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### Sustainability Spotlight statement

This article presents a novel approach to the smart design of circular biocomposites by combining low-cost and abundant biopolymers, producing keratin-based films either pure or blended with cellulose and  $\alpha$ -chitin, each of which is derived from waste. A bio-based solvent was used for biopolymer dissolution, enabling a sustainable and cost-effective approach. As an exemplar application, the films were used as adsorbents for contaminated water, achieving higher adsorption capacity than common adsorbents reported in the literature. This marks the first reported results on developing keratin-chitin films, offering a more sustainable alternative to chitosan. This work aligns with the UN Sustainable Development Goals: decent work, economic growth (SDG 8), and responsible consumption and production (SDG 12).



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### Data availability statements

The authors of the manuscript “Ionic-liquid-processed keratin-based biocomposite films with cellulose and chitin for sustainable dye removal” confirm that the data supporting the findings of this study are available within the article and/or its Supplementary Information.

Sincerely yours,

Jason Hallett

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## ARTICLE

**Ionic-liquid-processed keratin-based biocomposite films with cellulose and chitin for sustainable dye removal**Cariny Polesca<sup>a,b</sup>, Helena Passos<sup>a,c,d</sup>, Pedro Y. S. Nakasu<sup>b</sup>, João A. P. Coutinho<sup>a</sup>, Mara G. Freire<sup>a\*</sup>, Jason P. Hallett<sup>b\*</sup>Received 00th January 20xx,  
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Poultry is a widely consumed meat worldwide; however, its industrial processing generates a significant amount of feather waste. Since the major component of chicken feathers is keratin (90 wt. %), this study focused on using acetate-based ionic liquids (ILs) to fully dissolve chicken feathers and recover keratin, using a sustainable and cost-effective approach, ultimately allowing waste valorisation. The recovered keratin was processed into films, either pure or blended with cellulose and  $\alpha$ -chitin, aiming to develop a structural polymer biocomposite with improved mechanical properties. Experimental parameters were evaluated using different blend ratios, altering the pH, and adding glycerol as a plasticiser. Physico-chemical analysis revealed that all films exhibited hydrophilic behaviour and are stable up to 160 °C. Furthermore, the tensile strength of the keratin-based films significantly increased by adding chitin (achieving up to 66 MPa). Considering the growing significance of biopolymer-based films in wastewater treatment applications, the keratin-based films were evaluated as adsorbents for dye removal. Reactive Blue 4 (RB4) was used as a model dye, and the adsorption kinetics and isotherms were investigated. Between the studied films, the maximum adsorption capacity (55.7 mg·g<sup>-1</sup>) was obtained for the keratin film, emphasising the potential of this biomaterial in wastewater treatment.

**1 Introduction**

The global meat industry produces around 15 million tons of chicken feather waste annually<sup>1</sup>, posing challenges such as negative impacts on the land composition and environmental pollution (affecting nitrogen and phosphorous cycles), and a high cost for disposal<sup>2</sup>. Chicken feathers contain nearly 90 wt. % keratin, a valuable protein source for sustainable and environmentally friendly resource development<sup>1,3</sup>. However, keratin recovery is challenging, mainly because of the inter- and intramolecular disulphide bonds between sulphur-containing amino acid residues and extensive cross-linking, which are resistant to water, weak acids, and organic solvents<sup>2,4,5</sup>. Ionic liquids (ILs) have been investigated to overcome such issues due to their excellent dissolution capability and high efficiency for protein recovery<sup>6–11</sup>. ILs are salts formed by a large organic cation and an organic or inorganic anion, with lower melting temperatures than inorganic salts<sup>5,8,12</sup>. Recently, we demonstrated the ability of acetate-based ILs for feather

dissolution, which is due to the anion's high hydrogen-bonding acceptor ability, further providing an efficient keratin recovery (up to 93 wt. %) and a more sustainable pathway for producing keratin films<sup>11,13</sup>. The IL recovery (over at least four cycles) was successfully achieved, and a techno-economic assessment of the process was performed, demonstrating the potential for a cost-effective and environmentally friendly process<sup>13</sup>. Keratin applications involve several areas, including use in the biomedical field and as an adsorbent for water treatment/remediation<sup>14–16</sup>. Water contamination is a global concern, with the textile industry generating more than 7 million tons of toxic, carcinogenic, and nonbiodegradable dyes annually<sup>15</sup>. These substances pose threats to human health and aquatic life<sup>3,16</sup>. In the textile industry, reactive dyes (e.g. reactive blue 4, RB4) are widely used (around 700,000 tons of dyes are annually produced) and present acute toxicity (LD50 oral 8.98 mg·kg<sup>-1</sup>)<sup>17,18</sup>. The environmental impact of RB4 assessed by EPI Suite™ confirms its slow environmental degradation (150 days in water and soil and 600 days in sediment)<sup>19</sup>, highlighting the urgency to develop strategies to mitigate this concern.

Several water treatment methods have been investigated to address this issue, including chemical oxidation, electrochemical oxidation, ion exchange, and adsorption<sup>14</sup>. The typical water treatment technologies are energy-intensive and involve unsustainable synthetic materials. However, adsorption, particularly using biomaterials like keratin<sup>20–22</sup>, has shown promising results as a clean and effective alternative method<sup>3</sup>. Keratin-based adsorbents are attractive because their functional groups (e.g. hydrophobic and hydrophilic amino

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acids and carboxyl groups, such as  $-NH_2$ ,  $-COOH$ ,  $-SH$ , and  $-OH$  act as effective adsorption sites to a broad range of aqueous contaminants, accentuating their performance. Keratin is a fibrous protein with a high surface area, enhancing its adsorption capacity<sup>14,23,24</sup>. Moreover, it is an available, biodegradable, non-toxic, low-cost biomaterial<sup>3,15,21</sup>. While previous studies have demonstrated the feasibility of keratin-based adsorbents<sup>25,26</sup>, challenges remain in cost-effectiveness processes for its recovery and poor tensile strength of the produced films<sup>27</sup>. To overcome some of these challenges, we propose the use of the previously developed cost-effective process for keratin recovery using ILs<sup>11,13</sup>, focusing now on preparing keratin-based films with improved mechanical properties by creating blends with cellulose and chitin for water purification. The idea is to employ the smart design of biocomposites by combining different types of low-cost and relatively abundant biopolymers. To blend with keratin, two structural biopolymers were chosen to enhance the mechanical properties of the films without compromising their biodegradability<sup>24,28</sup>: cellulose — an abundant biopolymer with good mechanical and thermal properties<sup>29,30</sup> — and chitin — an acetylated polysaccharide with outstanding mechanical properties, such as high strength and high toughness<sup>31,32</sup>. The strong interaction between keratin and these polymers is attributed to their strong hydrogen bonding network between their functional groups ( $CH_3$ ,  $CONH$ ,  $NH_2$  and  $OH$ )<sup>33</sup>. The processing conditions for the preparation of keratin films (e.g. keratin concentration, pH, addition of glycerol as a plasticiser, effect of three acetate-based ILs, and the biopolymer ratio) were varied by means of the design experiments to evaluate their influence on the film properties. All keratin-based films were characterised using physicochemical, mechanical, and morphological analyses, and their potential as adsorbent materials for dye removal was explored using RB4. The adsorption kinetics and isotherms were examined. In summary, this work focuses on developing keratin-based films with adequate properties for application in wastewater treatment, while contributing to waste valorisation.

## 40 Materials and Methods

### 41 Materials

42 Chicken feathers were collected from Campoaves Company in  
43 Oliveira de Frades, Portugal, and pre-treated before dissolution  
44 as reported in the literature<sup>11</sup>. The ILs used, viz. 1-butyl-3-  
45 methylimidazolium acetate ( $[C_4C_1im][C_1CO_2]$ ) (>95 wt. % pure)  
46 and 1-ethyl-3-methylimidazolium acetate ( $[C_2C_1im][C_1CO_2]$ ) (>95  
47 wt. % pure) were purchased from Sigma-Aldrich. Ethanol (99.8  
48 wt. % pure), acetic acid (>99.7 wt. % pure), sodium hydroxide

(NaOH), and ethylene glycol were acquired from Fisher Scientific. Glycerol (>99 wt. % pure) was purchased from Acros  
Organics. Cholinium bicarbonate, cellulose highly purified, hydrochloric acid (HCl) (>37 wt. % pure) and RB4 (35 wt. % pure) were obtained from Sigma-Aldrich.  $\alpha$ -chitin from shrimp was purchased from Apollo Scientific. Cholinium acetate ( $[N_{111(20H)}][C_1CO_2]$ ) was synthesised as previously described by Muhammad et al.<sup>34</sup>. Briefly, acetic acid was added dropwise to cold cholinium bicarbonate in a round-bottom flask and stirred overnight. The moisture content in the synthesised IL was removed using a rotatory evaporator consisting of rotavapor R-10, heating bath B-491, vacuum pump V-700 and vacuum controller V-850 (all from Buchi, Switzerland). The water content was determined using a V20 Volumetric Karl-Fischer titrator (Mettler Toledo). The purity of the IL, confirmed by proton nuclear magnetic resonance ( $^1H$ -NMR), is available in the Supporting Information (Figure S1).

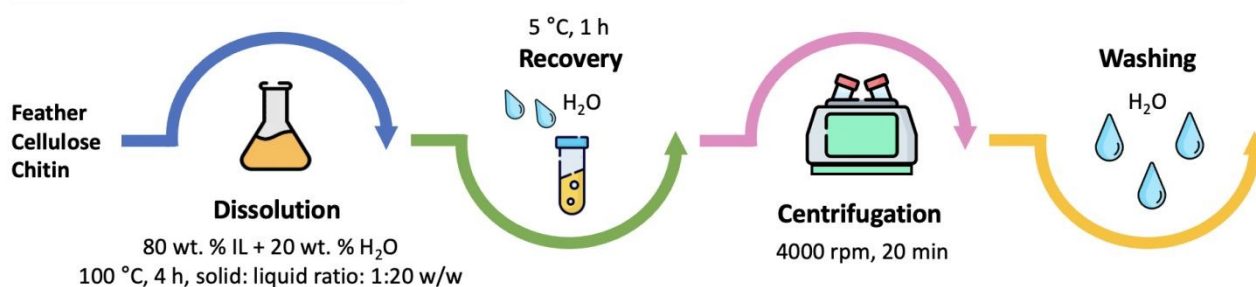
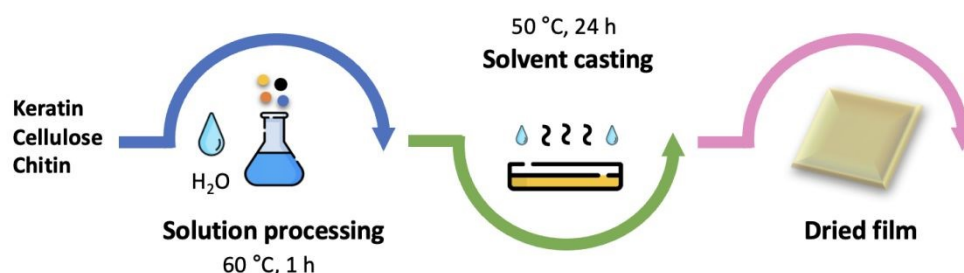
### Chicken feather dissolution and keratin recovery

Chicken feathers were dissolved, as previously reported by our research group<sup>11,13</sup>. Different aqueous solutions of  $[C_4C_1im][C_1CO_2]$ ,  $[C_2C_1im][C_1CO_2]$ , and  $[N_{111(20H)}][C_1CO_2]$  (80 wt. % IL + 20 wt. % water) were used for feather dissolution at 100 °C for 4 h in a solid:liquid (chicken feather:solvent) ratio of 1:20 w/w. After dissolution, keratin was recovered by adding water as a coagulant solvent in a solution:coagulant ratio of 1:2 w/w at 5 °C for 1 h. Then, the solution was centrifuged for 20 min at 4000 rpm in a refrigerator centrifuge machine (VWR® Mega Star 4.0), promoting the separation of the precipitated keratin. The protein was then washed with DI water to remove any residual IL and centrifuged at the previously described conditions. The wet keratin was used for film processing. Keratin recovery yield of up to 93 wt. % was obtained, in agreement with previous assays<sup>11,13</sup>.

### Keratin-based film processing

Wet keratin recovered by  $[C_4C_1im][C_1CO_2]$  was used to investigate the film processing conditions: protein concentration (on distilled water), solution pH (adjusted with NaOH 1 M), and addition of glycerol as a plasticiser. The mixture was mixed under constant magnetic stirring at 60 °C for 1 h. The solution was cast on a silicone moulding and placed in an air oven at 50 °C for 24 h. A control keratin solution (KER-15; 15 wt. % keratin, without glycerol and pH modification) was used to prepare films using keratin recovered by  $[C_2C_1im][C_1CO_2]$  and  $[N_{111(20H)}][C_1CO_2]$ , aiming to investigate the IL influence on the keratin film properties. Then, keratin-cellulose and keratin-chitin biocomposite films were processed, aiming to understand the impact of the blend composition on the film properties. Keratin-cellulose (75:25 and 50:50 w/w) and keratin-chitin (75:25 and 50:50 w/w) films were processed by adding keratin and cellulose or chitin in distilled water (15 wt. % of biopolymers). The film processing followed the previously described procedure for pure keratin films. The keratin-based film processing is schematically summarized in Figure 1, and the conditions used for the processing and their sample names are presented in Table 1.



**(i) Biopolymer dissolution****(ii) Bio-based film processing**

**Figure 1.** Schematic representation of the keratin-based film processing proposed in this work.

**Table 1.** Sample names and conditions used for preparing keratin-based films.

Sample	IL	Biopolymer (wt. %)	pH	Glycerol (wt. %)
KER-5		5		
KER-15		15	6.4	-
KER-20		20		
KER-pH9	[C <sub>4</sub> C <sub>1</sub> im][C <sub>1</sub> CO <sub>2</sub> ]	15	9.0	-
KER-pH12		15	12.0	-
KER-gly5		15	6.4	5
KER-gly10		15		10
KER-C2	[C <sub>2</sub> C <sub>1</sub> im][C <sub>1</sub> CO <sub>2</sub> ]	15	6.4	-
KER-N111	[N <sub>111(20H)</sub> ][C <sub>1</sub> CO <sub>2</sub> ]	15		
KER-CELL-C2 75:25		15 *		
KER-CELL-C2 50:50		15 **		
KER-CHI-C2 75:25	[C <sub>2</sub> C <sub>1</sub> im][C <sub>1</sub> CO <sub>2</sub> ]	15 *	6.4	10
KER-CHI-C2 50:50		15 **		
KER-CELL-N111 75:25		15 *		
KER-CELL-N111 50:50		15 **		
KER-CHI-N111 75:25	[N <sub>111(20H)</sub> ][C <sub>1</sub> CO <sub>2</sub> ]	15 *	6.4	10
KER-CHI-N111 50:50		15 **		

\* = 75 wt. % keratin + 25 wt. % cellulose or chitin; \*\* = 50 wt. % keratin + 50 wt. % cellulose or chitin

1 **Characterisation of keratin-based films**

2 The Fourier transform infrared attenuated total reflectance (FTIR)  
3 ATR) spectra of the films processed with different conditions were  
4 acquired in a FTIR spectroscopy (Spectrum One FTIR system  
5 PerkinElmer, Wellesley, MA). The functional groups available on films

6 were analysed at room temperature, in a frequency range of 4000-  
7 400 cm<sup>-1</sup>, by accumulating 16 scans, with a resolution of 4 cm<sup>-1</sup>, and  
8 an interval of 1 cm<sup>-1</sup>.

9 Thermogravimetric analysis (TGA) was carried out in a TA Q500 (TA  
10 Instruments, USA) TGA analyser. Keratin-based films were placed in



1 an aluminium pan and further analysed under a nitrogen gas at a flow  
2 rate of 25 mL·min<sup>-1</sup>. The samples were heated at a rate of 20 °C·min<sup>-1</sup>  
3 in the temperature range from 30 to 600 °C.

4 To investigate the hydrophilicity of the films, the contact angle was  
5 determined using a semi-automatic wettability analysis with high  
6 dosing precision (DSA25S, Krüss). Adding a drop of 7 µL of ethylene  
7 glycol at a rate of 7 µL·s<sup>-1</sup>, multiple measurements were made on  
8 keratin-based films. Further details on the procedure can be found  
9 elsewhere <sup>11</sup>.

10 Using a Lloyd EZ 50 testing machine, the tensile strength of the film  
11 was determined. Keratin-based films were cut into rectangular  
12 shapes (4 cm × 1 cm), and three replicates were carried out. The  
13 tensile strength (MPa) was calculated by dividing the obtained value  
14 (N) by the cross-sectional area of the films.

15 Scanning electron microscopy (SEM) was performed using a high  
16 resolution field-emission Zeiss Auriga Cross Beam. Aiming to ensure  
17 the conductivity of the films, they were coated with chromium (5  
18 nm) in a Q150 TS machine before the sample analysis. Images were  
19 obtained using an accelerating voltage of 5 kV and a working distance  
20 of 5 nm.

### 21 Adsorption properties of keratin-based films

22 The adsorption of RB4 was addressed using keratin-based films with  
23 the best achieved properties (high thermal degradation and high  
24 tensile strength). First, the adsorption kinetic was investigated using  
25 10 mL of RB4 solution (60 ppm) at pH 2.0 with 5 mg of adsorbent for  
26 0-360 min. The effect of pH on adsorption performance was studied  
27 in a pH range of 2.0-8.0 using 10 mL of RB4 solution (60 ppm) with  
28 mg of adsorbent for 300 min. Then, the effect of the initial  
29 concentration of RB4 (10-60 ppm) was investigated for all keratin-  
30 based films using 5 mg of adsorbent and 10 mL of RB4 solution at  
31 pH 2.0 for 300 min. The samples were maintained by stirring (150 rpm)  
32 at 30 °C. The dye concentration was determined by a UV-Vis  
33 spectrophotometer UV-2600 (Shimadzu) at a wavelength of 595 nm  
34 using a previously established calibration curve. The pH modification  
35 on dye solutions did not alter the chemical structure of the dye  
36 Supporting Information, Figure S2)<sup>35</sup>. To determine the amount of  
37 dye adsorbed, Equation 1 was used:

$$38 \quad q_e = \frac{C_0 - C_e}{m} \times V$$

39 where  $q_e$  is the equilibrium adsorption capacity (i.e. the amount of  
40 dye-adsorbed (mg·g<sup>-1</sup>) on the adsorbent at equilibrium),  $C_0$  and  $C_e$  are  
41 the initial and equilibrium concentrations of dye (mg·L<sup>-1</sup>)  
42 respectively,  $m$  is the mass of adsorbent used (mg), and  $V$  is the  
43 volume of dye solution (L).

44 The adsorption kinetic data generally follow one of the two kinetic  
45 models, namely the Pseudo First-Order (PFO) or the Pseudo Second-  
46 Order (PSO) models, which are described by the following equations  
47 respectively:

$$48 \quad \frac{dq_t}{dt} = k_1 \times (q_e - q_t)$$

$$49 \quad \frac{dq_t}{dt} = k_2 \times (q_e - q_t)^2$$

where  $k_1$  is the PFO constant (min<sup>-1</sup>) and  $k_2$  is the PSO constant (min<sup>-1</sup>·  
g<sup>2</sup>·mg<sup>-1</sup>),  $q_e$  is the amount of sorbent bound to the sorbate at the  
equilibrium (mg·g<sup>-1</sup>),  $q_t$  is the amount of sorbent bound to the  
sorbate at a given time (mg·g<sup>-1</sup>), and  $t$  is the time (min).

## Results and discussion

### Keratin-based film characterisation

The FTIR-ATR spectra are shown in Figure 2, showing the  
essential absorption bands representative of keratin-based  
films. The presence of keratin is confirmed by the stretching  
vibrations of O–H and N–H (Amide A) at 3670-2800 cm<sup>-1</sup>, C=O  
stretching (Amide I) at 1700-1600 cm<sup>-1</sup>, N–H bending and C–H  
stretching (Amide II) at 1590-1470 cm<sup>-1</sup>, and amide III (1280-  
1200 cm<sup>-1</sup>). In general, all keratin films present the same bands,  
except for the keratin films obtained by adding glycerol as a  
plasticiser, where a band at 1070-1000 cm<sup>-1</sup> is also observed,  
being indicative of the presence of the added alcohol <sup>36</sup>.  
Regarding the blends, the hydroxyl group broad stretching at  
3300 cm<sup>-1</sup> and 1650 cm<sup>-1</sup> are related to the water absorption  
and hydroxyl groups present in the cellulose <sup>37</sup>, while the band  
at 1060 cm<sup>-1</sup> is attributed to the C-O asymmetric stretching  
vibration of the glycosidic ring in cellulose <sup>38</sup>. The bands at 1650  
cm<sup>-1</sup> and 1560 cm<sup>-1</sup> are related to chitin's amide vibrations,  
assigned to the C=O stretching and N-H bending <sup>39</sup>. Overall,  
these results reveal that the biopolymers are well blended.

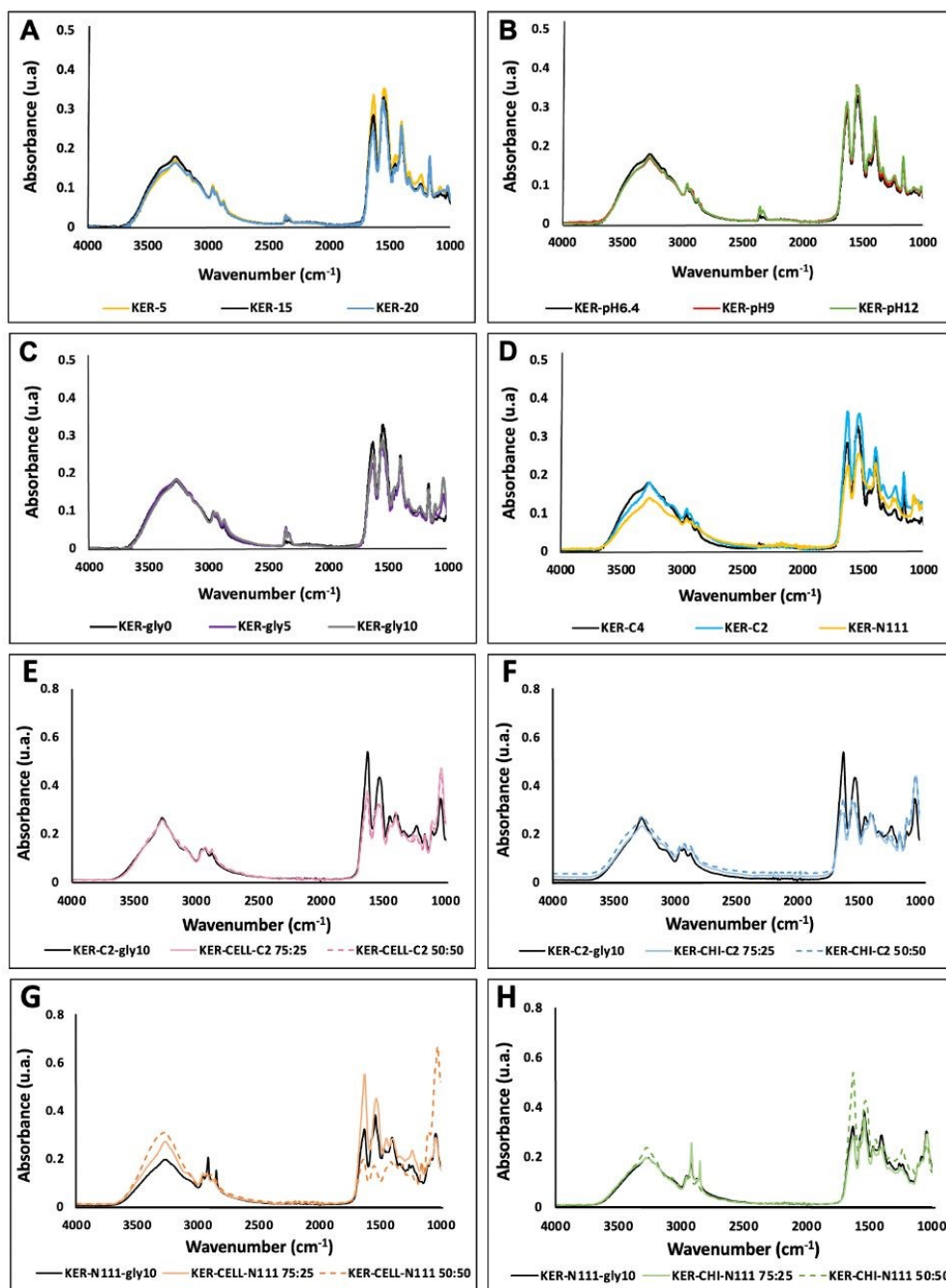
Regarding the thermal behaviour (Supporting Information,  
Figure S3), all keratin-based films presented more than one step  
of degradation, with the first degradation step, likely related to  
moisture, occurring at below 100 °C. The second stage  
corresponds to keratin decomposition for pure keratin films  
(without adding cellulose or chitin). All keratin film samples  
(prepared with different ILs, solution pH, keratin concentration,  
and glycerol added) were stable up to 215 °C and revealed a  
similar behaviour. The biocomposite blends (with different  
ratios of keratin-cellulose and keratin-chitin) exhibited similar  
behaviour and were stable up to 160 °C. The samples were  
decomposed between 160 °C and 360 °C, with a slow  
degradation, divided into steps, thus indicating physical  
interactions, and no chemical interactions, between the  
biopolymers (the thermal degradation of cellulose and chitin  
correspond to 335 °C <sup>40</sup> and 253 °C <sup>41</sup>, respectively). The starting  
decomposition at 160 °C can be related to glycerol evaporation  
(boiling temperature = 182 °C). In general, the results are within  
the range of the thermal degradation obtained for keratin <sup>6,11,42</sup>  
and other protein films, such as soy protein films (180 °C) <sup>43</sup> and  
whey protein films (295 °C) <sup>44</sup>.

The wettability of keratin-based films was assessed by  
measuring the contact angle of ethylene glycol drops on the  
films (Table 2). The contact angle of all keratin-based films was  
found to be lower than 90°, confirming their hydrophilic nature  
due to the presence of hydrogen bond donor and acceptor  
groups, such as amino, carboxylic and hydroxyl groups.  
Furthermore, no significant variances in the contact angle were  
observed across different IL films, ranging from (55 ± 1)° to (60  
± 5)°, meaning that there is no IL residue on the keratin-based  
films and that their use in keratin processing does not cause  
significant differences in keratin properties. Nevertheless, when  
keratin films were prepared with higher pH, the contact angle  
value increased from (55 ± 1)° to (84 ± 1)°, indicating that



1 increasing pH can promote a more a hydrophobic film. This  
 2 behaviour is attributed to the alkaline pH, which results in  
 3 stronger intramolecular electrostatic repulsion and unfolding of  
 4 proteins, exposing their hydrophobic groups and amino acid  
 5 residues. Furthermore, the films with glycerol were more  
 6 hydrophilic, which is due to its plasticiser effect and the  
 7 presence of additional polar hydroxyl groups. The addition of  
 8 wt. % glycerol decreased the contact angle from  $(55 \pm 1)^\circ$  to  $(46$

$\pm 5)^\circ$ . The same behaviour was observed by Cerqueira et al.<sup>45</sup>  
 who investigated the influence of glycerol on chitosan films.  
 The wettability of the keratin-cellulose and keratin-chitin films  
 range from  $(47 \pm 2)^\circ$  to  $(63 \pm 3)^\circ$ , supporting their hydrophilic  
 nature. For the keratin-cellulose films, increasing the cellulose  
 content from 25 wt. % to 50 wt. % resulted in a rise of  $9^\circ$  in the  
 contact angle for films produced with both ILs. This is a direct  
 result of the higher cellulose hydrophobic nature. A contrasting



**Figure 2.** FTIR spectra of keratin-based film samples processed using different conditions: varying protein concentration (A); pH of the solution (B); addition of glycerol (C); using different acetate-based ILs (D); keratin-cellulose blend films processed by  $[C_2C_1im][C_1CO_2]$  (E); keratin-chitin blend films processed by  $[C_2C_1im][C_1CO_2]$  (F); keratin-cellulose blend films processed by  $[N_{111(20H)}][C_1CO_2]$  (G); and keratin-chitin blend films processed by  $[N_{111(20H)}][C_1CO_2]$  (H).





## ARTICLE

behaviour was observed for keratin-chitin films, with the contact angle decreasing by 16° when chitin addition increased from 25 wt. % to 50 wt. %. This behaviour can be attributed to the interaction between the biopolymers, leading to a high exposure of their hydrophilic amino acids. Deng et al.<sup>46</sup>, who processed wool keratin-cellulose membranes with different ratios, also obtained hydrophilic biomaterials, with changes in the contact angle from 61° to 80°. To the best of our knowledge, no results regarding keratin-chitin films have been reported. Tomihata et al.<sup>47</sup> evaluated the contact angle of chitin films and obtained 69.5°, thus confirming the hydrophilicity of chitin films.

Tensile strength refers to the ability of a film to resist tensile stress before breaking, being the results obtained for the investigated keratin-based films summarised in Table 2. The tensile strength of keratin films increased 26 times by increasing keratin concentration from 5 wt. % to 15 wt. %, ranging from (0.53 ± 0.04) MPa (KER-5) to (14 ± 2) MPa (KER-15). No further increase in the tensile strength was observed when increasing

keratin concentration from 15 wt. % (KER-15) to 20 wt. % (KER-20). Concerning the pH and use of various ILs, no significant differences were observed. The addition of glycerol as a plasticiser increased the tensile strength of the film 3.5 times, going from (14 ± 2) MPa (KER-gly0) to (50 ± 1) MPa (KER-gly10). Therefore, among the variables evaluated in this work, the keratin concentration and glycerol addition presented the highest impact on the films' tensile strength. Shahrim et al.<sup>48</sup> also observed this glycerol effect while investigating the tensile strength of starch films. The results obtained by the authors revealed an increase in tensile strength of 7 % when increasing the glycerol from 5 % to 10 %<sup>48</sup>, which is lower than the increase observed with the keratin films studied here.

For the blends, the tensile strength of keratin-cellulose films did not present a significant increase when the addition of cellulose increased from 25 wt. % to 50 wt. %. Furthermore, increases of 1.1 and 1.4 times were observed for the films processed with keratin dissolved by [N<sub>111</sub>(2OH)][C<sub>1</sub>CO<sub>2</sub>] and [C<sub>2</sub>C<sub>1</sub>im][C<sub>1</sub>CO<sub>2</sub>], respectively.

**Table 2.** Contact angle and tensile strength of keratin-based films.

Samples	Contact angle (°)	Tensile strength (MPa)
KER-5	23 ± 2	0.53 ± 0.04
KER-15*	55 ± 1	14 ± 2
KER-20	62 ± 1	14 ± 2
KER-pH6.4*	55 ± 1	14 ± 2
KER-pH9	86 ± 2	10 ± 1
KER-pH12	84 ± 1	11 ± 2
KER-gly0*	55 ± 1	14 ± 2
KER-gly5	49 ± 2	36 ± 1
KER-gly10	47 ± 5	50 ± 1
KER-C4*	55 ± 1	14 ± 2
KER-C2	58 ± 5	12 ± 1
KER-N111	60 ± 5	11 ± 1
KER-CELL-C2 75:25	54 ± 2	21 ± 1
KER-CELL-C2 50:50	63 ± 3	30 ± 3
KER-CHI-C2 75:25	63 ± 1	53 ± 2
KER-CHI-C2 50:50	47 ± 2	66 ± 1
KER-CELL-N111 75:25	54 ± 3	23 ± 1
KER-CELL-N111 50:50	62 ± 2	26 ± 3
KER-CHI-N111 75:25	59 ± 2	34 ± 2
KER-CHI-N111 50:50	48 ± 2	41 ± 5

\* KER-15 = KER-pH6.4 = KER-gly0 = KER-C4

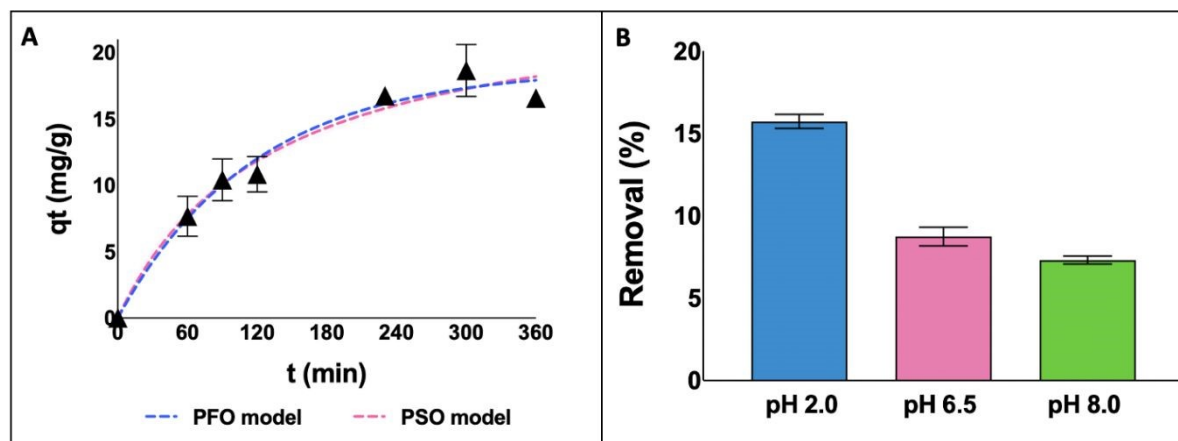


1 These results suggest that using  $[C_2C_1im][C_1CO_2]$  for dissolution  
 2 leads to more resistant films. The maximum tensile strength  
 3 obtained for the keratin-cellulose blend was  $(30 \pm 3)$  MPa  
 4 achieved with KER-CELL-C2 50:50. On the other hand  
 5 considering blends with chitin, the highest tensile strength of  
 6  $\pm 1$  MPa was obtained for KER-CHI-C2 50:50, demonstrating the  
 7 benefit of adding chitin due to its more promising mechanical  
 8 properties<sup>49</sup>.  
 9 Ma et al.<sup>50</sup> prepared keratin-cellulose films with various ratios  
 10 and showed that the blends have poor mechanical properties  
 11 compared to pure cellulose films. According to the authors, the  
 12 pure cellulose film had a higher tensile strength (44 MPa) than  
 13 a keratin-cellulose 40:60 film (28 MPa)<sup>50</sup>. The authors did not  
 14 report films with higher amounts of keratin; however, their  
 15 results show that more cellulose could be essential to create a  
 16 stronger film. Concerning the authors' process, keratin was  
 17 recovered from wool using urea, sodium dodecyl sulphate, and  
 18 sodium bisulphite. After protein dialysis, the liquid was cast  
 19 a polypropylene mould, dried, and dissolved in formic acid  
 20 to prepare a keratin solution. Another solution was prepared for  
 21 cellulose by dissolving the polysaccharide in NMMO solution  
 22 under heating. On the other hand, to the best of our knowledge,  
 23 no results have yet been reported for keratin-chitin films.  
 24 However, looking for pure chitin films, Wu et al.<sup>49</sup> obtained a  
 25 high tensile strength value for their films (320 MPa), confirming  
 26 chitin's high tensile strength.  
 27 The morphological properties of keratin films (Supporting  
 28 Information, Figure S4) were appraised using SEM. All keratin  
 29 films, except KER-pH12 and KER-gly10, present a smooth  
 30 surface. In KER-pH12, a particular behaviour can be noticed due  
 31 to the presence of salt (in this case, NaOH, used to adjust the

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 solution pH). In contrast, the roughness in KER-gly10 may be  
 due to the addition of glycerol and solution destabilization  
 during casting and drying. SEM analysis was also performed to  
 illustrate the morphological effects of keratin-blend films, being  
 shown in Supporting Information, Figure S5. In general, the  
 films are homogeneous, suggesting an appropriate blending;  
 however, there were some irregularities observed in the  
 blended films, indicating the presence of biopolymer particles  
 on the film's surface, which can be related to possible  
 differences in coagulation for each biopolymer.

### Dye adsorption using keratin-based films

With the aim of preparing stronger keratin-based films for dye adsorption studies, the films processed with  $[C_2C_1im][C_1CO_2]$  were selected due to their superior mechanical properties, specifically tensile strength (see Table 1). Keratin (KER-gly10), keratin-cellulose (KER-CEL-C2 50:50), and keratin-chitin films (KER-CHI-C2 50:50) were applied to investigate the effects of the biopolymers on dye adsorption. Different adsorption experiments were carried out by varying the RB4 initial concentration, pH, time and adsorbent type. First, aiming to understand the adsorption process and identify the contact time necessary to achieve the equilibrium stage, adsorption kinetic experiments were performed for the KER-CHI-C2 film at pH 2.0, from 0 to 360 min (Figure 3A). Then, the pH effect (from 2.0 to 8.0) was evaluated for the same adsorbent at 300 min (Figure 3B). The respective parameters from the fitting of adsorption kinetic experimental data with PFO and PSO models are given in Table 3.



**Figure 3.** Adsorption kinetics curves for KER-CHI-C2 film as a function of time (A) and the effect of pH on the adsorption of RB4 by KER-CHI-C2 (B).

**Table 3.** Adsorption parameters obtained from the fitting of adsorption kinetic experimental data with PFO and PSO models alongside the respective correlation coefficients.

PFO model			PSO model		
$q_e$ ( $mg \cdot g^{-1}$ )	$k_1$ ( $min^{-1}$ )	$R^2$	$q_e$ ( $mg \cdot g^{-1}$ )	$k_2$ ( $min^{-1}$ )	$R^2$
18.82	0.0084	0.96	24.86	0.0003	0.95



## ARTICLE

1 According to the adsorption kinetic curves (Figure 3A), the  
2 equilibrium stage was achieved between 240 and 360 min and  
3 maintained for 300 min in the next experiments. Looking at  
4 Table 3, despite both models presenting a good fit to the  
5 experimental data, PFO presented the best fit ( $R^2 = 0.96$ )  
6 suggesting that the adsorption process occurs on localised sites  
7 and does not involve interactions with the adsorbed molecules  
8<sup>51</sup>.

9 By decreasing the pH from 8.0 to 2.0 (Figure 3B), a significant  
10 increase in RB4 removal is obtained for KER-CHI-C2, moving  
11 from 7.3 % to 15.7 %. This behaviour can be explained by the pH  
12 value at zero potential point of the keratin-based films (4 - 5),  
13 which means that in acidic conditions the adsorbent is  
14 protonated, promoting electrostatic interactions with the dye.  
15 RB4 has two sulfonate groups and a primary amino group, with  
16 pKa values at 0.8 and 7.0, respectively. These groups can be  
17 easily dissociated; thus, the dye molecule has negative and  
18 positive charges under the working experimental conditions.  
19 The diverse keratin functional groups (e.g.  $-NH_2$ ,  $-COOH$ ,  $-SH$   
20 and  $-OH$ ) make it a promising adsorbent<sup>52</sup>. Accordingly, the  
21 adsorption mechanism of keratin-based films for RB4 dye is  
22 mainly ruled by electrostatic attractions (between the  $-NH_2$   
23 group of keratin and the sulfonate ( $-SO_3^-$ ) group on RB4  
24 structure) and hydrogen bonding interactions (between the  $-OH$   
25 group of keratin and the nitrogen group on RB4 structure)<sup>24,53</sup>.

## 26 Adsorption isotherms

27 Adsorption isotherms were determined to investigate the  
28 relationship between the adsorbent and the dye adsorbed at  
29 equilibrium conditions. The two models most widely used to  
30 represent equilibrium isotherms for adsorbent materials were  
31 applied: the Langmuir and Freundlich models<sup>54</sup>.

32 Langmuir (Equation 4) is a model based on homogeneous  
33 monolayer adsorption on a homogeneous surface, while  
34 Freundlich (Equation 5) is based on heterogeneous adsorption  
35<sup>54</sup>.

$$q_e = q_{max} \times \frac{k_L \times C_e}{1 + k_L \times C_e} \quad (4)$$

where  $q_{max}$  is the maximum adsorption capacity of the adsorbent ( $mg \cdot g^{-1}$ ),  $k_L$  is the Langmuir constant ( $L \cdot mg^{-1}$ ),  $C_e$  corresponds to the dye concentration in the solution ( $mg \cdot L^{-1}$ ), and  $q_e$  is the adsorption capacity of the adsorbent ( $mg \cdot g^{-1}$ ), at the equilibrium.

$$q_e = k_f \times C_e^{\frac{1}{n}} \quad (5)$$

where  $k_f$  is the Freundlich constant related to the adsorption capacity ( $mg \cdot g^{-1} \cdot (L \cdot mg^{-1})^{1/n}$ ) and  $n$  is an empirical parameter related to adsorption intensity.

Figure 4 shows the Langmuir and Freundlich's fittings for all keratin-based films at pH 2.0. The parameter values of the Langmuir and Freundlich isotherms and the related correlation coefficients ( $R^2$ ) are reported in Table 4. Both models presented satisfactory fittings; however, the Langmuir model yielded the better fitting, showing  $R^2$  values higher than 0.92. This trend indicates that the dye adsorption on the adsorbent occurs in a homogeneous surface with monolayer sorption<sup>54</sup> and that the adsorption is of chemical nature<sup>55</sup>. These results are in accordance with the previously discussed results, where it was shown the relevance of the pH and of electrostatic interactions between the dye and the polymers. Furthermore, the maximum adsorption capacity obtained in this work ( $55.71 mg \cdot g^{-1}$ ) for KER-C2 film confirms that keratin is a fibrous protein with diverse functional groups (e.g. hydrophobic and hydrophilic amino acids and carboxyl groups)<sup>14,23,24</sup>, acting as an efficient adsorbent for RB4.

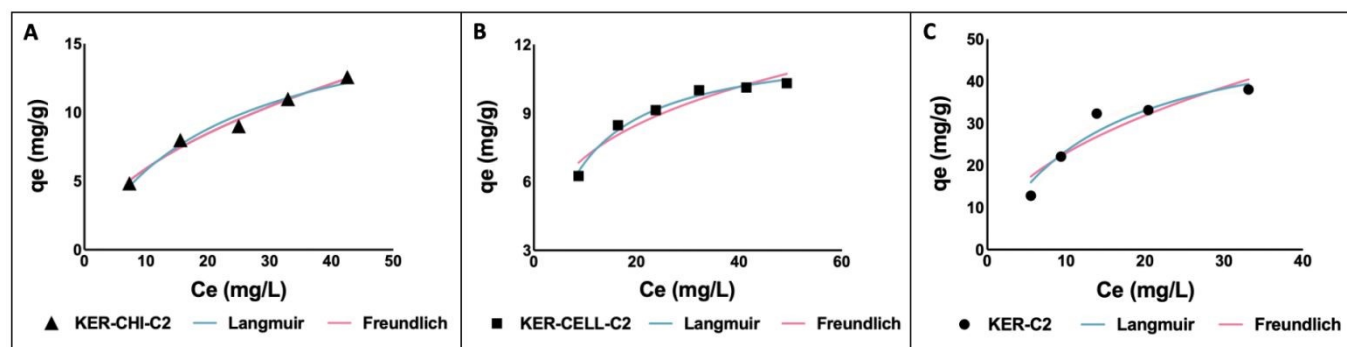


Figure 4. Experimental adsorption isotherms of RB4 at pH 2.0 using different keratin-based films: KER-CHI-C2 (A); KER-CELL-C2 (B); and KER-C2 film (C).



**Table 4.** Parameters obtained for RB4 adsorption using the Langmuir and Freundlich models.

Samples	Model	Parameter	Value	R <sup>2</sup>
KER-CHI-C2	Langmuir	$q_{max}$ (mg·g <sup>-1</sup> )	18.35	0.97
		$k_L$ (L·mg <sup>-1</sup> )	0.05	
	Freundlich	$k_f$ ((mg·g <sup>-1</sup> )·(L·mg <sup>-1</sup> ) <sup>1/n</sup> )	1.81	0.98
		$n$	1.95	
KER-CELL-C2	Langmuir	$q_{max}$ (mg·g <sup>-1</sup> )	12.09	0.99
		$k_L$ (L·mg <sup>-1</sup> )	0.13	
	Freundlich	$k_f$ ((mg·g <sup>-1</sup> )·(L·mg <sup>-1</sup> ) <sup>1/n</sup> )	3.89	0.92
		$n$	3.85	
KER-C2	Langmuir	$q_{max}$ (mg·g <sup>-1</sup> )	55.71	0.92
		$k_L$ (L·mg <sup>-1</sup> )	0.07	
	Freundlich	$k_f$ ((mg·g <sup>-1</sup> )·(L·mg <sup>-1</sup> ) <sup>1/n</sup> )	7.76	0.86
		$n$	2.1	

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1 According to the results depicted in Figure 4, the high adsorption capacity (55.71 mg·g<sup>-1</sup>) obtained in this work for RB4 dye is comparable to other adsorbents reported in the literature, such as chitosan-glutaraldehyde beads (1.8 mg·g<sup>-1</sup>)<sup>45</sup> and cellulose-epichlorohydrin polymers (69.8 mg·g<sup>-1</sup>)<sup>57</sup>.

## 6 Conclusions

7 In this study, a sustainable approach for keratin recovery from chicken feather waste was employed, allowing to prepare keratin-based films, either in their pure form or blended with chitin and cellulose. Various processing conditions were investigated to improve the film mechanical properties. Our findings confirmed that keratin-based films' mechanical properties, particularly the tensile strength, can be improved, principally by increasing the protein concentration and by adding glycerol or with keratin-chitin blends.

16 To evaluate the use of keratin-based films as adsorbent materials, RB4 adsorption tests were performed at different conditions. RB4, a toxic and widely used dye in the textile industry, was efficiently removed by decreasing the pH. Moreover, the Langmuir isotherm better described the obtained experimental results, indicating that the RB4 adsorption occurs in a monolayer and is of a chemical nature. Overall, the maximum adsorption capacity obtained in this work (55.71 mg·g<sup>-1</sup>) for KER-C2 film confirms the efficiency of keratin as an adsorbent due to its functional groups (e.g. e.g. -NH<sub>2</sub>, -COOH, -SH, and -OH). As future steps, evaluating the recycling and reuse of keratin-based films (e.g. by desorption) is crucial to attend to a more sustainable process.

29 Overall, this work brings new perspectives for chicken feather waste valorisation, recovering keratin and processing keratin-based films (pure or blend with cellulose or chitin) that can be successfully used as adsorption biomaterials. This attempt shows the promise of keratin-based films for dye removal, addressing the possibility of chicken feather' economic valorisation and overcoming the challenging removal of water-soluble dyes using a renewable, sustainable, and low-cost biomaterial. Notably, our investigation also marks the first reported results on the development of keratin-chitin films, proving that chitin can be used instead of chitosan, and whose industrial production still produces high amounts of liquid effluents from the deacetylation of chitin with concentrated NaOH<sup>58</sup>.

## 43 Author Contributions

43 Conceptualisation, C. P., J. H., H. P., P. Y. S. N., J. A. P. and M. G. F.; methodology, C. P. and P. Y. S. N.; writing – original draft preparation, C. P.; writing – review and editing, J. H., H. P., P. Y. S. N., J. A. P. C. and M. G. F.; supervision, J. H., H. P., and M. G. F.; funding acquisition, J. H. and M. G. F.; project administration, J. H. and M. G. F. All authors listed have made a substantial, direct, and intellectual contribution to the work and agreed to the published version of the manuscript.

## 47 Conflicts of interest

47 There are no conflicts to declare.

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## 61 Notes and References

61 NMR spectra of [N<sub>111(20H)</sub>][C<sub>1</sub>CO<sub>2</sub>], speciation of the RB4 dye according to pH changes, and physicochemical properties of keratin-based films.

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