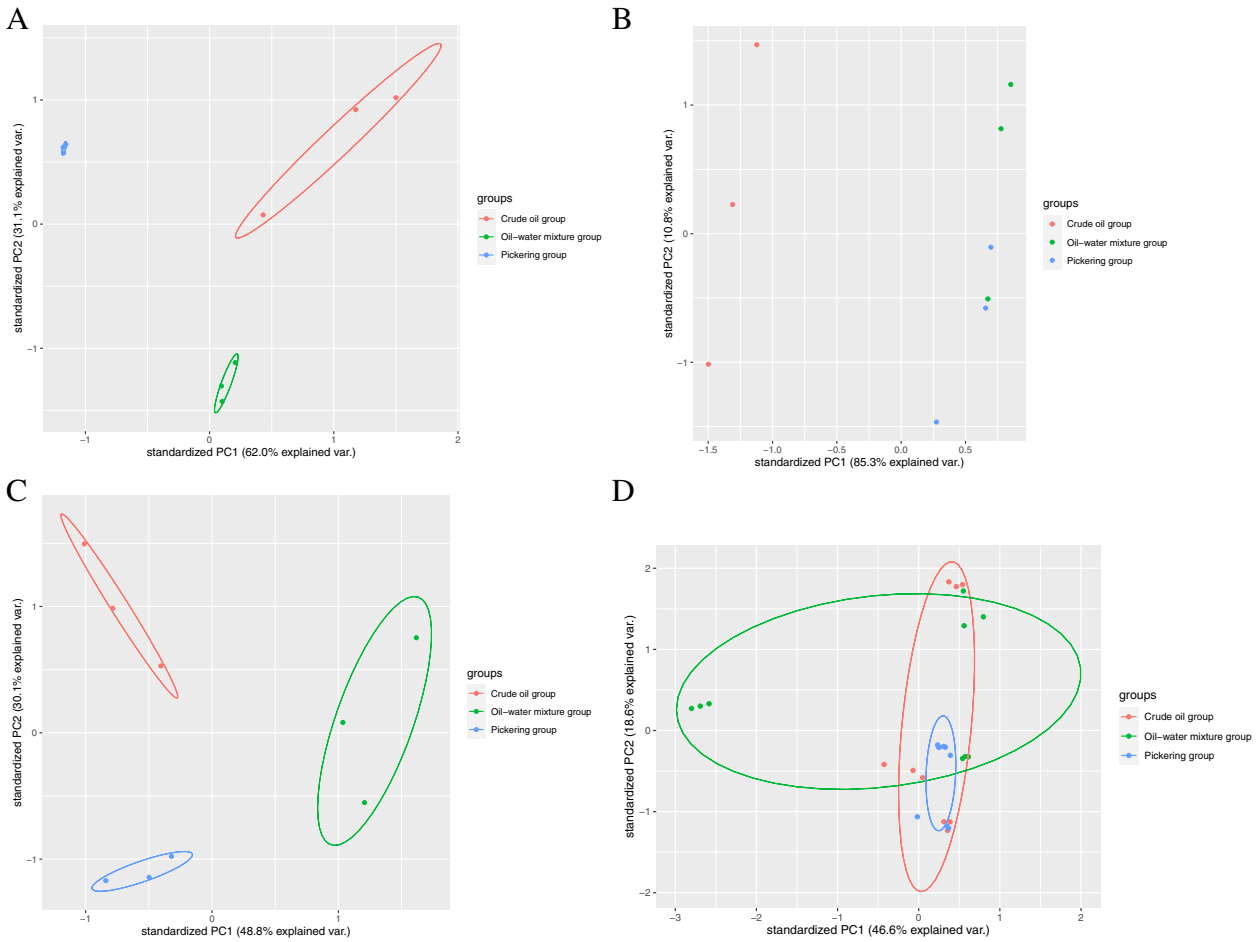

Fig. 5 The total ion current zmap, the volcano map for each pH segment, and the heat map of the different components of the volatile oil of *Acorus calamus*

emulsions are nano-sized monomeric compounds, while the stabilizers used in this study were a mixture of pearl powder with larger particle size. The pH stability study showed that the Pickering emulsion prepared in this study showed a significant reduction in both malondialdehyde and peroxide compared to the crude oil group, and the trend of the screened differential components was able to slow down, fully demonstrating the technical advantage of the Pickering emulsion to enhance the stability of the oil of *Acorus calamus* at a larger particle size. Since the amount of the oil of *Acorus calamus* in the prescription of Lingzhu Pulvis pearl powder is very small (1% V/m), the water introduced during the preparation of Pickering emulsion is also very small compared with the full prescription, which will not affect the quality of the powder. At the same time, Pickering emulsion is a small solid particle formed by spontaneous wrapping of pearl powder fine powder in the oil of Acorus calamus at the oil-water interface, in which calamus oil is stably wrapped, and after mixing with other powders, the water phase will be adsorbed, and the solid particles containing volatile oil will be dispersed in the whole formula in a state similar to that of solid dispersion, which is more stable than the simple mixing of traditional preparation methods. In the future, clinical use can better play its efficacy. The stability of the oil of *Acorus calamus* was improved after the introduction of Pickering emulsion technology, which shows that Pickering emulsion can be used to improve the stability of volatile components of oil-containing solid formulations under the concept of "combination of medicine and adjuvant" and provides a reference for other volatile oil-containing bulk formulations to improve their stability.

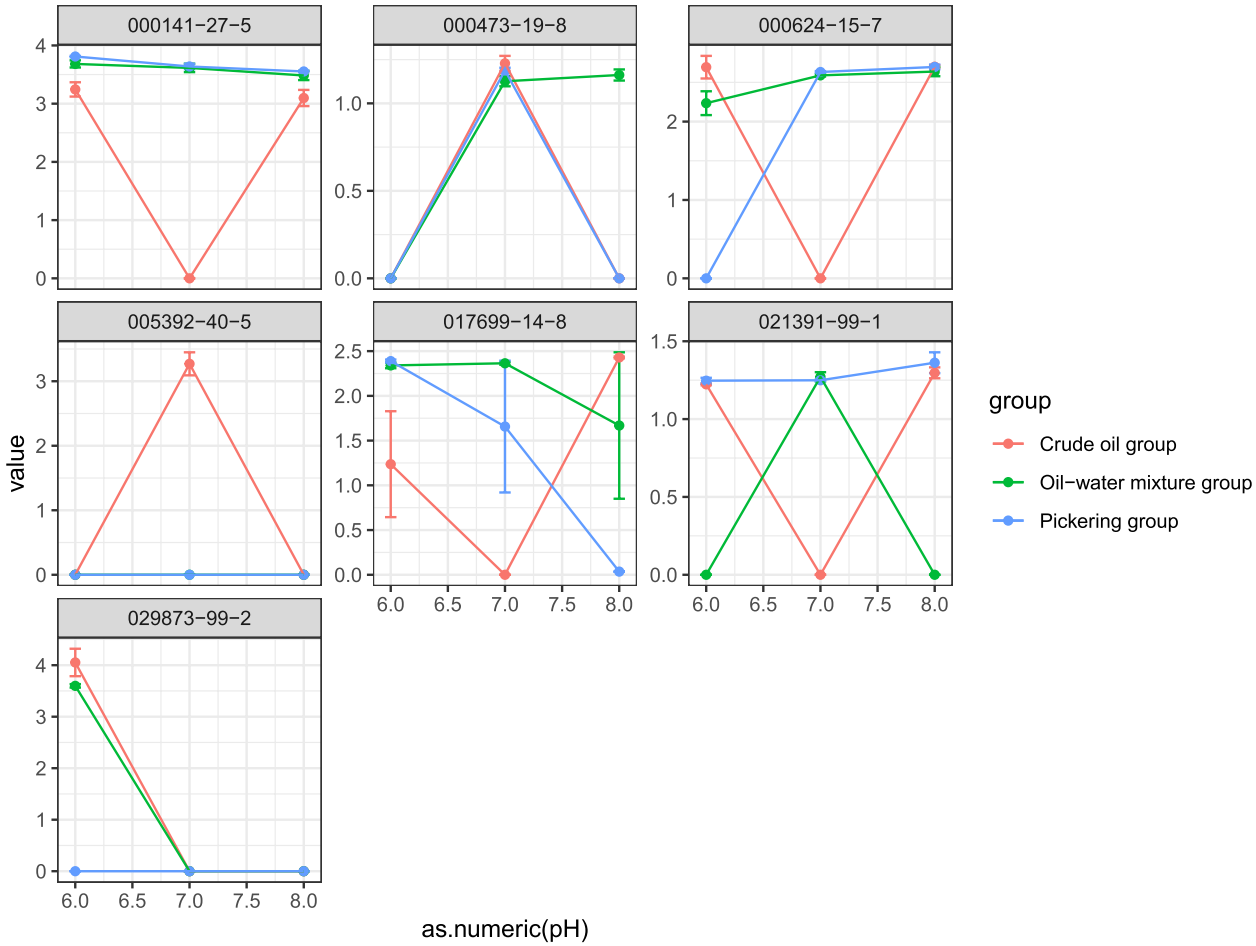

Fig. 6 Line graph of the difference components of each group

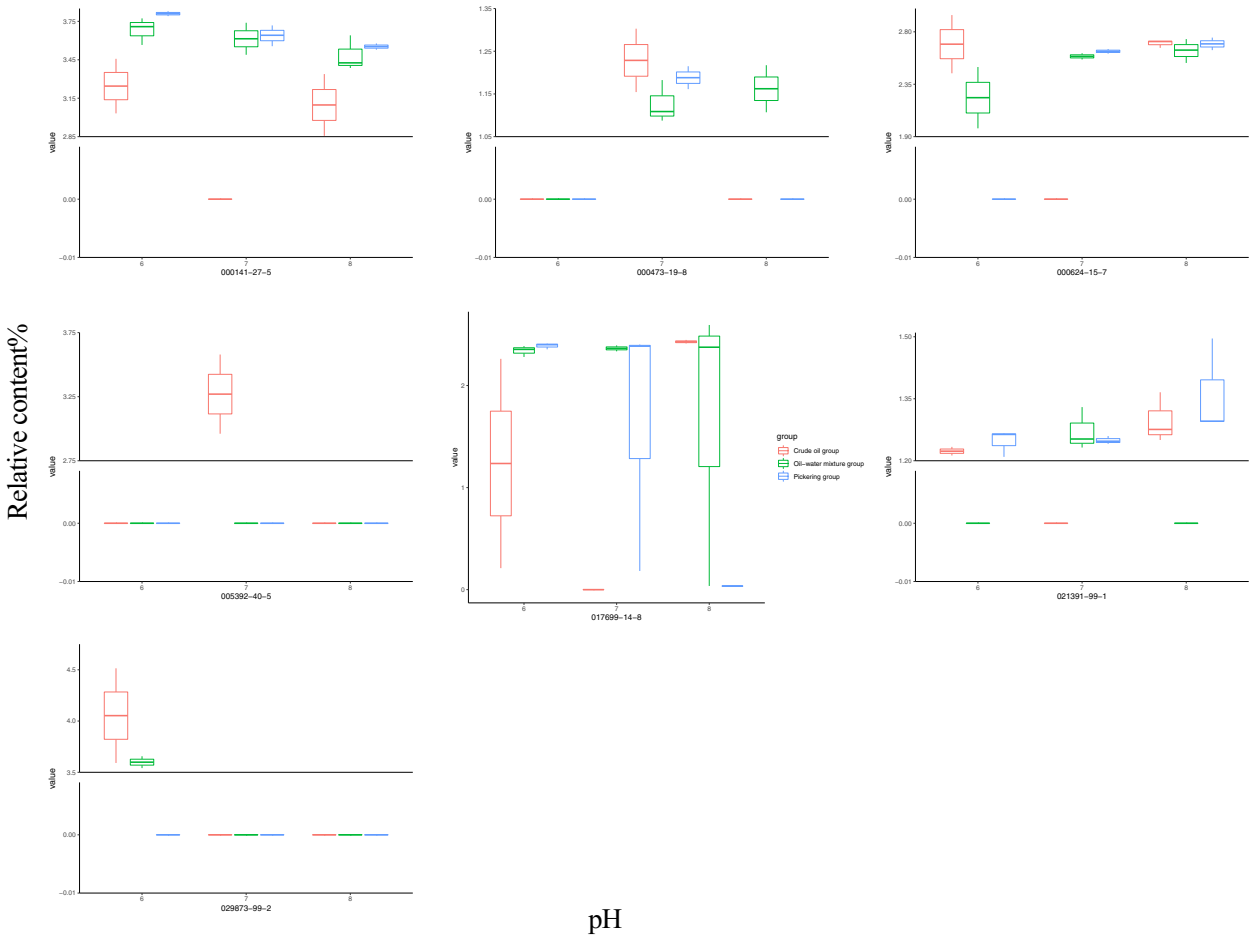

Fig. 7 Box plot of different components of each group

Supplementary Information

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Additional file 1.

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Authors' contributions
L. Peng and M. Wang wrote the main manuscript text. X. Zhang, D. Guo, and B. Zhai analyzed the data. J. Zou and Y. Shi designed the study, and all the authors amended the paper. The authors read and approved the final manuscript.

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Availability of data and materials
The data and materials have been shown in the diagrams and attachments. If you need other materials, please send an email to the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors of the article have agreed to publish this paper in your journal.

Competing interests
The authors declare that they have no competing interests.

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