Collagen and Leather

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Synergistically enhancing hydrogen bonding, hydrophobic interaction and electrostatic association of collagen fiber to flavonoid aglycones for their effective separation by polyethyleneimine modification

Qixian Zhang[1,2], Rui Wang[2] and Bi Shi[1,2*]

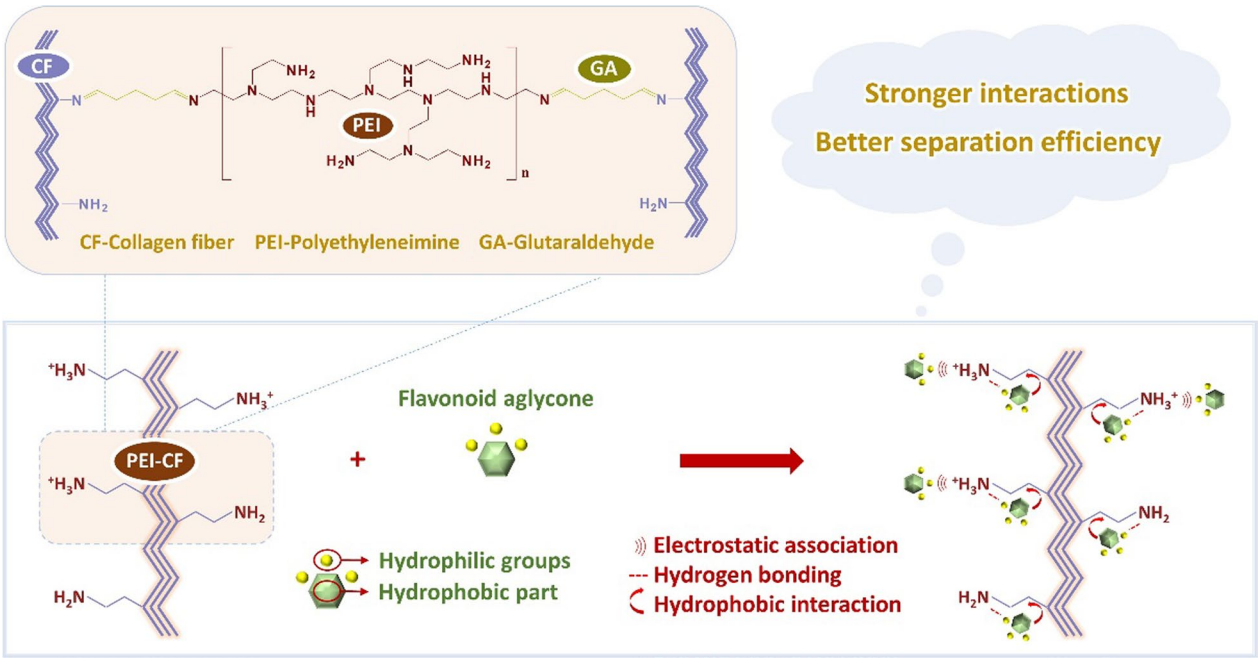
Abstract

Compared with flavonoid glycosides, flavonoid aglycones are difficult to be separated since they have less hydroxyls. Collagen fiber (CF), a natural polymer, was once used as packing material for separation of kaempferol and quercetin (the typical flavonoid aglycones) after crosslinking by glutaraldehyde mainly based on hydrogen bonding and hydrophobic interaction in column length-diameter ratio of 60:1. Hydrophobic modification by grafting alkyl chains was then employed to enhance the hydrophobic interaction between CF and flavonoid aglycones, which can improve the separation efficiency and decrease column length-diameter ratio to 19:1. In order to further improve the adsorption capacity and separation efficiency, the strategy of simultaneously grafting hydrophobic alkyl chains ($-CH_2-CH_2-$) and alkali groups ($-NH_2$) was adopted in this work to enhance hydrophobic interaction, hydrogen bonding and electrostatic association to flavonoid aglycones at the same time through grafting polyethyleneimine (PEI). PEI modified CF (PEI-CF) maintained the fiber structure of CF, and had higher adsorption extent and rate to flavonoid aglycones through the enhanced synergetic effect of hydrophobic interaction, hydrogen bonding and electrostatic association. As a result, PEI-CF presented a satisfactory column separation efficiency for kaempferol and quercetin even the length-diameter ratio of column was decreased to 11:1, which was much better than previously developed glutaradehyde-crosslinked collagen fiber and isobutyl-grafted collagen fiber, as well as commonly used polyamide and Sephadex LH-20.

Keywords Collagen, Packing material, Separation of flavonoids, Separation of flavonoid aglycones

*Correspondence:
Bi Shi
sibitannin@vip.163.com
Full list of author information is available at the end of the article

Graphical abstract



1 Introduction

Flavonoids widely exist in plant and present excellent bioactivities. The basic structure of flavonoids is C_6–C_3–C_6, in which two benzene rings are connected by a chain with three carbons, and it has hydroxyls on it. Flavonoid aglycones without glucosyls as well as flavonoid glycosides combined with one or more glucosyls, are the main forms of flavonoids [1, 2]. Flavonoid aglycones have much higher bioavailability, and thus are superior to flavonoid glycosides in bioactivities like oxidation resistance. Flavonoid aglycones can be absorbed into blood directly through the walls of small intestine, whereas most flavonoid glycosides can not be absorbed directly, instead they are degraded and metabolized by the microorganisms in enteric cavity. The rest flavonoid glycosides are absorbed into blood mainly through hydrolyzation into flavonoid aglycones by hydrolase from probiotics in colon [3–5].

Usually, flavonoids with similar structure but different bioactivities coexist in plant [6], and need effective separation before fine utilization. Alkali extraction and acid precipitation are commonly used for crude separation because flavonoids present weak acidity owing to the phenol hydroxyls, so they have high solubility in alkali solution but low solubility in acid solution [7, 8]. As for fine separation in preparative scale, column chromatographic separation is widely used, in which column packing material is the key point. It influences the separation performance and determines the quality of target flavonoids [9, 10]. The packing materials specially for separation of various flavonoid aglycones should be significantly developed not only due to the superior bioactivities of flavonoid aglycones, but also the difficulties in separation caused by their high structure similarity and insufficient hydroxyls and phenyls to combine with packing materials. The coexisted flavonoid aglycones are usually different only in content or/and location of hydroxyl. Figure 1 shows two typical coexisted flavonoid aglycones (kaempferol and quercetin), which are different only in one hydroxyl number on B ring. Their similar retention times on HPLC also indicate the difficulty in separation [11, 12].

Collagen fiber is one of the richest renewable biomasses [13, 14], and traditionally used as raw material in leather industry. Because of the fullness of hydrophilic groups like peptide bond, $-NH_2$, $-COOH$, $-OH$ and hydrophobic groups like alkyl and benzyl, as well as the inherently excellent biocompatibility, biodegradability and mechanical strength, collagen fiber could be also developed for other utilizations. It was previously developed as a packing material for separating flavonoids mainly through the hydrogen bond and hydrophobic interactions [15–21]. Before practical

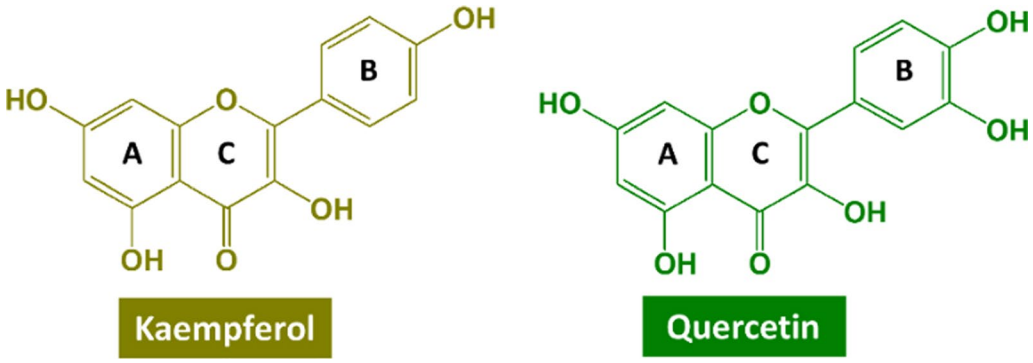


Fig. 1 Molecular structures of typical flavonoid aglycones

application, collagen fiber needs crosslinking to further improve the mechanical strength and thermal stability. Collagen fiber crosslinked by glutaraldehyde (GCF) is able to effectively separate flavonoid glycosides and the corresponding aglycones due to their obvious difference in structure [16], and different flavonoid glycosides due to their strong interaction with collagen fiber on account of glucosyls [18]. However, GCF presented weak adsorption capacity to flavonoid aglycones [17, 19]. Consequently, only a GCF column with much higher dosage and length-diameter ratio can effectively separate flavonoid aglycones. For example, a GCF column with length-diameter ratio of 60:1 can separate kaempferol and quercetin [19]. Hydrophobic modification by using silane coupling agent with alkyl chains was once adopted to enhance the hydrophobic interaction, and thus improve the separation efficiency to flavonoid aglycones, and it could reduce the length-diameter ratio of collagen fiber-based column for separation of kaempferol and quercetin to 19:1 [21]. Interestingly, when the grafted alkyl chain is short like isobutyl, it is also beneficial to the formation of hydrogen bond, showing excellent synergistic effect of hydrophobic and hydrogen bond interactions. However, increasing the grafted alkyl chain length to octyl and dodecyl can further enhance the hydrophobic interaction, but the hydrogen bond interaction was largely weakened due to the steric hindrance of long carbon chains [21]. So, further increasing the hydrophobicity of collagen fiber could not be considered as a good way to further improve the separation ability to flavonoid aglycones. As mentioned above, flavonoids have somewhat weak acidity owing to phenol hydroxyls. Therefore, simultaneously grafting the hydrophobic groups and hydrophilic alkali groups like $-NH_2$ on collagen fiber may be conducive to the formation of hydrophobic interaction, hydrogen bonding [22] and electrostatic association [23, 24], and is considered as a more efficient strategy to improve the adsorption and separation effects for flavonoid aglycones. In addition, electrostatic interaction is a kind of long range force [25, 26] and is expected to improve both the adsorption strength and adsorption rate.

Herein, collagen fiber was modified by using polyethyleneimine (PEI) and glutaraldehyde through the mode shown in Fig. 2, to obtain the modified collagen fiber (PEI-CF) with more hydrophobic short carbon chains ($-CH_2-CH_2-$) and $-NH_2$, and is expected to synergistically improve the hydrophobic, hydrogen bonding and electrostatic interactions to flavonoid aglycones. The static and dynamic adsorptions of PEI-CF to kaempferol and quercetin, and the adsorption mechanism were investigated. Then PEI-CF was packed as a shorter chromatographic column (length-diameter ratio of 11:1) for separation of kaempferol and quercetin, and GCF, isobutyl grafted collagen fiber (isobutyl-CF), as well as polyamide and Sephadex LH-20 were used for comparison.

2 Experimental section

2.1 Materials

GCF [15, 16] and isobutyl-CF [21] were prepared based on our previously established method. Quercetin and kaempferol (purity higher than 98%) were purchased from Shaanxi Huike Botanical Development Co. Ltd. (Shaanxi, China). Sephadex LH-20 was obtained from General Electric Company (Boston, USA). Polyethyleneimine (PEI) with M.W. 600 was analytical grade from Aladdin Co., Ltd. (Shanghai, China). Polyamide in 100 mesh was purchased from Kelong Co., Ltd. (Chengdu, China).

2.2 Preparation of PEI-CF

750 mL of PEI solutions were prepared by dissolving 5, 10, 25, 50 g PEI in deionized water and adjusted to pH 6.5. 50 g of CF was added into the solution with constant stirring at 30 °C for 24 h and then washed by 750 mL ethanol twice. After filtration, 500 mL of 1% (w/w) glutaraldehyde solution was added with constant stirring at 40 °C

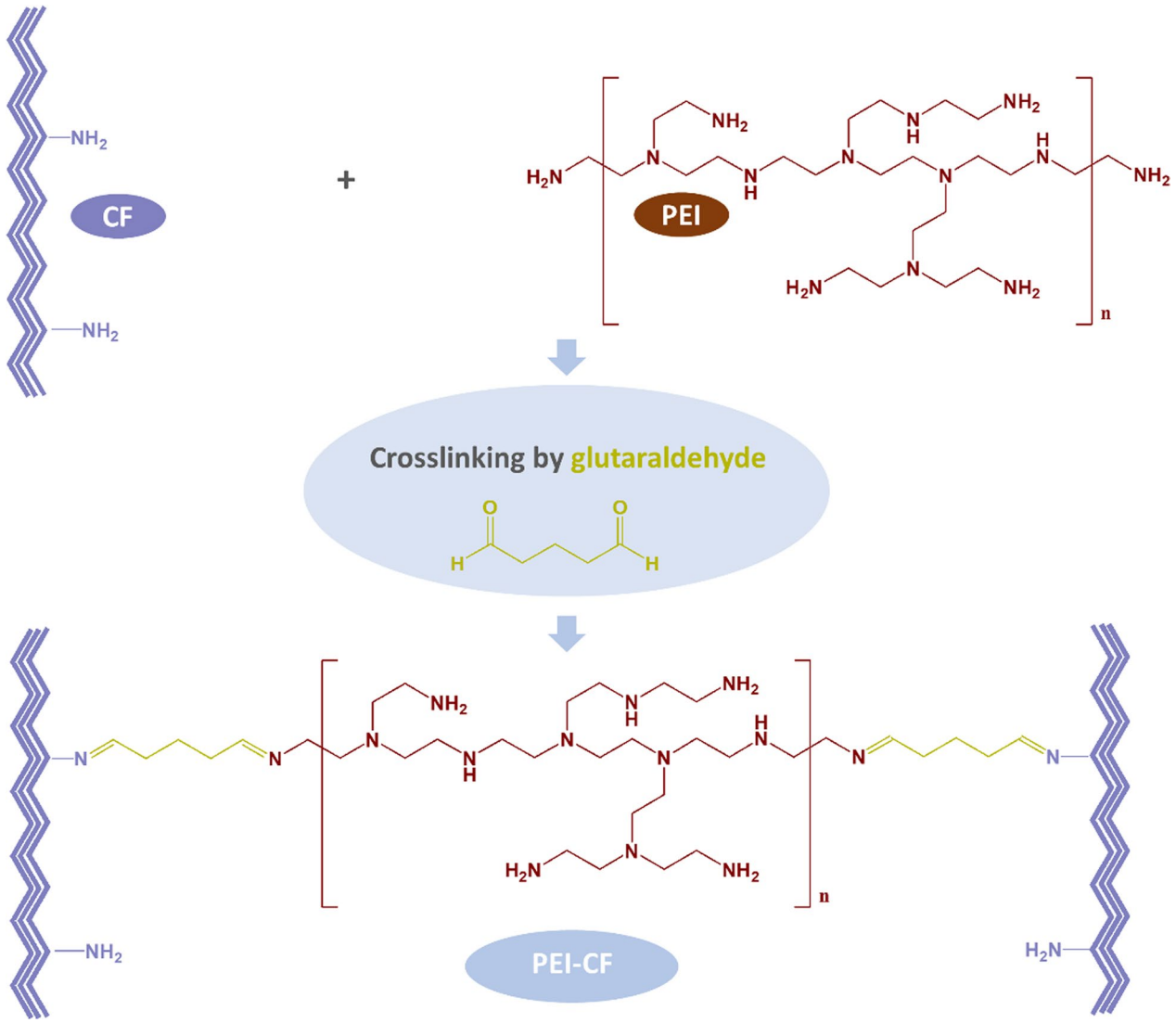


Fig. 2 Schematic illustration of PEI-CF preparation by using PEI and glutaraldehyde

for 2 h and then the pH of reaction solution was adjusted to 6.5 followed by further stirring at 40 °C for 2 h. The PEI-CF was finally obtained by successive filtration, washing with deionized water, dehydration with ethanol and drying at 50 °C for 24 h.

The PEI-CFs with PEI dosages of 5, 10, 25, 50 g were named as PEI-CF-5, PEI-CF-10 and PEI-CF-25 and PEI-CF-50, respectively.

2.3 Characterization of PEI-CF

Zeta potentials of PEI-CF were determined under different pHs by Zeta potential analyzer (Mütek TM SZP-10, Germany), and the pH at zero potential was obtained from Zeta potential-pH graph and set as the isoelectric point (pI). The FT-IR spectra were detected by FT-IR spectrophotometer (Nicolet iS10, Thermo Fisher Scientific, USA) in 4000–400 cm^{-1} by using the samples mixed with KBr and pressed into a pellet. The elemental analysis (EL cube, Elementar, Germany) was used to determine the nitrogen content of collagen fiber before and after grafting of PEI, and then the PEI grafting rate of PEI-CF-10 was calculated. Thermal denaturation and decomposition temperatures were measured using DSC 204 F1 and TG 209 F1 (NETZSCH, Germany), respectively. The morphology was observed by field emission scanning electron microscopy (FEI Inspect F50, USA).

2.4 Static adsorption

Equal mass of kaempferol and quercetin was dissolved in aqueous ethanol solutions with ethanol content

of 40–80% (v/v), and the concentration of each component was 20 mg/L. 0.1 g of PEI-CF was added to 10 mL of above solutions followed by constant shaking at 120 rpm for 24 h under 25 °C. HPLC (Agilent 1260 infinity, USA), Inertsil ODS-3 C18 column (Shimadzu, Japan) were combined to detect the flavonoids concentration. The adsorption extent (q, %) was calculated by Eq. (1).

$$q = \frac{C_0 - C_e}{C_0} \times 100 \quad (1)$$

where c_0 and c_e are the concentrations of each component in solutions before and after adsorption for 24 h (mg/L).

2.5 Adsorption mechanism

Urea, n-propanol and NaCl were added into 20 mg/L quercetin solution, and their contents were 0–0.75 mol/L, 0–40% (v/v) and 0–0.20 mol/L respectively. The adsorptions were conducted as the same conditions in Sect. 2.4.

2.6 Dynamic adsorption and desorption

Kaempferol and quercetin mixture solution was prepared in pure ethanol, where the concentration of each component was 20 mg/L. 1 g of PEI-CF-10 was added into 100 mL of the above solution and then constant shaking was conducted at 120 rpm, 25 °C. 1 mL of the solution was sampled at a certain interval and analyzed by HPLC. The adsorption quantity (q_t, mg/g) was calculated by Eq. (2).

$$q_t = \frac{(c_0 - c_t)V}{m} \times 10^{-3} \quad (2)$$

where c_0 and c_t are the concentrations of kaempferol/quercetin in solutions before and during adsorption (mg/L); V is the volume of adsorption solution (mL); m is the mass of PEI-CF-10 used for adsorption (g).

The above adsorbent was collected after adsorption by filtration and set for desorption in 100 mL of 80% aqueous ethanol solution (v/v) at 25 °C, 120 rpm. 1 mL of the desorption solution was collected at a certain interval and analyzed by HPLC. The desorption rate (E_d, %) was calculated by Eq. (3).

$$E_d = \frac{C_d \times V_d}{q_t \times m} \times 10^{-3} \times 100\% \quad (3)$$

where c_d is the concentration of each component in the desorption solution (mg/L); V_d is the volume of desorption solution (mL); q_t is the adsorption quantity (mg/g) obtained from Eq. (2); m is the mass of PEI-CF used for adsorption (g).

2.7 Column chromatographic separation

6 g of PEI-CF-10 in ethanol was packed into a glass column with diameter of 1.6 cm. The column height was 18 cm, and the length-diameter ratio was 11:1. 1 mL of the mixture solution of kaempferol and quercetin in the same amount of 5 mg/mL was loaded, and stepwise elution of ethanol and 80% aqueous ethanol solutions was then conducted under the flow rates of 0.6 and 1.0 mL/min. 10 mL of the effluent was collected and analyzed by HPLC. The purity (P, %) was calculated by Eq. (4).

$$P = \frac{m_1}{m_1 + m_2} \times 100 \quad (4)$$

where m_1 is the mass of objective component after separation (mg); m_2 is the mass of non-objective component after separation (mg).

The columns packed with the same amount of GCF, isobutyl-CF, polyamide, Sephadex LH-20 were used for comparison.

3 Results and discussion

Grafting charged groups on collagen fiber would influence the Zeta potential, thus change the isoelectric point (pI) of collagen fiber. As shown in Fig. 3a, when the PEI dosage was 0–25 g, the pI of collagen fiber increased with PEI dosage, indicating that more PEI reacted with collagen fiber and more $-NH_2$ grafted. On further increasing the PEI dosage to 50 g, pI decreased unpredictably, which may be due to the reason that too much PEI caused strong adhesion of collagen fiber, resulting in the plastic look, as well as limited exposure of $-NH_2$. It is also indicated that, at the same pH, the collagen fiber with more $-NH_2$ grafted showed higher Zeta potential and positivity, which is beneficial for the adsorption of acidic flavonoid aglycones. Reaction with PEI also influences the color of collagen fiber. As shown in Fig. 3 (b), the pristine collagen fiber (CF) was white. After crosslinking by glutaraldehyde (GCF), it changed to light yellow. After grafted with PEI and crosslinked by glutaraldehyde, it turned to deep yellow, and the color presented deeper with increasing PEI dosage, proving the successful grafting of PEI on collagen fiber. The FT-IR spectra in Additional file 1: Fig. S1 show the enhanced peak at 3420 cm^{-1} of PEI-CF-10, which is attributed to the increased amount of amino groups [27], further indicating the successful grafting. The grafting rate of PEI was 8.57% in PEI-CF-10 obtained from elemental analysis. The denaturation temperature of PEI-CF-10 was 3.3 °C higher than that of GCF, as shown in Fig. 4a, which is benefitted from

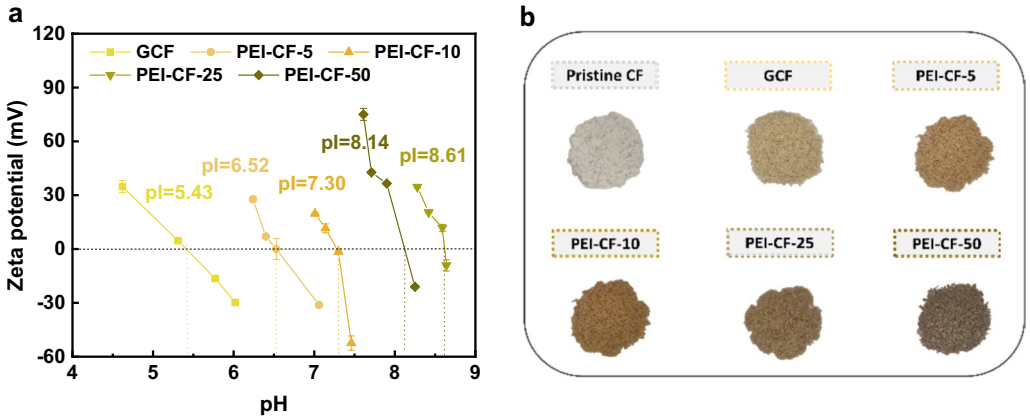


Fig. 3 Zeta potential (**a**) and color (**b**) of collagen fibers

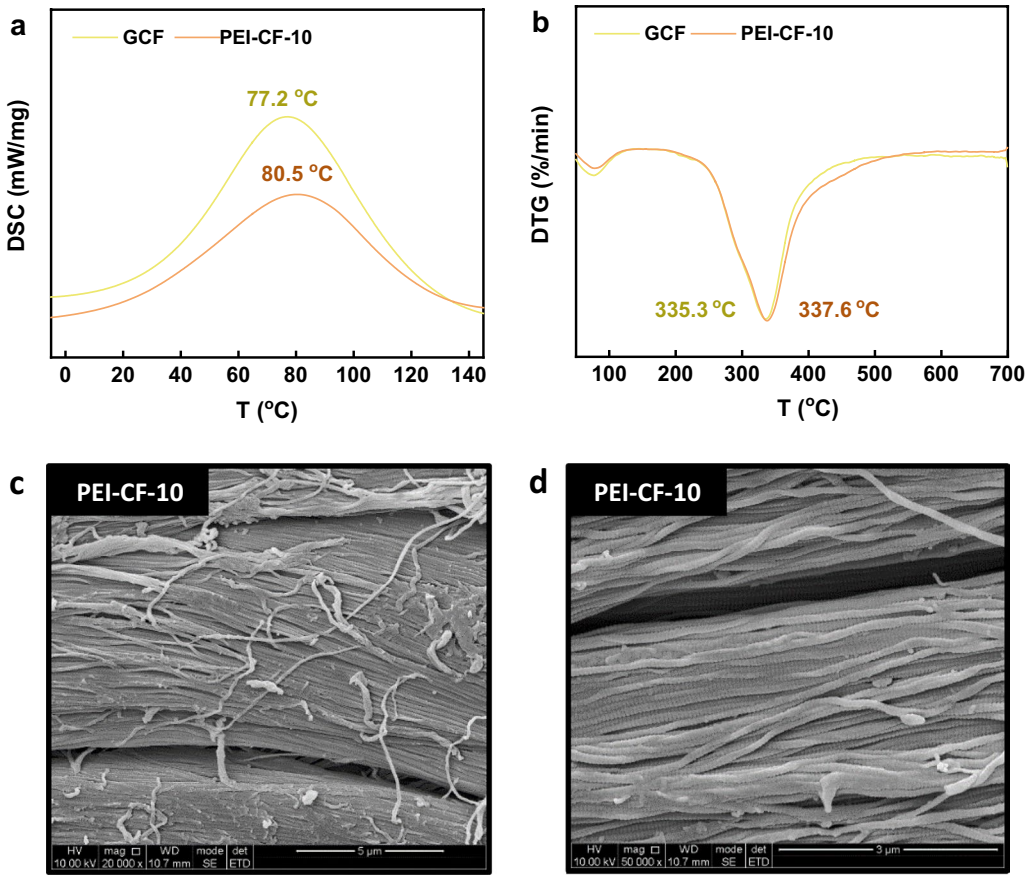


Fig. 4 DSC curves (**a**), DTG curves (**b**), SEMs of mag. 20,000 × (**c**) and mag. 50,000 × (**d**) of PEI-CF-10

the hydrogen bonds donated by PEI, and it favors the practical application of collagen fiber. The decomposition temperature of PEI-CF-10 was 2.3 °C higher than that of GCF, as shown in Fig. 4b, which also favors the practical application. The SEM images of PEI-CF-10 in Fig. 4c and d show that the fiber structure of collagen fiber was retained after grafting.

The equilibrium adsorption capacity of PEI modified collagen fibers (PEI-CF-5, PEI-CF-10, PEI-CF-25) to flavonoid aglycones in different aqueous ethanol

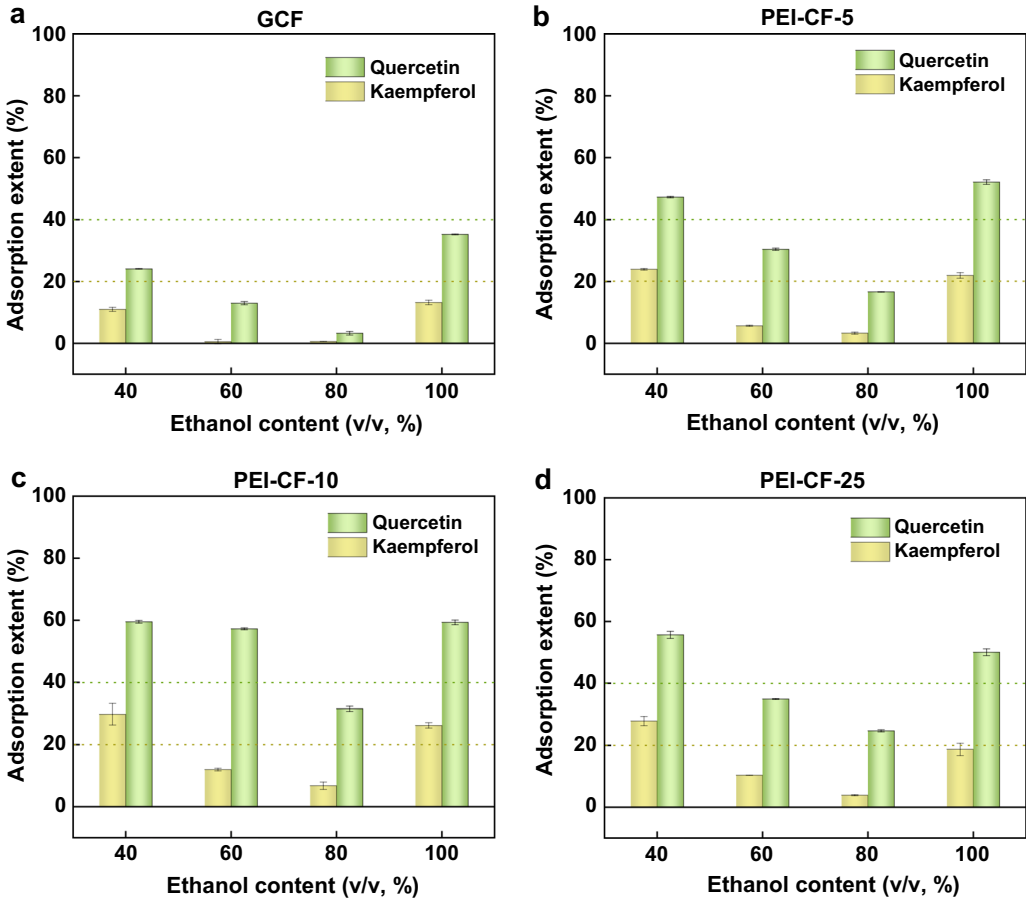


Fig. 5 Static adsorption of GCF (**a**), PEI-CF-5 (**b**), PEI-CF-10 (**c**), PEI-CF-25 (**d**) to quercetin and kaempferol

solutions was investigated, and GCF was used as control. As shown in Fig. 5, all the investigated PEI-CF showed higher adsorption extents to quercetin and kaempferol than GCF, and the adsorption extents increased when PEI dosage increased from 5 to 10 g, and then decreased when PEI dosage further increased to 25 g, which may be due to the fiber adhesion caused by PEI in higher content. Owing to the serious deformation and denaturation of PEI-CF-50, it was not used for adsorption and separation.

Ethanol concentration played an important role on the adsorption of flavonoid aglycones by PEI-CF. The adsorption extents of quercetin and kaempferol decreased with ethanol concentration in the ethanol concentration range of 40–80% and increased with ethanol concentration in the ethanol concentration range of 80–100%. Flavonoid aglycones are highly hydrophobic, and their solubility is limited when ethanol content is below 40%, so the static adsorption was not conducted in this condition.

It was previously considered that hydrogen bond and hydrophobic interactions were the main forces between collagen fiber and flavonoids due to the phenol hydroxyls and phenyls of flavonoids [15–21]. The phenol hydroxyls also give flavonoids weak acidity, and thus the electrostatic interaction was thought to be another interaction between PEI-CF and flavonoids. In order to investigate the adsorption mechanism, the effects of urea, n-propanol and NaCl, used as breaking agents of hydrogen bonding [28], hydrophobic interaction [29] and electrostatic association [30], on adsorption extents of PEI-CF-10 to quercetin were investigated in pure ethanol, 80% and 40% aqueous ethanol solutions, and shown in Fig. 6a–c. The three breaking agents all influenced the adsorption to some extent, meaning hydrogen bonding, hydrophobic interaction and electrostatic association were the main forces for adsorption. But the dominant adsorption interaction was changed in different aqueous ethanol solutions. The adsorption extent in 100% ethanol was mainly negatively influenced by urea, whereas the adsorption extent in 40% ethanol was mainly limited by n-propanol. The effect of NaCl on adsorption was only investigated in

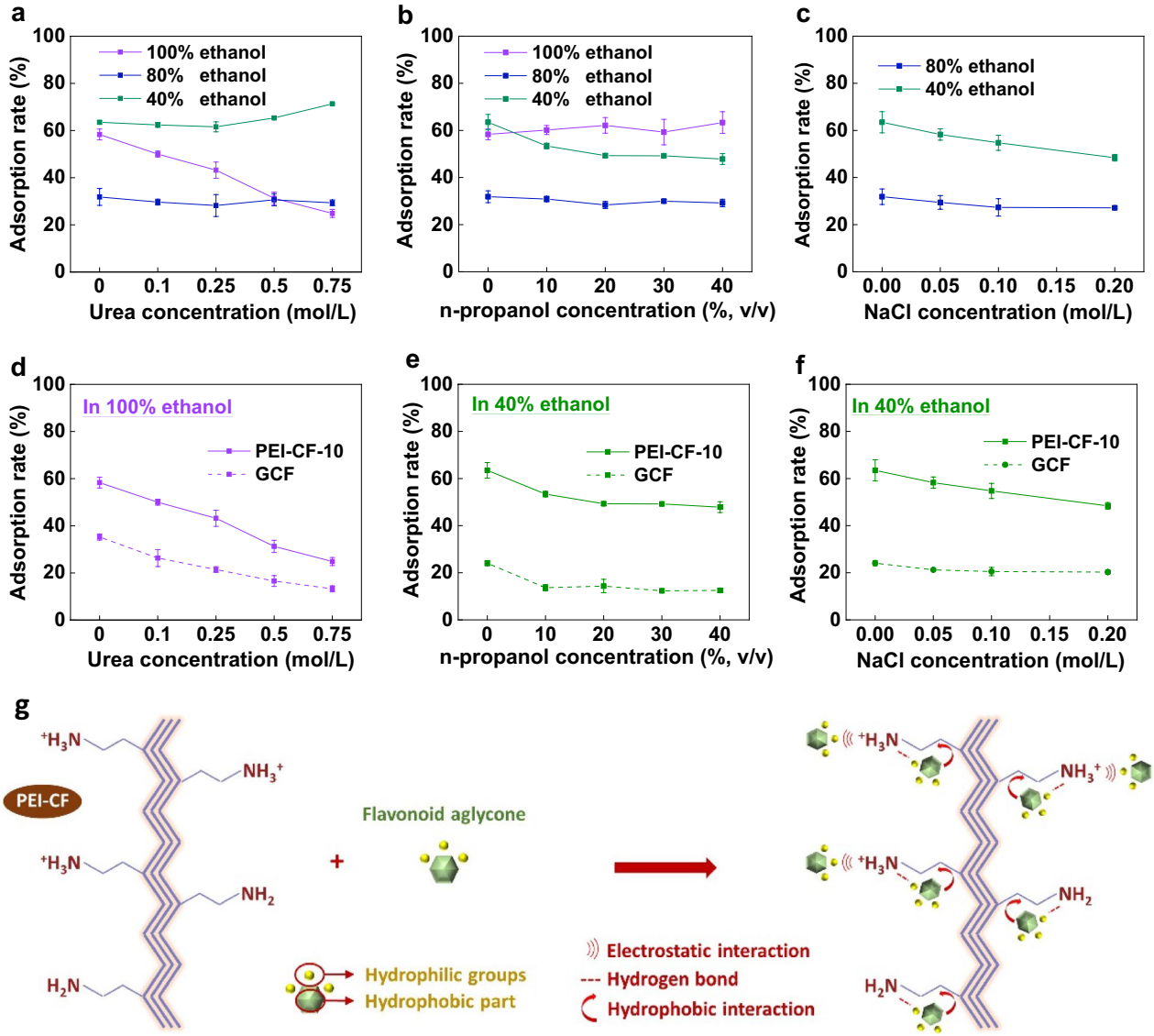


Fig. 6 Effect of urea (**a**, **d**), n-propanol (**b**, **e**) and NaCl (**c**, **f**) on adsorption extents of PEI-CF-10 and GCF to quercetin; schematic diagram of the interaction (**g**)

80% and 40% aqueous ethanol solutions, because NaCl could not be dissolved in pure ethanol. NaCl negatively influenced adsorption extent to some extent in both 80% and 40% aqueous ethanol solutions, but the influence in 40% ethanol was much stronger due to the reason that water is beneficial for ionization and electrostatic interaction compared with ethanol. Therefore, the dominant force for PEI-CF to adsorb flavonoid aglycones was hydrogen bond in pure ethanol, synergistic hydrophobic and electrostatic interactions in 40% aqueous ethanol solution, and electrostatic interaction in 80% aqueous ethanol solution. So, the reason why PEI-CF had higher adsorption extents to kaempferol and quercetin in all aqueous ethanol solutions may be that short carbon chains and $-NH_2$ donated from PEI improve the hydrogen bonding, hydrophobic interaction and electrostatic association between collagen fiber and flavonoid aglycones.

The adsorption mechanism was further verified by comparing the effects of urea, n-propanol and NaCl on the adsorption of GCF and PEI-CF-10 to quercetin, and the results are shown in Fig. 6d–f. The effect of urea was conducted in pure ethanol to compare the hydrogen bond interaction of GCF and PEI-CF-10. When urea content increased from 0 to 0.75 mol/L, the adsorption extent of PEI-CF-10 decreased by 33.5%, whereas the adsorption extent of GCF only decreased by 22.0%, indicating that PEI-CF has stronger hydrogen bond

interaction with flavonoid aglycones. The effects of n-propanol and NaCl were conducted in 40% ethanol to compare the hydrophobic and electrostatic interactions of GCF and PEI-CF-10. The adsorption extent of PEI-CF-10 decreased by 15.6% as n-propanol content increased from 0 to 40%, and 15.1% as NaCl content increased from 0 to 0.20 mol/L. But under the same circumstances, the adsorption extent of GCF decreased only by 11.6% and 3.8%, respectively, implying that PEI-CF also has stronger hydrophobic and electrostatic interactions with flavonoid aglycones. Therefore, it is further verified that reaction with PEI improves the synergistic effects of hydrogen bond, hydrophobic and electrostatic interactions between collagen fiber and flavonoid aglycones, as the illustration in Fig. 6g.

Furthermore, the effect of ethanol and water in aqueous ethanol solution on adsorption of PEI-CF to kaempferol and quercetin can be reasonably explained. Ethanol has weaker polarity than water, which is conducive to the formation of hydrogen bond between flavonoids and PEI-CF, but limits hydrophobic and electrostatic interactions. Water has stronger polarity than ethanol, and thus tends to weaken hydrogen bond interaction, but improves hydrophobic and electrostatic interactions. All our previous investigations demonstrated that hydrogen bonding always makes the greatest contribution in the interaction between flavonoids and collagen fiber [15–21]. Therefore, in 40–80% aqueous ethanol solutions where hydrogen bonding is suppressed and hydrophobic and electrostatic interactions are dominant, adsorption extent decreased with the increase of ethanol concentration. When ethanol concentration further increased from 80 to 100% where hydrogen bond interaction becomes the dominant force and hydrophobic / electrostatic interactions are suppressed, the adsorption extent increased with ethanol concentration. This also implies that the adsorption and desorption of flavonoid aglycones on PEI-CF could be effectively adjusted by changing the ethanol content in aqueous ethanol solution.

In addition, the adsorption extent of quercetin was always higher than kaempferol, as shown in Fig. 5a–d, because quercetin has one more hydroxyl on B-ring, leading to stronger hydrogen bond and electrostatic interactions. Both quercetin and kaempferol have two phenyls and one heterocyclic ring, so they are similar in hydrophobicity and hydrophobic interaction. The difference in adsorption may be mainly based on the different hydrogen bond and electrostatic interactions benefitted from the hydroxyl, which favors the separation of them. PEI-CF-10 presented the highest adsorption extents for flavonoid aglycones and biggest adsorption difference between quercetin and kaempferol, so it was used for subsequent adsorption and separation.

The adsorption rate and elution potential of PEI-CF to flavonoid aglycones could be evaluated by dynamic adsorption and desorption. Figure 7 shows the dynamic adsorptions and desorptions of GCF and PEI-CF-10 to quercetin and kaempferol. Compared with GCF, PEI-CF-10 had higher adsorption extent and adsorption rate, which is beneficial to the separation of quercetin and kaempferol. As for the desorption in 80% aqueous ethanol solution, the desorption went on quickly and turned to equilibrium within 30–60 min, implying the effective elution and recovering of flavonoid aglycones in subsequent column separation. It is worth noting that, the difference in desorption rate between quercetin and kaempferol on PEI-CF-10 was bigger than that on GCF, which implied that separation of flavonoid aglycones by PEI-CF-10 may be much easier due to the fact that column chromatographic separation is consisted of constant adsorption and desorption processes, and bigger differences in both adsorption and desorption could favor the effective separation.

Separation of kaempferol and quercetin on PEI-CF-10 column with length-diameter ratio of 11:1 was conducted by stepwise elution of pure ethanol and 80% aqueous ethanol solution at flow rates of 0.6 and 1.0 mL/min, and the chromatograms as well as the purity were presented in Fig. 8a and b. Kaempferol and quercetin were well separated on PEI-CF-10 column at both flow rates, meaning that PEI-CF-10 has a considerable adsorption rate to flavonoid glycones so that it presented satisfactory separation efficiency even under a higher flow rate with a shorter separation period. In addition, the repeat separations under these two flow rates were conducted on the same PEI-CF-10 column, and the similar elution curves and separation performance indicated an excellent reusability of PEI-CF-10. However, as shown in Fig. 8c and d, GCF and isobutyl-CF were not able to separate kaempferol and quercetin at a flow rate of 1.0 mL/min under the same dosage of packing material and elution condition, because they have less adsorption ability and retention capacity to flavonoid aglycones compared with PEI-CF. It was reported that GCF column with length-diameter ratio of 60:1 can well separate kaempferol and quercetin [19], and isobutyl-CF with hydrophobic modification to enhance the hydrophobic interaction can well separate kaempferol and quercetin in the column with length-diameter ratio of 19:1 [21]. So PEI-CF had better separation efficiency to flavonoid aglycones in a shorter column owing to more $-NH_2$ and hydrophobic short carbon chains grafted on collagen fiber, which favors the electrostatic, hydrogen bond and hydrophobic interactions of collagen fiber to flavonoid aglycones.

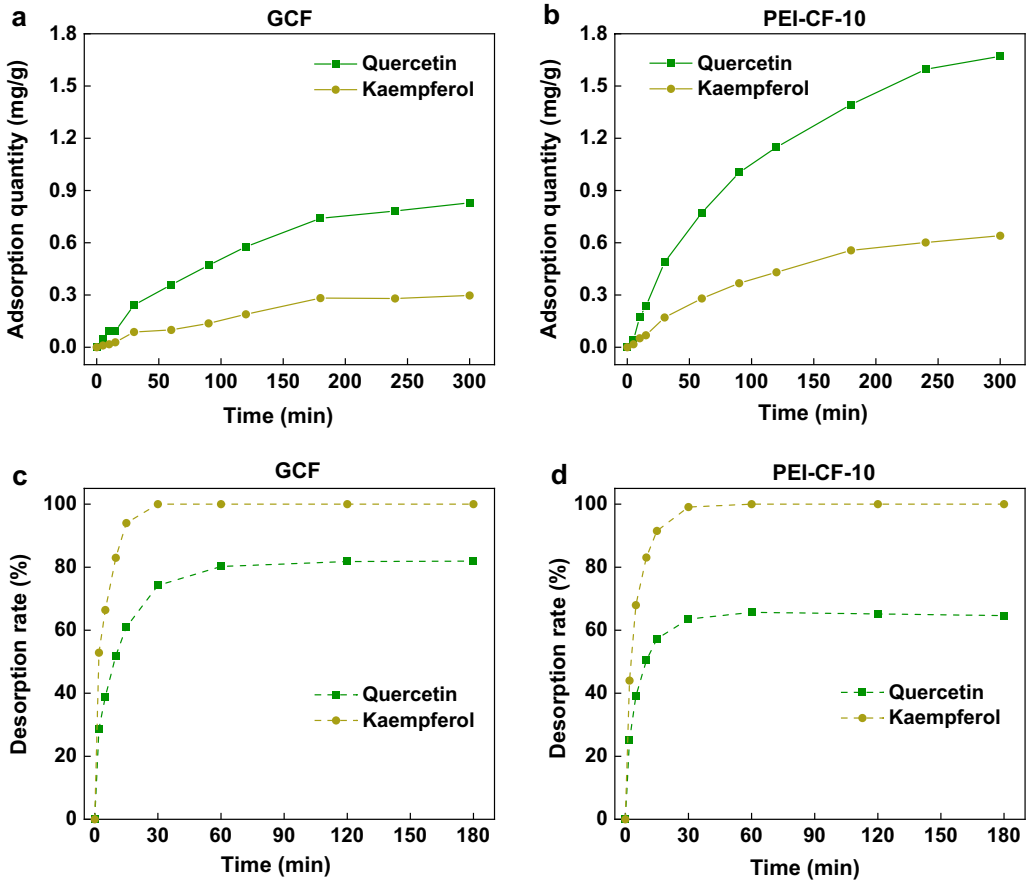


Fig. 7 Dynamic adsorption of GCF (**a**) and PEI-CF-10 (**b**) to quercetin and kaempferol in pure ethanol; dynamic desorption of quercetin and kaempferol on GCF (**c**) and PEI-CF-10 (**d**) in 80% aqueous ethanol solution

Polyamide and Sephadex LH-20 are the commonly used separation materials for flavonoids, and the chromatograms of kaempferol and quercetin on these columns under the same packing material dosage are shown in Fig. 8e and f. Owing to the reverse-phase characteristic of polyamide and Sephadex LH-20 in reverse mobile phase like water, the stepwise elution was the aqueous ethanol solution changed from lower ethanol content to higher ethanol content. The kaempferol and quercetin were eluted together from polyamide and Sephadex LH-20 columns by the first step of elution (60% aqueous ethanol solution). As a result, the purities of kaempferol and quercetin obtained from polyamide and Sephadex LH-20 were much lower than those from PEI-CF-10 column.

4 Conclusion

Collagen fiber (CF) was previously developed as a packing material for column chromatographic separation of flavonoids based on the hydrogen bond interaction benefitted from its hydrophilic groups like peptide bond, $-NH_2$, –COOH, –OH, as well as the hydrophobic interaction benefitted from the hydrophobic amino acids. Flavonoid aglycones lack sufficient hydroxyls and phenyls to combine with CF, so it was then grafted with short alkyl chains to enhance the hydrophobic interaction for more efficient separation of flavonoid aglycones. Simultaneously grafting hydrophobic groups ($-CH_2-CH_2-$) and alkali groups ($-NH_2$) by using polyethyleneimine (PEI) is a better strategy to further improve the adsorption capacity and separation efficiency, because it can synergistically enhance hydrophobic interaction, hydrogen bonding and electrostatic association to flavonoid aglycones. As a result, the modified CF presents a higher column separation efficiency for flavonoid aglycones, with a smaller column length-diameter ratio (11:1), and even better performance than commercial polyamide and Sephadex LH-20. Therefore, collagen fiber as a natural polymer with low cost is highly expected for practical use in separation of flavonoid aglycones by grafting short carbon chains and $-NH_2$ groups.

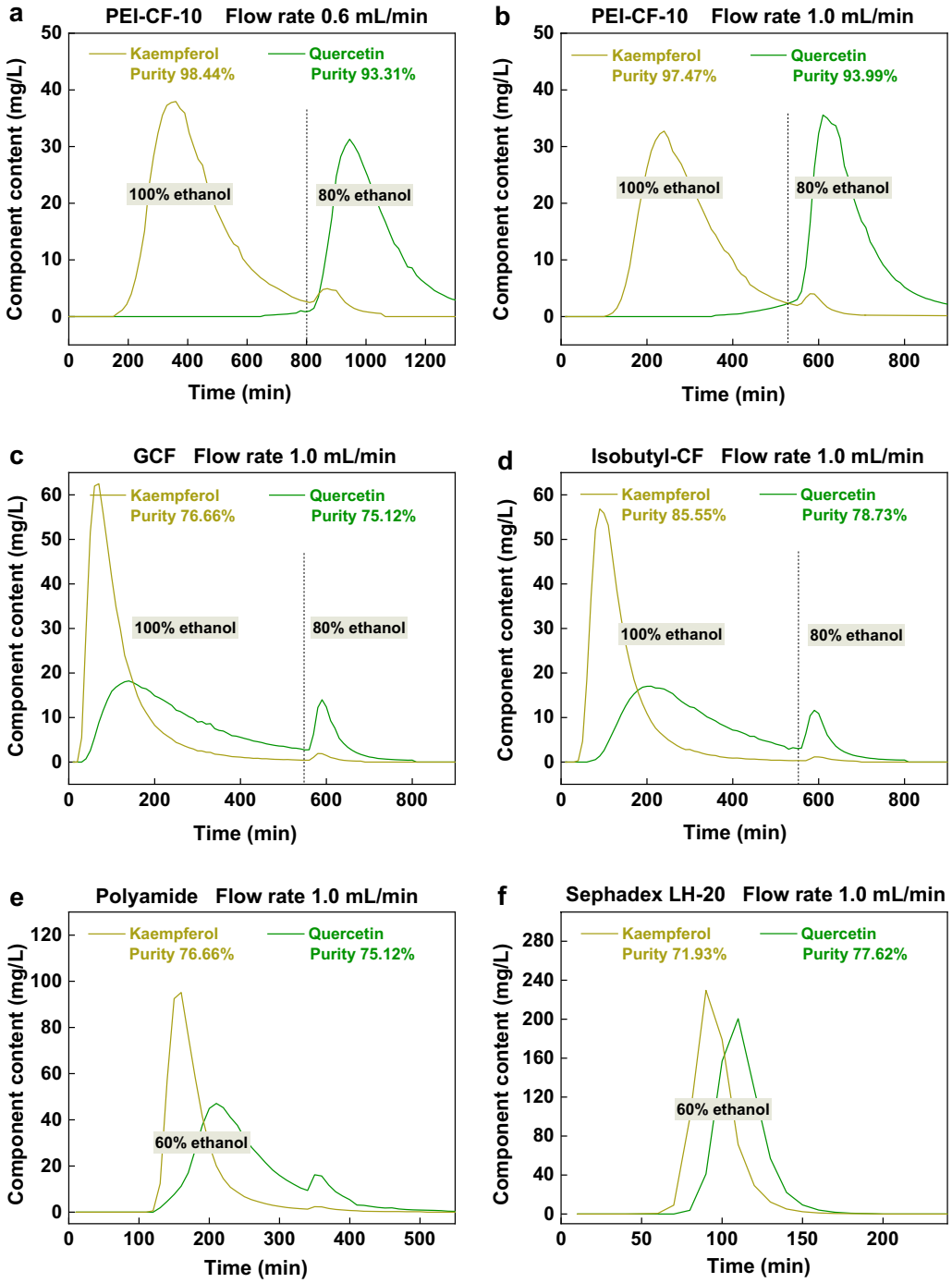


Fig. 8 Chromatograms of kaempferol and quercetin on PEI-CF (**a**, **b**), GCF (**c**), isobutyl-CF (**d**), polyamide (**e**), Sephadex LH-20 (**f**) columns

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42825-023-00138-2.

Additional file 1: **Fig. S1**. FT-IR spectra of PEI-CF-10.

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Author contributions
QXZ: Investigation, visualization, writing-original draft preparation, methodology; RW: Software, validation; BS: writing—resources, writing-review and editing, supervision, funding acquisition.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
Bi Shi serves as the Editor-in-Chief of Collagen and Leather. Qixian Zhang serves as the Managing Editor of Collagen and Leather. They were not involved in the editorial review, or the decision to publish this article. All authors declare that there are no competing interests.

Author details
[1]College of Biomass Science and Engineering, Sichuan University, Chengdu 610065, People's Republic of China. [2]National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, People's Republic of China.

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