Studies on Adsorption Kinetics and Thermodynamics of Macroporous Resin for Rosmarinic Acid
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Abstract: This experiment treated perilla seeds with different concentrations of NaCl solution to enrich and purify their rosmarinic acid (RosA). The results showed that low concentrations of salt (0-20 mmol/L) promoted seed germination, while high concentrations (> 20 mmol/L) inhibited germination. When the salt concentration was 20 mmol/L, the germination rate was the highest. The content of RosA in germinated perilla seeds was 3.5 mg/g, which was 3.5 times as much as that in the seeds without germination. The RosA was purified using NK-109 macroporous resin and its adsorption kinetics, isotherms and thermodynamics were determined. The adsorption kinetics showed that the adsorption behavior of RosA in NK-109 resin conformed to the pseudo-second-order kinetic model. The model for RosA in the NK-109 resin exhibited Langmuir adsorption based on a spontaneous exothermic process according to its adsorption thermodynamics, which included both physical and chemical adsorption. The optimized process conditions were as follows: the loading concentration of 0.04 mg/mL, loading volume of 40 mL, 70% methanol as the eluent with the volume of 60 mL, and the purity of RosA was 42.1%.

Key words: salt stress, macroporous resin, thermodynamics, kinetics, rosmarinic acid

1 Introduction
Rosmarinic acid (RosA) has a variety of physiological activities, such as antioxidant, antibacterial, anticancer, antitumor, antiviral, antidepressant and other functions. It can be used as food antioxidants, anti-aging cosmetics, natural food additives, health products and medicines. So RosA has a very important relationship with human diet and health. RosA is the most abundant phenolic acids in perilla, an important Asiatic crop, accounting for more than 80% of the total phenolic acids content, but its content is still relatively small compared with the other phenolic substances in perilla. There have been few studies on enriching RosA content but salt stress has been reported to enrich phenolics in soybean. Salt stress is an abiotic stress that affects seed germination. One study has reported that one type of water-soluble ammonium salt can promote the germination of corn and Arabidopsis seeds. However, salt stress can affect the osmotic pressure in cells and destroy their structure. Spraying salt solution at different concentrations enables the seeds to absorb the water from the solution and swell, which will rupture the seed coat and encourage germination to begin. However, if the salt concentration is too low, the moisture content in the external environment will be too high, so the seed cells will absorb too much water. This may cause the cells to rupture and die, thus affect seed germination. If the salt concentration is too high, the seed cells may undergo plasmolytic separation, so the germination rate will also be reduced. Therefore, an appropriate salt concentration is required to promote seed germination. Salt stress can also enhance the activity of amylase, lipase, protease and saccharifying enzymes in the cotyledons and endosperm where the nutrients and energy required for seed germination are located. Therefore, this will efficiently hydrolyze the starch, fat, protein and sugar in the seed, providing nutrients and energy for seed germination. The plants themselves also have a self-defense system, which can resist some of the harmful stimuli produced by high concentration salt, such as the regulation of photosynthesis and the synthesis of osmotic regulators. However, if the stimulation is too strong and exceeds the plant’s tolerance, it will irreversibly damage the plant, and can destroy the struc-
structure and function of the chloroplasts, and the structure of the proteins and nucleic acids. Low concentrations of salt can also increase the activity of some antioxidant enzymes, such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT), which remove the active oxygen accumulating under salt stress thus reducing damage to the seeds. High concentrations of salt may inhibit the activities of POD, SOD and CAT, thus allowing an excess of active oxygen to accumulate in the cell and exceed the plant’s ability to repair itself, thus affecting the germination of perilla seeds. Seed germination is a complex process, which includes respiration, photosynthesis, the conversion of organic matter and changes in enzyme activities, plant hormones and phytic acid. These processes may be closely related to enriching the content of phenolic substances in germinated seeds. This will promote cell respiration during germination, which will lead to the synthesis of certain substances. During seed germination, monosaccharides will also be converted to polysaccharides.

Some porous materials, such as activated carbon, zeolite, silica gel, macroporous resin, etc., play an important role in adsorbing substances. Activated carbon has a strong adsorption capacity due to its well-developed microporous structure, large specific surface area and surface chemical properties. Shi-wei, Z. et al. studied the adsorption performance of the activated carbon for phenol. The adsorption capacity was 606.88 mg/g under optimal conditions, so it could be used to treat phenol in sewage. As a porous material, zeolite molecular sieve has the characteristics of regular and uniform pore structure, high specific surface area and high hydrothermal stability. Liu, Y. et al. used ZSM-5 molecular sieve to adsorb berberine in Phellodendron amurense extract and found that the elution rate of berberine was 98.73%, and ZSM-5 had greater purification capacity and reusability. This research had laid a theoretical foundation for the application of zeolite molecular sieves in the field of enrichment and purification of natural product active ingredients. Silica gel has an open porous structure and a large specific surface area, and is mostly used for gas adsorption. Li, L.M. et al. found that aminobonded silica gel could effectively adsorb heavy metal Pb⁺⁺. Macroporous resin is a polymeric material with a porous skeleton structure comprising polymerized monomers and some crosslinking agents and porogenic agents. The adsorption capacity is generated by the force between the resin and the adsorbent, such as the vander Waals and hydrogen bonding forces. The porous structure of macroporous resins also plays a particular role: different macroporous resins have different pore sizes and specific surface areas, so can retain different molecules, which determine their adsorption selectivity. Macroporous resins are now a new form of purification technology, and widely used for the separation of natural products in the chemical, pharmaceutical and industrial water treatment industries. Yang et al. used AB-8 resin to separate and purify tea seed polysaccharide and tea seed saponin from camellia cake to obtain a product purity of 89.2% and 96.0%. Limwachira et al. used S8 polar resin to recover phenolic compounds, flavonoids, and proanthocyanidins from lotus seed pods with purities of 85.69%, 95.57%, and 89.85%, respectively. Macroporous resins have the advantages of simple operation, low cost, selective adsorption, easy elution and high reusability. They can also provide better purification than some traditional purification technologies, such as activated carbon, membrane filtration, gel resin and ion exchange resin. Methods for studying the purification of RosA include high-speed counter-current chromatography, normal-phase silica gel column chromatography and macroporous resins, the latter being effective and thus widely used. A suitable macroporous resin must be chosen based on the molecular size and structural characteristics of RosA. Lin et al. compared the purification of RosA using HP-20 and XAD-7HP resins, and found that both could purify RosA with similar adsorption and desorption behaviors. Xiong et al. used LX-60 to obtain RosA from lavender with a purity of 15.6%. However, the purification of RosA reported in the literature is relatively low.

The aim of this study was to increase the content of RosA in Perilla seeds under salt stress, and then to purify RosA with NK-109 resin. The adsorption mechanism was studied systematically, which provided a new method for the enrichment and purification of RosA, and laid a theoretical foundation for industrial production of RosA.

2 Materials and Methods

2.1 Materials and instruments

The perilla seed was provided by Baicaosheng Biotechnology Co., Ltd. (Shanxi, China). The RosA standard (purity >99.46%) was purchased from Ronghe Pharmaceutical Technology Development Co., Ltd. (Shanghai, China), the NK-109, XDA-8, AB-8, D101, HPD450, HPD750, HPD950, NKA-9 and X-5 macroporous resins from Xilebo Biochemical Technology Co., Ltd. (Shanxi, China) and the resin column from Xiamei Biochemical Technology Development Co., Ltd. (Shanghai, China).

2.2 Determining the effect of salinity on germination

This experiment was carried out as described by Ghaderi et al., with modifications. Perilla seeds with full grains were selected, soaked in tap water for 24 h, then sprayed with NaCl solutions of different concentrations (0, 10, 20, 30, 40 and 50 mmol/L) and germinated at 25-30°C. The seeds were sprayed with the saline solution every 1 h to maintain a sufficient degree of wetness. The experiments were repeated three times for each treatment to reduce errors. After germination was completed, 40 germinated
perilla seeds were randomly selected then their bud length, fresh weight and dry weight were measured. The germination rate (GP) and germination potential (GE) in the first 4 days were calculated as follows:

\[
GP = \frac{n}{N} \times 100\% \quad (1)
\]

\[
GE = \frac{n_4}{N} \times 100\% \quad (2)
\]

where \(n\) is the number of all normally germinated seeds at the end of germination, \(n_4\) the number of germinated seeds during the first 4 days, and \(N\) the total number of seeds tested.

2.3 Sample preparation
The germinated perilla seeds were dried and crushed, then passed through a 20-mesh sieve and stored in a 4°C refrigerator for later use.

2.4 RosA extraction and detection
The extraction conditions of the crude extract of RosA were as follows: liquid ratio of 1:60 (w/v), using an extraction solvent of 40% ethanol solution, a water bath temperature of 55°C, and a heating time of 35 min. The chromatographic conditions for the HPLC were: an TC-C18 column (Agilent Technologies Inc., Santa Clara, CA, USA: 4.6 mm \(\times 250\) mm, 5 \(\mu\)m); detection wavelength, 330 nm; column temperature, 30°C; mobile phase acetonitrile: 0.1% phosphoric acid (30:70); and flow rate 1.0 mL/min.

2.5 Total flavonoid extraction
The sample (1 g) and 50 mL 60% ethanol were placed in a centrifuge tube, ultrasonic extraction at 65°C for 40 min, extraction of 2 times, centrifuged in a 3622 g centrifuge for 20 min, took the supernatant and volumed it with 60 mL of absolute ethanol to 100 mL for later use. The total flavonoid content of the crude extract was determined by the \(\text{NaNO}_2\)-Al (\(\text{NO}_3\)_3-NaOH colorimetric method\(^{29}\). The standard curve was established as:

\[
y = 16.07714x - 0.0102 (R^2 = 0.99964) \quad (3)
\]

where \(y\) is total flavonoid content (mg rutin equivalent (RUT)/g sample) and \(x\) is absorbance value at 510 nm.

2.6 Total phenolics extraction
The extraction method of total phenolics was the same as total flavonoid. Took 0.2 mL extract to determine the total phenol content. The total phenolics content of the extract was determined using the Folin–Ciocalteu method\(^{25}\). The standard curve was established as:

\[
y = 124.9x + 0.0235 (R^2 = 0.99923) \quad (4)
\]

where \(y\) = total phenolics content (mg gallic acid equivalent (GAE)/g sample) and \(x\) = absorbance value at 765 nm.

2.7 Polysaccharides extraction
The sample (0.5 g) and 25 mL of distilled water were placed in a erlenmeyer flask, extracted for 30 min in a water bath at 80°C, extraction of 2 times. After centrifugation in a 3622 g centrifuge for 15 min, the supernatant was concentrated to 25 mL. After adding 50 mL of absolute ethanol, the sample was placed in a refrigerator at 4°C for 12 h, and then centrifuged again. After centrifugation, the precipitate was dissolved in 25 mL of distilled water for later use. 1 mL of the remaining liquid, 1 mL of 5% phenol solution, 5 mL of concentrated sulfuric acid were mixed by shaking and then left to stand for 20 min at room temperature. The absorbance was then measured at 485 nm. The glucose standard curve was obtained according to the Chinese National Standard GB/T9695.31-2008 glucose standard curve), was:

\[
y = 0.00729x + 0.00548 (R^2 = 0.99964) \quad (5)
\]

where \(y\) is glucose content (mg/g sample) and \(x\) is absorbance value at 485 nm.

2.8 Pretreatment and packing of macroporous resins
Nine resins (NK-109, XDA-8, AB-8, D101, HPD450, HPD750, HPD950, NKA-9 and X-5) were pretreated according to the method described by Zhou et al. with modifications\(^{26}\). To remove the monomers and porogenic agents trapped inside the pores during their synthesis, all resins were soaked in 95% ethanol for 24 h then washed with distilled water until the eluent was clear. The resins were then soaked in 5% HCl for 2 h, and washed with distilled water until the pH of the filtrate was 7.0, followed by soaking in 4% NaOH for 2 h, and finally washed with distilled water until the pH of the filtrate was 7.0. The column was packed using the wet method, where 50 mL of the resin treated with water was slowly loaded into the 1.5 cm \(\times 42\) cm resin column along the column wall to avoid air bubbles.

2.9 Selection of macroporous resin
Each of the pretreated resins (5.00 g) was put into a 100 mL conical flask with a stopper. 30 mL of RosA extract at a concentration of 0.01 mg/mL were added, then the flasks were placed in a constant temperature incubator at 30°C for 24 h. After adsorption equilibrium was reached, the supernatants were discarded, then the adsorbate-laden resins were washed twice with distilled water. After loading the resins into a small purification column, 30 mL of 50% methanol solution were added for desorption. The adsorption/desorption ratios and capacities were calculated using the following equations\(^{27}\).

Adsorption capacity:

\[
A \left( \frac{mg}{g} \right) = \frac{(c_0 - c_1) \times v_1}{m} \quad (6)
\]

Adsorption ratio:
\[ B(\%) = \frac{c_0 - c_1}{c_0} \]  \hspace{1cm} (7)

Desorption capacity:
\[ C(\frac{mg}{g}) = \frac{c_2v_3}{m} \]  \hspace{1cm} (8)

Desorption ratio:
\[ D(\%) = \frac{c_2v_3}{(c_0 - c_1)v_1} \]  \hspace{1cm} (9)

Here, \( c_0, c_1, \) and \( c_2 \) are the RosA concentrations (mg/mL) initially, after reaching adsorption equilibrium, and in the eluent, respectively; \( V_1 \) and \( V_2 \) are the volumes of the loading solution and the eluent, respectively; and \( m \) (g) is the mass of the resin.

2.10 Adsorption kinetics study

The adsorption kinetics were studied as described by Limwachiranon et al. with slight modifications. Pretreated NK-109 resin (5.0 g) was mixed with 20 mL of RosA extract solution (0.025 mg/mL) in a flask then placed in incubators at temperatures of 30, 35 or 40 °C for 24 h. The extract solution was sampled once every 2 h to determine the concentration of RosA. The kinetic curve for RosA on NK-109 was plotted with time as the abscissa and the concentration of RosA. The kinetic curve for RosA on NK-109 was plotted with time as the abscissa and the concentration of RosA.

The Langmuir model can be expressed by the following mathematical formula:
\[ q_e = \frac{q_m k_L c_0}{1 + k_L c_0} \]  \hspace{1cm} (14)

A linearized formula of Freundlich can be expressed as:
\[ \ln q_e = \ln k_F + \frac{1}{n} \ln c_0 \]  \hspace{1cm} (15)

The equilibrium constant \( R_L \) is given by:
\[ R_L = \frac{1}{1 + k_L c_0} \]  \hspace{1cm} (16)

The Freundlich model can be expressed by the following equation:
\[ q_e = k_F c_0^{1/n} \]  \hspace{1cm} (17)

The Gibbs free energy change can be calculated as follows:
\[ \Delta G = \Delta H - T \Delta S \]  \hspace{1cm} (20)

Here, \( q_e \) (mg/g) is the adsorption capacity at equilibrium; \( c_0 \) (mg/mL) is the concentration of solute in solution at equilibrium; \( q_m \) (mg/g) is the maximum adsorption capacity; \( c_0 \) is the highest initial concentration; \( k_F \) is the association Langmuir constant; \( 0 < R_L < 1 \) is considered optimal; \( k_F \) is the Freundlich constant that measures the adsorption capacity; \( n \) is related to the sorption driving force and the energy distribution of the sorption sites, with the value of \( 1/n \) indicating the type of isotherm (1/n > 1 indicates difficult adsorption); \( \Delta H \) (kJ/mol) is the enthalpy change; \( \Delta S \) (J/mol·K) is the entropy change; \( \Delta G \) (kJ/mol·K) is the change in Gibbs free energy; \( R \) (8.314 J/mol·K) is the ideal gas constant; \( T \) (K) is the temperature; and \( k_F \) is the equilibrium distribution coefficient.

2.11 Adsorption isotherms study

The adsorption isotherms of the selected resin were analyzed at five temperatures (20, 25, 30, 35 and 40 °C). Pretreated NK-109 resin (5.0 g) was mixed with 30 mL of RosA extract solution at varying concentrations (0.01, 0.015, 0.02, 0.025 and 0.03 mg/mL). The Langmuir and Freundlich equations were evaluated to analyze their degrees of fitness: the Langmuir isotherm model describes monolayer adsorption onto a homogeneous surface, with no interactions between adjacent adsorbed molecules and the Freundlich isotherm model is suitable for describing multilayer adsorption. The Clausius-Clapeyron equation reflects the relationship between the thermodynamic parameters, \( \Delta H \), \( \Delta S \), and \( \Delta G \), during the adsorption process to study the mechanism of adsorption.

The Langmuir model can be expressed by the following mathematical formula:
\[ q_e = \frac{q_m k_L c_0}{1 + k_L c_0} \]  \hspace{1cm} (14)

Equation (14) can be linearized as follows:
\[ \frac{c_e}{q_e} = \frac{c_0}{q_m} + \frac{1}{k_L q_m} \]  \hspace{1cm} (15)

The equilibrium parameter \( R_L \) is given by:
\[ R_L = \frac{1}{1 + k_L c_0} \]  \hspace{1cm} (16)

The Freundlich model can be expressed by the following equation:
\[ q_e = k_F c_0^{1/n} \]  \hspace{1cm} (17)

2.12 Resin purification process

2.12.1 Effect of loading volume on RosA recovery

Enrichment experiments were carried out in glass columns (1.5 cm × 42 cm) packed with NK-109. Different volumes of extract solution (20, 30, 40, 50 and 60 mL) at a...
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concentration of 0.04 mg/mL were applied to the column, eluted with 2 BV (bed volume) 50% methanol, then the recovery rate of RosA in the eluent was measured.

The recovery rate was calculated as follows:

\[ E(\%) = \frac{m_1}{m_2} \]  

where \( m_1 \) and \( m_2 \) are the amounts of RosA in the extract and the eluent, respectively.

2.12.2 Selection of eluting solvent

Forty mL of RosA extract solution (0.04 mg/mL) were applied to the column, then desorbed with 2 BV methanol, ethanol, acetone, and ethyl acetate, then the desorption capacity and desorption rate of RosA were calculated.

2.12.3 Effect of eluent concentration on RosA recovery

The adsorption experiment was the same as in 2.12.2, but eluted with different concentrations of methanol (40%, 50%, 60%, 70%, 80%), then the recovery of RosA was calculated.

2.12.4 Effect of eluent volume on RosA recovery

The adsorption experiment was the same as in 2.12.2. The eluent was 70% methanol, and the eluent collected in ten 10-mL tubes in sequence, the content of RosA were measured.

2.13 Statistical analysis

All experiments were performed in triplicate, and the experimental results obtained were expressed as means ± S.D. Statistical analysis was performed using Excel (Microsoft Corp., Redmond, WA, USA). All plots and fitted curves in the experiment were made using Origin 9.1 (OriginLab Corp., Northampton, MA, USA).

3 Results

3.1 Effect of salinity on germination

At low salt concentrations (0-20 mmol/L), the germination rate and germination potential gradually increased with increasing salt concentration, reaching a maximum at 20 mmol/L (Fig. 1A). As the salt concentration increased further, the germination rate and germination potential decreased. Figure 1B shows that the fresh weight and dry weight of the buds on the germinated seeds also increased at first then decreased with increasing salt concentration, reaching maximum values at a salt concentration of 20 mmol/L. In Fig. 1C, the length of the buds at salt concentrations of 10 mmol/L were longest. Therefore, a low concentration of salt enhanced the germination of perilla seeds, and a high concentration salt inhibited it. Overall, 20 mmol/L was the optimum concentration for perilla seed germination. Seed germination was closely related to the osmotic pressure of the solution. When the salt concentration was 0-20 mmol/L, the osmotic pressure of the solution was low, the seed coat absorbed water and expanded, fresh weight increased, and the germination was accelerated. When the salt concentration was 20-50 mmol/L, the osmotic pressure of the solution was high and the seed coat lost water, fresh weight decreased, which hindered the germination of seeds. During seed germination, the increase of organic matter was greater than the decrease of organic matter, and the dry weight of seeds increased.

3.2 Effects of salt stress on the active components in perilla seeds

The contents of the active components of perilla seed after germination were shown in Fig. 2A. The contents of

![Fig. 1](image_url)

Fig. 1 Effects of salt stress on the germination rate and germination potential (a), fresh weight and dry weight (b), and bud length of the seeds (c) of Perilla seeds.
RosA and polysaccharides first increased and then decreased with the increase of salt concentration. The salt concentration was 0-10 mmol/L, and the contents of both increased significantly. When the concentration was 20 mmol/L, the contents of RosA and polysaccharides reached the maximum. As the salt concentration continued to increase, the content of both of them presented a large decline trend. Flavonoids and polyphenols had the same trend, the salt concentration was 0-10 mmol/L, the concentration of both was increasing, but the increase was inapparent, the concentration of both reached the maximum at 20 mmol/L, and then decreased slowly with the increase of salt concentration. This might be that low concentration of salt promoted the expression of organic matter-related synthase genes, and high concentration of salt inhibited the expression of organic matter-related synthase genes.

Figure 2B shows the changes of the content of RosA in Perilla seeds at different germination stages on the concentration of 20 mmol/L with 0 d as the control group (the non-germination group). The figure also shows that the content of RosA increased slightly at the initial stage of germination. The content of RosA in Perilla seeds increased significantly after 3 days of germination, and reached the maximum value of 3.5 mg/g on the sixth day when the seeds had fully germinated. Therefore, germinating perilla seed at a salt concentration of 20 mmol/L can increase the content of RosA, and also enrich other active ingredients.

### 3.3 Selection of macroporous resin

Macroporous resins have different adsorption effects on different substances due to their unique polar size, pore size, and specific surface area. The resin with different pore size can prevent the free entry of larger molecules and make the resin have selective adsorption. The physical properties of the different resins were shown in Table 1. In the static adsorption tests of RosA, the adsorptive properties of nine different resins were studied at 30°C (Fig. 3A). RosA is a polar molecule with a molecular weight of 360, which is easily adsorbed by polar resins. NK-109, NKA-9 and XDA-8 are polar resins, so the adsorption capacity and adsorption rate of RosA were high. The adsorption capacity and adsorption rate of NK-109 were 0.041 mg/g and 68.3%, which were higher than that of NKA-9 (0.025 mg/g, 41.7%) and XDA-8 (0.037 mg/g, 61.7%). This might be because the specific surface area of NK-109 is higher than that of NKA-9 and XDA-8. Therefore, NK-109 had the strongest adsorption capacity for RosA. HPD450, HPD750 and AB-8 are medium-polar resins, so the adsorption capacity and adsorption rate of RosA were small. D101 is a weakly polar resin, but due to its large pore size, the adsorption capacity and rate

### Table 1  Properties of nine macroporous resins.

| Resins  | Polarity | Specific surface area (m²/g) | Pore size (nm) |
|---------|----------|-----------------------------|---------------|
| NK-109  | +++      | 900-1200                     | 3-4           |
| XDA-8   | +++      | 480-550                      | 9.5-10        |
| AB-8    | ++       | 480-520                      | 130-140       |
| D101    | +        | 500-550                      | 90-100        |
| HPD450  | ++       | 500-550                      | 9-11          |
| HPD750  | ++       | 650-700                      | 8.5-9         |
| HPD950  | -        | 1200-1500                    | 85-105        |
| X-5     | -        | 500-600                      | 29-30         |
| NKA-9   | +++      | 250-290                      | 15.5-16.5     |

Note: "+++" means Strong Polarity, "++" means Medium Polarity, "+" means Weak Polarity, "-" means Non-polar.
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of RosA were slightly stronger than that of HPD450, HPD750 and AB-8. HPD950 and X-5 are non-polar resins, so they did not adsorb RosA easily (Fig. 3A).

The purification performance of a resin is not only related to its adsorption capacity, but also to its desorption capacity. The level of RosA in the desorption solution reflects the desorption capacity of the resin. In the desorption figure, the desorption capacity and desorption rate of NK-109 were the highest, 0.03 mg/g and 73.2%, respectively. The desorption capacity of NKA-9 and XDA-8 were not significantly different. However, as the adsorption capacity of XDA-8 was higher than that of NKA-9, the desorption rate was lower than that of NKA-9. The desorption rates of HPD750, AB-8 and HPD450 were 44.8%, 31.6% and 25%, respectively. The desorption rates of HPD950 and D101 were lower (Fig. 3B). From these results on the static adsorption and desorption of the resins, NK-109 resin was finally selected for purifying RosA from germinated perilla seeds.

3.4 Adsorption kinetics study

The adsorption kinetics of RosA on the NK-109 resin at three different temperatures were shown in Fig. 4. The adsorption capacity of RosA increased with time in the range of 2-16 h. The adsorption capacity of RosA was the largest at 30°C, followed by 40°C and the minimum at 35°C. In the range of 16-24 h, the adsorption capacity of RosA increased slowly and reached equilibrium gradually. Overall, the adsorption capacity of RosA was the highest at 40°C, followed by 30°C and the lowest at 35°C. The reason might be that there was both physical adsorption and chemical adsorption in the entire adsorption process (Table 4). At 30°C-35°C, the entire adsorption process was dominated by chemical adsorption, and the amount of adsorption was less affected by temperature. Therefore, the rise of temperature would not cause the increase of adsorption capacity. At 35°C-40°C, the physical adsorption gradually took the dominant position with the increase of temperature, and adsorption capacity had a greater influence on the temperature. So, with the increase of temperature, the adsorption capacity was also increasing, and the adsorption capacity reaches the maximum at 40°C.

The equations for the pseudo-first-order, pseudo-second-order and particle diffusion kinetics models and associated kinetic parameters are shown in Table 2. The R² values from the pseudo-second-order kinetic model (0.98378, 0.95915, 0.98392) at the three temperatures of 30, 35 and 40°C, respectively, which were higher than those from the pseudo-first-order model (0.91613, 0.90042, 0.92612) and the particle diffusion kinetics model (0.95416, 0.93581, 0.97675). The kinetic parameters from the pseudo-second-order model for RosA (q_e = 0.097, 0.106 and 0.11 mg/g, at 30, 35 and 40°C, respectively) were also close to the experimental data (q_e = 0.087, 0.081 and 0.088 mg/g, respectively). Therefore, in comparison with the pseudo-first-order dynamic and particle diffusion kinetics models, the pseudo-second-order dynamic model was more suitable for describing the adsorption of RosA on the NK-109 resin. Although the particle diffusion kinetics model could not represent the whole adsorption process, its coefficient of determination (R²) was still high. Table 2 shows that the curves from the fitting equations at the three temperatures did not pass through the origin, which indicates that the adsorption process was a combination of internal and external diffusion.
3.5 Adsorption thermodynamic studies

The adsorption isotherms of RosA on the NK-109 resin and their isothermal parameters at 20, 25, 30, 35, and 40°C were shown in Fig. 5 and Table 3. The equilibrium adsorption at 25°C was the highest, followed by those at 20, 30, and 35°C, with that at 40°C being the lowest. The reason may be that at 20°C-25°C, the temperature of the entire system was relatively low, the rise of temperature accelerated the absorption rate, so the equilibrium adsorption capacity also increased, and the equilibrium adsorption capacity reached the maximum at 25°C. However, as the temperatures continued to rise, due to the adsorption process was exothermic (Table 4), the rise of temperature was not conducive to the adsorption, so the equilibrium adsorption capacity decreased continuously at 25°C-40°C. To determine the optimum model, the experimental equilibrium data were fitted to two isotherm models, the Langmuir and the Freundlich. The Langmuir adsorption model showed that as the temperature increased, the \( k_L \) value decreased (Table 3), indicating that increasing the temperature did not enhance the adsorption of RosA. The value of \( R_L \) between 0 and 1 meant that RosA had good adsorption on the NK-109 resin. The maximum adsorption capacity was influenced by the temperature. For the Freundlich model, the value of \( 1/n \) was between 0.1 and 0.5, which indicated that the adsorption was easy. Thus, it was easier to adsorb RosA at 20, 25 and 30°C than at 35 and 40°C, which was consistent with the adsorption isotherms in Fig. 5.

Overall, the Langmuir model was considered as a better model for describing the adsorption of RosA on NK-109 resin on the basis of comparing correlation coefficients. Table 4 shows the thermodynamic parameters of NK-109 resin adsorption, where \( \Delta H \) was obtained by fitting the linear equation of \( \ln k_c \) and \( 1/T \) in the Clausius-Clapeyron equation. The slope and intercept of the fitted equation are the values for \( -\Delta H/R \) and \( \Delta S/R \), respectively. The value of \( \Delta G \) was then calculated according to equation (20). As the
### Table 3  Parameters of the Langmuir and Freundlich adsorption models for RosA.

| T(°C) | Langmuir Adsorption | Freundlich Adsorption |
|-------|----------------------|-----------------------|
|       | equation             | R²                    | qₘ (mg/g) | Kₘ (mg/mL) | Rₑ | equation             | R² | 1/n | K_f (mg/g) (mL/mg)¹/a |
| 20    | \( \frac{c_e}{q_e} = 17.19c_e + 0.03 \) | 0.99195 | 0.06 | 555.6 | 0.06 | ln \( q_e = 0.15 ln c_e - 2.36 \) | 0.95459 | 0.15 | 0.094 |
| 25    | \( \frac{c_e}{q_e} = 12.62c_e + 0.055 \) | 0.99049 | 0.08 | 227.3 | 0.13 | ln \( q_e = 0.37 ln c_e - 1.17 \) | 0.97376 | 0.37 | 0.31 |
| 30    | \( \frac{c_e}{q_e} = 16.18c_e + 0.12 \) | 0.96249 | 0.062 | 134.4 | 0.2 | ln \( q_e = 0.41 ln c_e - 1.47 \) | 0.90152 | 0.41 | 0.23 |
| 35    | \( \frac{c_e}{q_e} = 14.3c_e + 0.21 \) | 0.992 | 0.07 | 68 | 0.3 | ln \( q_e = 0.55 ln c_e - 1.05 \) | 0.98464 | 0.55 | 0.35 |
| 40    | \( \frac{c_e}{q_e} = 12.5c_e + 0.3 \) | 0.99718 | 0.08 | 41.7 | 0.4 | ln \( q_e = 0.71 ln c_e - 0.57 \) | 0.99537 | 0.71 | 0.57 |

### Table 4  Thermodynamic parameters of adsorption of RosA using NK-109 resin.

| T(°C) | Clausius–Clapeyron equation | ΔH (kJ/mol) | ΔS (kJ/mol·K) | ΔG (kJ/mol) |
|-------|-------------------------------|-------------|---------------|-------------|
|       | equation                      | R²          | -15.35        | -13.38      | -12.34      | -10.75      | -9.63       |
| 20    | \( \ln k_e = 11592 \frac{1}{T} - 33.4 \) | 0.97705     | -96.4         | -0.28       | -9.63       |
Langmuir adsorption model was more suitable for fitting RosA adsorption on the NK-109 resin, the $k_c$ value here was taken as the $k_L$ value. $\Delta H$ was a negative value, indicating that resin adsorption was an exothermic process so the temperature rise was unfavorable to resin adsorption, which was consistent with the results shown by the adsorption isotherms. The absolute value of $\Delta H$ was greater than 43 KJ/mol, therefore, the absorption of RosA on NK-109 resin was attributed to a physico-chemical adsorption process rather than purely physical or chemical processes. The negative value of $\Delta G$ indicated that the process was spontaneous. The negative value of $\Delta S$ seemed to violate the principle of entropy increase, but this principle is only applicable in a thermodynamically isolated system, so this also indicated that heat was exchanged between the adsorption process and the external environment. Therefore, the adsorption of RosA on NK-109 resin was a spontaneous exothermic process, with both physical and chemical adsorption.

### 3.6 Resin purification process

Figure 6A shown that the recovery of RosA was highest, 49.1%, when the loading volume was 40 mL. As the volume of loading solution increased, the content of RosA also increased, so more RosA was adsorbed by the resin. When resin adsorption reaches saturation, the RosA in the loading solution will leak out, resulting in reduced RosA recovery. Figure 6B shows that when the methanol concentration was 70%, the recovery rate reached a maximum of 72.5%. When eluted with 80% methanol, the recovery rate of RosA decreased slightly, possibly because the methanol concentration was too high and other substances were eluted, which affected the desorption rate of RosA. Therefore, the optimal eluent concentration was 70%. Figure 6C shows that RosA was detected in the first six tubes, but not in the seventh to tenth tubes, which indicated that RosA had been completely eluted, so the optimal volume of the eluate was 60 mL.

The liquid chromatogram of the purified RosA was shown in Fig. 7. Before purification, there were more miscellaneous peaks in the chromatogram, and the contents were relatively high. After purification, the chromatogram had fewer miscellaneous peaks and lower contents. The results showed that the NK-109 resin had better purification effect. The eluent was freeze-dried to obtain RosA.
with a purity of 42.1%, which was 41.75% higher than the value before purification of 0.35%.

4 Conclusions
This paper reported the experimental study on the enrichment and purification of RosA from perilla seeds by salt stress and macroporous resin. The perilla seeds exhibited the highest germination rate at salt concentrations of 20 mmol/L, with a RosA content of 3.5 mg/g, 3.5 times higher than that in the ungerminated seeds (1.0 mg/g). The adsorption kinetics, isotherm and thermodynamic data show that the adsorption of RosA on the macroporous resin NK-109 was a spontaneous exothermic process, including physical adsorption and chemical adsorption. Using the best purification process, the purity of RosA could be increased from 0.35% to 42.1%. This laid the foundation for the industrial production of RosA.

Macroporous resin adsorption technology is mainly used in wastewater treatment, pharmaceutical industry, chemical industry, analytical chemistry, clinical verification and treatment and other fields. In recent years, it has been widely used in the extraction, separation and purification of active ingredients of Chinese herbal medicines. With the deepening of research, highly selective resins will be developed, or combined with other purification technologies, to further improve the efficiency of extraction, separation and enrichment of the effective components of active ingredients. In addition, macroporous resin also has broad application prospects in wastewater treatment. It can purify high-concentration, difficult-to-degrade organic industrial wastewater, and can be used to make industrial wastewater purifiers in the future.

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Author Contributions
ZHANG Jin-hua designed the experimental scheme and ideas; ZHANG Min finished the experiment and the first draft of the article; BAI Bao-qing and JIA Huai-wang revised and improved the article; FAN san-hong provided all the reagents and equipment needed for the experiment.

Conflicts of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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