Chapter 12

Glandless Cottonseed Protein for Environmentally Friendly Bioplastics

Hangbo Yue, Guoqiang Yin and Yingde Cui

Additional information is available at the end of the chapter

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Abstract

Environmentally friendly bioplastics have attracted renewed attention over the last few decades due to the ever-growing awareness of the environmental impact of petroleum-based polymers and the rising costs of raw materials. Cottonseed protein (CP) extracted from cottonseed meal has abundant amino acid components and nutrition value, but are not carefully considered in non-feed industries. For the purpose of being explored, glandless cottonseed flour is utilized in this work, as a raw material, to prepare cottonseed protein bioplastics (CPBs) as environmentally friendly products. The optimum synthesis conditions of CPBs were firstly investigated, followed by the analysis of protein modification and cross-linking mechanism, with a close view on changes of their micro- or chemical structures. Detailed morphologies, element composition and biodegradabilities of CPBs were characterized via scanning electron microscopy, energy dispersive spectroscopy (EDS) and soil-burial test, respectively, pointing out its structural heterogeneity as well as nature of biodegradability. Characterizations of the thermal stability and thermomechanical relaxation of thermal-treated CPBs and its interaction with molecule revealed the presence of different thermal relaxation behaviours and different water states. Concluding remarks shortly summarize the importance of the work and point out possible solutions to addressing future potential challenges.

Keywords: biodegradability, bioplastics, cottonseed protein, synthesis, water states

1. Introduction

The soaring consumption of organically synthesized polymers in agriculture and a wide variety of industries around the globe poses a great threat to our living environment, mainly

because of the non-biodegradability of such polymers. The increased awareness of environmental impact of using petroleum-derived polymers and the rising costs of chemical raw materials shift people's attention to seeking out environmentally friendly polymers for sustainable development. In the course of this searching over the last few decades across the world, protein-based bioplastics (PBBs) have attracted renewed attention due to the following reasons: they can be completely degraded through regular composting, readily obtained from abundant natural resources (i.e. plant and animal proteins such as soy, corn zein, wheat gluten, sunflower, peanut, cottonseed, milk casein, fish gelatin, feather quill and serum albumin) [1–4], as well as easily modified and manufactured. All these advantages together make PBBs suitable alternatives to petroleum-based plastics, which are being considered or have already fabricated in many environmentally sensitive industries, including agriculture (e.g. mulch films, greenhouse films, flower pots, planting pots, etc.), green packaging (one-time or short-term use before disposal) and possible biomedical industries (e.g. soft-tissue scaffold, superabsorbent) [2, 5–7].

In addition, tons of wasted protein from cottonseed, feather quill, soya residue and animal keratin are being utilized to fabricate bioplastics, which have even emerged as a new family of renewable and sustainable plastics, known as 'Second-generation bioplastics' [1, 8, 9] owing again mostly to their low cost, availability and biodegradability.

Cottonseed proteins (CPs) extracted from cottonseed meal after degreasing and peeling process contain rich amino acid components enjoying nutrition value, thereby they were used mostly as dairy-cattle feeding products. However, this type of protein has not been considered as non-feed industries, especially being used as a raw material for producing biodegradable plastics. The main component of cottonseed protein is globulin, approximately 90%, which contains 60% of globulins with gossypin (11S) and congossypin (7S) and 30% of albumins (2S) [10], resembling soy protein, the most studied material for bioplastic synthesis. In addition, the processability of CP with respect to plasticizer efficiency (PE) calculated from the relative contents of amino acids was similar to that of other proteins that have already been synthesized into various bioplastics [9]. Good amino acid composition together with a relatively high PE value (>5) makes cottonseed protein a good candidate as a renewable raw material for bioplastics production. However, little research to date has been carried out on cottonseed protein bioplastics (CPBs) while most studies focus on soy protein and wheat gluten bioplastics. Casting of CP films has been achieved by Marquié [11], but this process is time consuming and film formation is very limited. On the contrary, hot compression moulding is a more efficient strategy in achieving protein films.

In this chapter, a series of CPBs were synthesized starting from glandless cottonseed flour purified from cottonseed meal that was subjected to the processes of protein denaturation, plasticizing, cross-linking and hot compression; they showed good biodegradability, thermal stability and a low degree of water absorption (WA), but they are structurally heterogeneous even after thorough mixing and dispersion. The results of this study should provide important theoretical guidance on the development of environmentally friendly protein-derived bioplastics with improved properties, and necessary steps to pave the way towards expanding non-feed industry of cottonseed protein after oil extraction.

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2. Synthesis of glandless CPBs

2.1. Optimum synthesis conditions

The synthesis route of the bioplastics is demonstrated in **Figure 1**. A typical synthesis experiment includes the following steps in sequences: (i) glandless cottonseed flour was placed into a beaker where deionized water was added at a solid-liquid weight ratio of 1:6, followed by the addition of 1-M urea solution. Denatured cottonseed protein (DCP) was obtained after the mixture was agitated by a magnetic stirrer for 4 h at room temperature; (ii) using 1 N NaOH solution, acidity/alkalinity of the DCP solution was adjusted to pH 11 with stirring for 10 min; (iii) the alkaline solution was kept in a water bath at 70°C, stirred for a further 30 min, and a cross-linking agent (formaldehyde (FA), glyoxal (GX) or glutaraldehyde (GA)) at 10 wt% of cottonseed flour was added; (iv) the resultant mixture was vacuum-dried for 10 h at 80°C prior to further fabrication; (v) glycerol was added to the dried denatured protein and then homogenized in a high-speed mixer (HR1704, PHILIPS Ltd.) for 5 min; (vi) the mixture was then ground and processed for three to five times using a three-roller (at a speed of 9:3:1) mill with the clearance of the outlet roller less than 1 mm; (vii) this mixture was then further conditioned in a desiccator at room temperature for 24 h; (viii) the conditioned mixture was placed on the surface of a stainless steel plate covered with aluminium (Al) foil with a layer of Al foil mounted on the back side; then the mixture was hot pressed at 20 MPa, 130°C for 5 min; and (ix) after cooling, the prepared CPBs were carefully removed from the mould, and then stored in desiccators at room temperature for further uses.

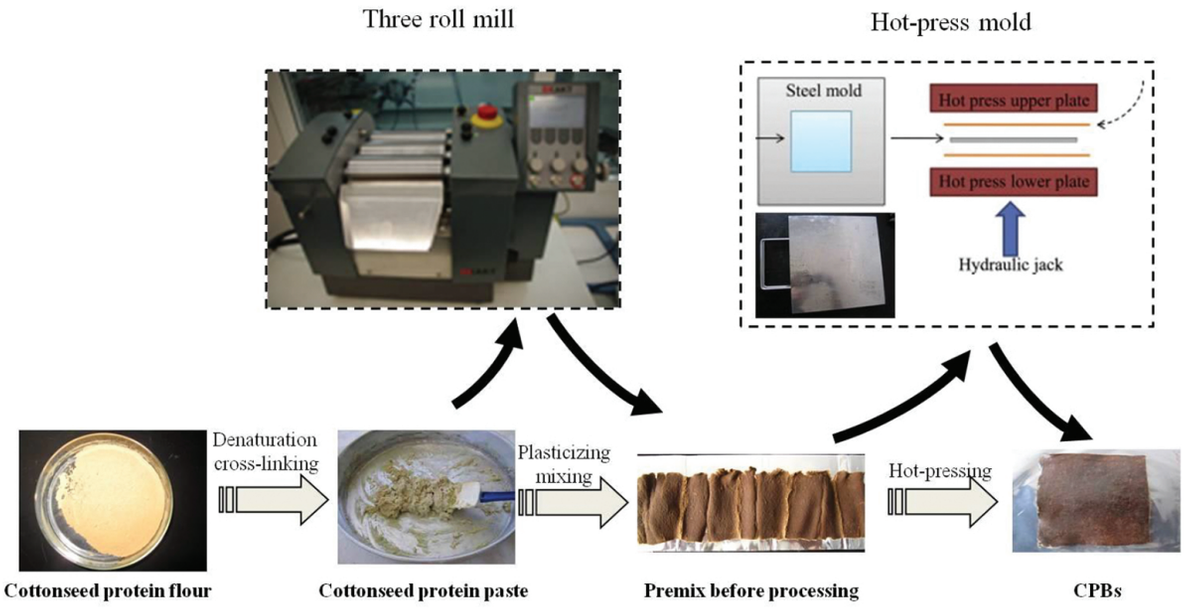

Figure 1. Diagram of cottonseed protein bioplastic preparation, primarily including protein denaturation and cross-linking, plasticizing, homogenization and hot-press moulding.

Regulation and optimization of synthesis and processing conditions are particularly important for improving the comprehensive properties of the bioplastic products. Macroscopic proper-

ties and/or microscopic structure of the CPBs are influenced by a series of experimental conditions: the CP flour dosage, solid-liquid ratio of protein solution, concentration of the denaturation agents (urea), cross-linker (aldehyde) content, concentration of the plasticizer (glycerol), reaction temperature and solution pH value. At the same time, a good mixing and homogeneous dispersion of the precursors are necessary while the hot-press processing parameters (temperature, pressure, time) are reasonably controlled. The optimum CPB synthesis conditions were obtained and summarized as follows: initially, cottonseed protein was denatured using a urea solution, which was then adjusted to pH 11 with NaOH solution, the aldehyde cross-linking agent added, and the mixture vacuum-dried for 10 h at 80°C. Glycerol as plasticizer was then added homogeneously to the dried solid using both a high-speed mixer and a three-roller mill. This mixture was conditioned in a desiccator at room temperature for 24 h and then hot pressed at 20 MPa, 130°C for 5 min.

2.2. Mechanism of protein modification and cross-linking

2.2.1. Protein denaturation induced by urea

A schematic illustrating the denaturation of cottonseed protein in the presence of urea is depicted in **Figure 2a**. It is shown that the process of protein denaturation involves both indirect and direct hydrogen bonding between urea molecules and water molecules as well as protein macromolecules, which has been confirmed by Bennion and Daggett [12] using atomic-resolution molecular dynamics simulation. In case of the indirect denaturation, the presence

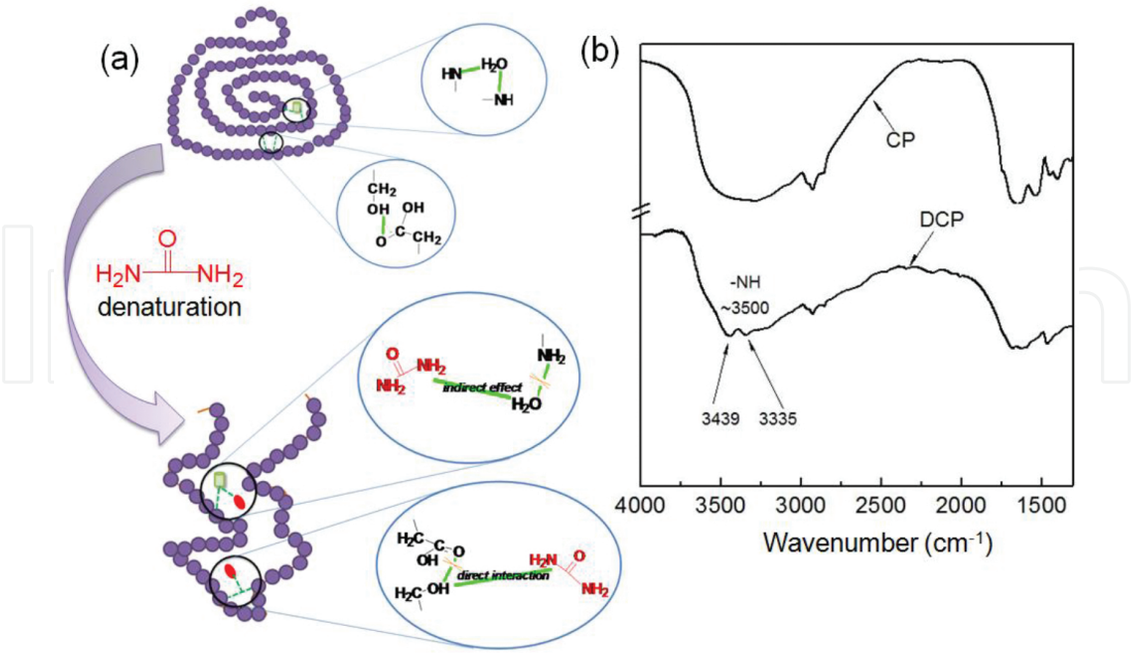

Figure 2. (a) Schematic illustration of the urea-induced CP denaturation via indirect and direct interactions and (b) FTIR spectra of pristine and denatured CP [15]. Reproduced with permission from The Royal Society of Chemistry (2012).

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of urea weakens the cohesion of water molecules by reducing the water-water and/or water-protein interactions; as a result, water diffusion is decreased, thus exposing more functional groups in side chains so as to increase water-urea hydrogen bonding. As for the direct denaturation, urea molecules interact with the peptide backbone as well as polar moieties of the protein via direct hydrogen bonding; in particular, the strength of hydrogen bonds between urea and the peptide backbone of CP will largely increase on the condition that the secondary structure of CP is disrupted.

Figure 2b shows Fourier transform infrared (FTIR) spectra of the cottonseed proteins (pristine and denatured CP). Clearly, amide N—H-stretching vibration bands for the CP appear at around 3500 cm^{-1} as a broad absorption, whereas two bands at similar region (3439 and 3335 cm^{-1}) are observed for the denatured cottonseed protein being assigned to the amide N—H-stretching vibration bands in CP and urea, respectively. In addition, the appearance of absorbing band ranging from 1630 to 1680 cm^{-1} can be assigned to the C═O stretch vibrations of the peptide linkages of amide I, the most sensitive spectral area of protein secondary structure [13].

On the contrary, the characteristic amide II band at 1480–1575 cm^{-1} is attributed to in-plane N—H bending and C—N-stretching vibrations. As the position of absorbing amide I and amide II bands is very sensitive to the secondary structure of the protein, the frequency of these bands would be mainly dependent on the hydrogen bonds between C═O and N—H groups. The presence of urea has a noticed impact on the characteristic absorbing band position. For instance, the amide I band shifts from 1648 cm^{-1} (CP) to a lower frequency, 1618 cm^{-1} (DCP), and the amide II band shifts from 1541 cm^{-1} (CP) to 1467 cm^{-1} (DCP), as shown in **Figure 2b**.

2.2.2. Protein cross-linking with aldehyde

Three aldehydes (formaldehyde, glyoxal and glutaraldehyde) react with cottonseed protein molecules in a quite different manner, creating the cross-linked structures. Specifically, methylene bridge formation is responsible for the formaldehyde cross-linked networks (**Figure 3a**) [14]; however, the formaldehyde polymer (in FA solution) cannot generate further methylene bridges. As for the glutaraldehyde cross-linked networks (**Figure 3b**), both GA monomer and GA polymer are able to react with protein molecules via Maillard-driven generation of the imine covalent bond formation [15]. Glyoxal maintains the same mechanism as GA.

This reaction can be accelerated with the increased nucleophilicity as long as the amino groups are deprotonated. In this work, the carbonyl groups in the aldehydes react with the amino groups within CP under an alkaline condition at elevated temperature, leading to the formation of an imine group (—CH═N—), ultimately improving the thermal stability, mechanical strength and moisture resistance of the CPB bioplastics. In addition, Maillard-driven reaction explains the changes in colour and odour [15] before and after the hot press. **Figure 1** shows the colour of the CPBs changing from yellow-brown before preparation to golden-brown after hot compression moulding. Interestingly, the odour was not the same after hot-press moulding. For example, CP-GA smelled like persimmon while the smell of the CP-FA CPBs was a bit malodourous.

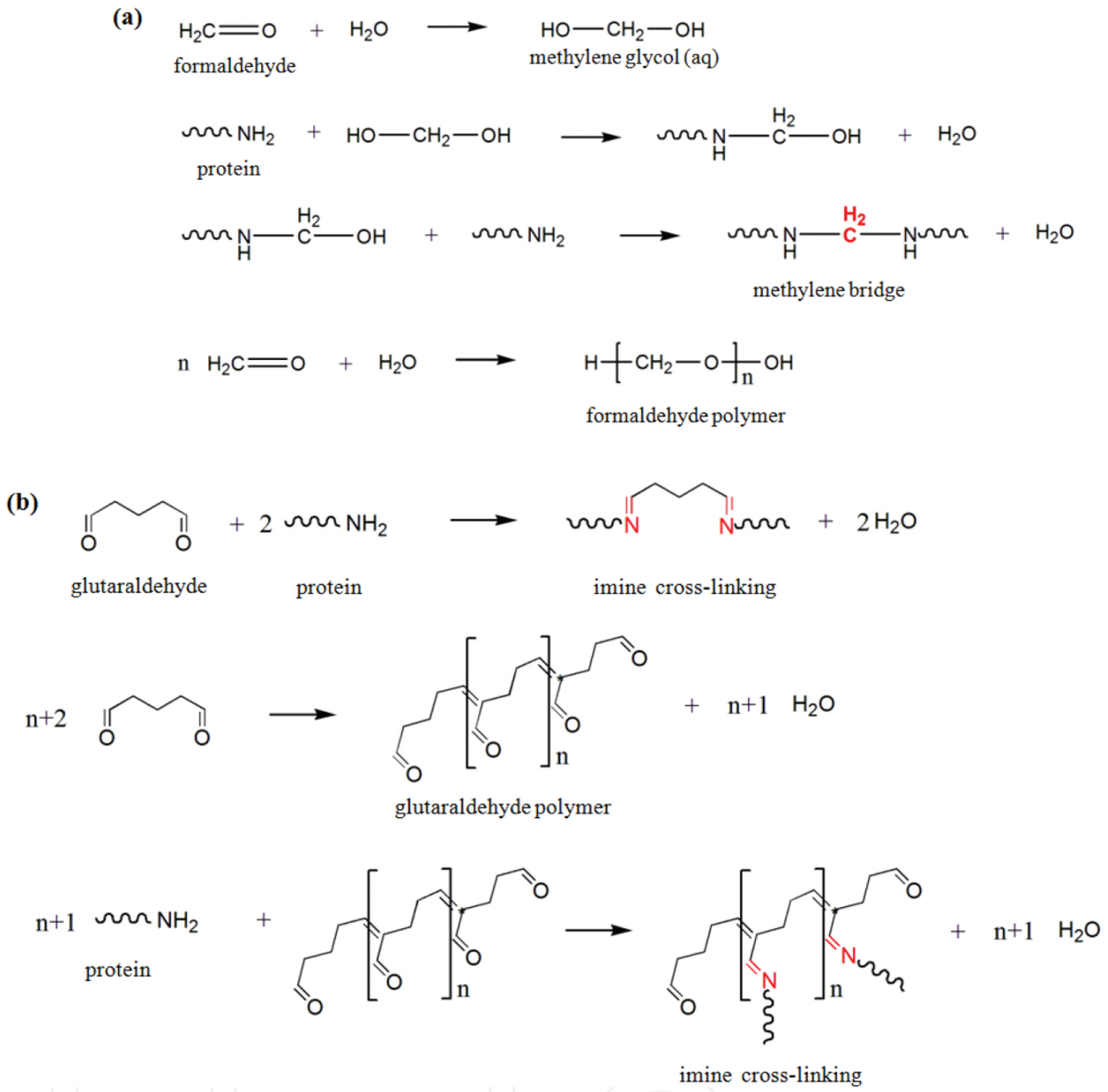

Figure 3. Cross-linking reactions of protein macromolecules with formaldehyde (a) showing the formation of methylene bridge and glutaraldehyde monomer/polymer, and (b) creating imine covalent bonds.

3. Morphology, element composition and biodegradability

Figure 4 shows scanning electron microscopic (SEM) micrographs of the CPBs, including their surface morphology and fracture surface. A fluctuated and continuous structure can be observed from the surface morphology of the cross-linked samples (CP-FA, CP-GX and CP-GA CPBs), compared to the cracks appeared at the smooth surface of CP-0CL indicating its brittle nature. Moreover, the height of the asperities at the fractured surface indicates a ductile

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failure behaviour for the cross-linked bioplastics, whereas it is discontinuous for the CP-0CL structure.

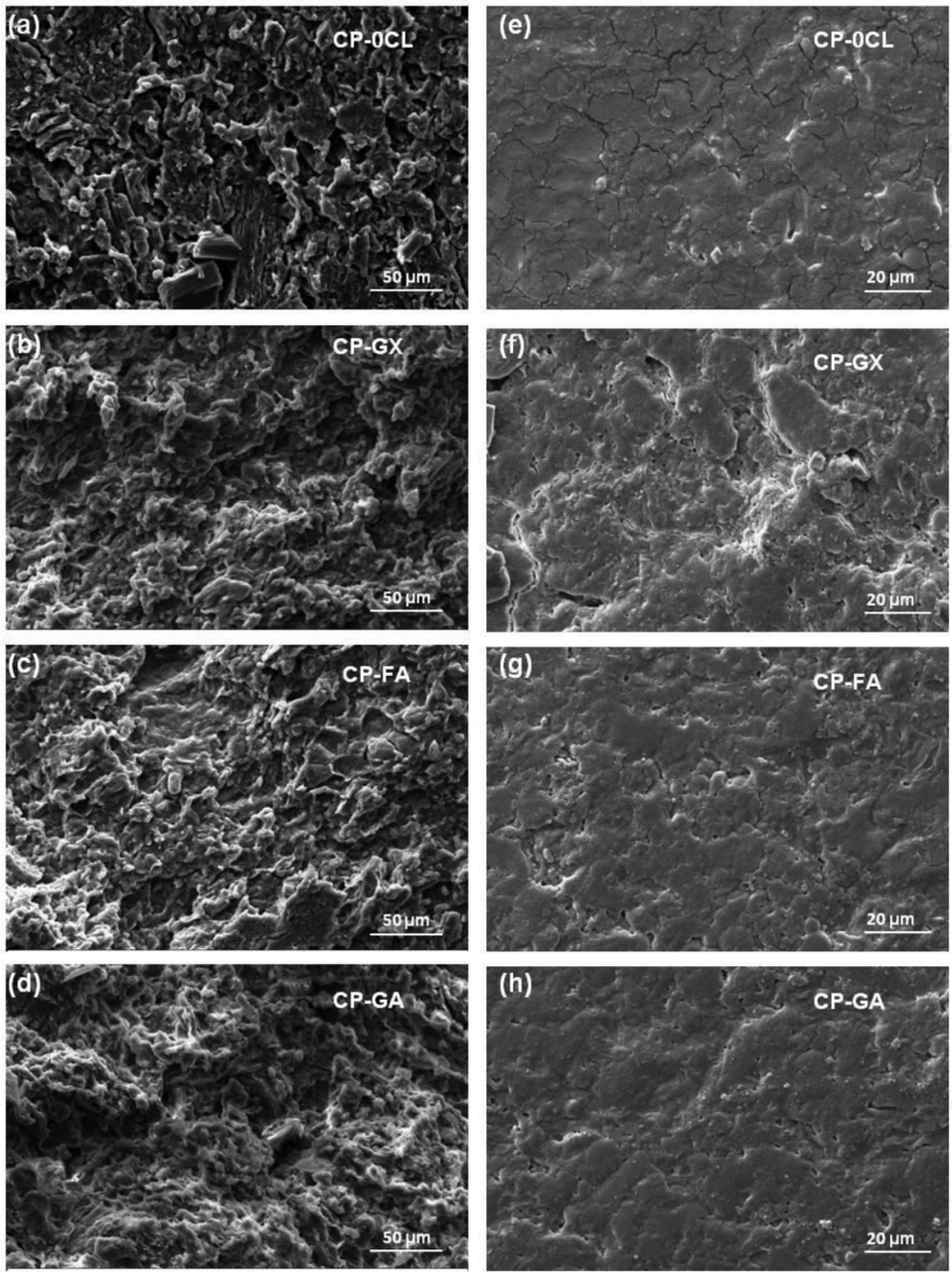

Figure 4. SEM images of the fractured microstructure (a–d) and the surface morphology (e–h) of the CPBs (CP-0CL, CP-FA, CP-GX and CP-GA, respectively) [15]. Reproduced with permission from The Royal Society of Chemistry (2012).

In addition to the ductile characteristics of the cross-linked samples, they exhibit a certain degree of heterogeneity within the whole networks with respect to their element composition distributed as well as the presence of unreacted or partially reacted aldehydes with variable concentration.

3.1. Element composition and heterogeneity feature

The element composition of the CPBs is detected by X-ray energy dispersive spectroscopy (EDS), as shown in **Figure 5**. The dominant presence of three elements, carbon C, nitrogen N and oxygen O, is at an atomic ratio of 4:1:1, or 3:1:1 in weight, while three other elements, magnesium Mg, sulphur S and phosphorus P, are present at a trace amount (weight percentage of <2%, atomic percentage of <1%). All the elements are randomly distributed on the measured surface, especially for Mg, S and P, as can be seen from element mapping (**Figure 6**), suggesting

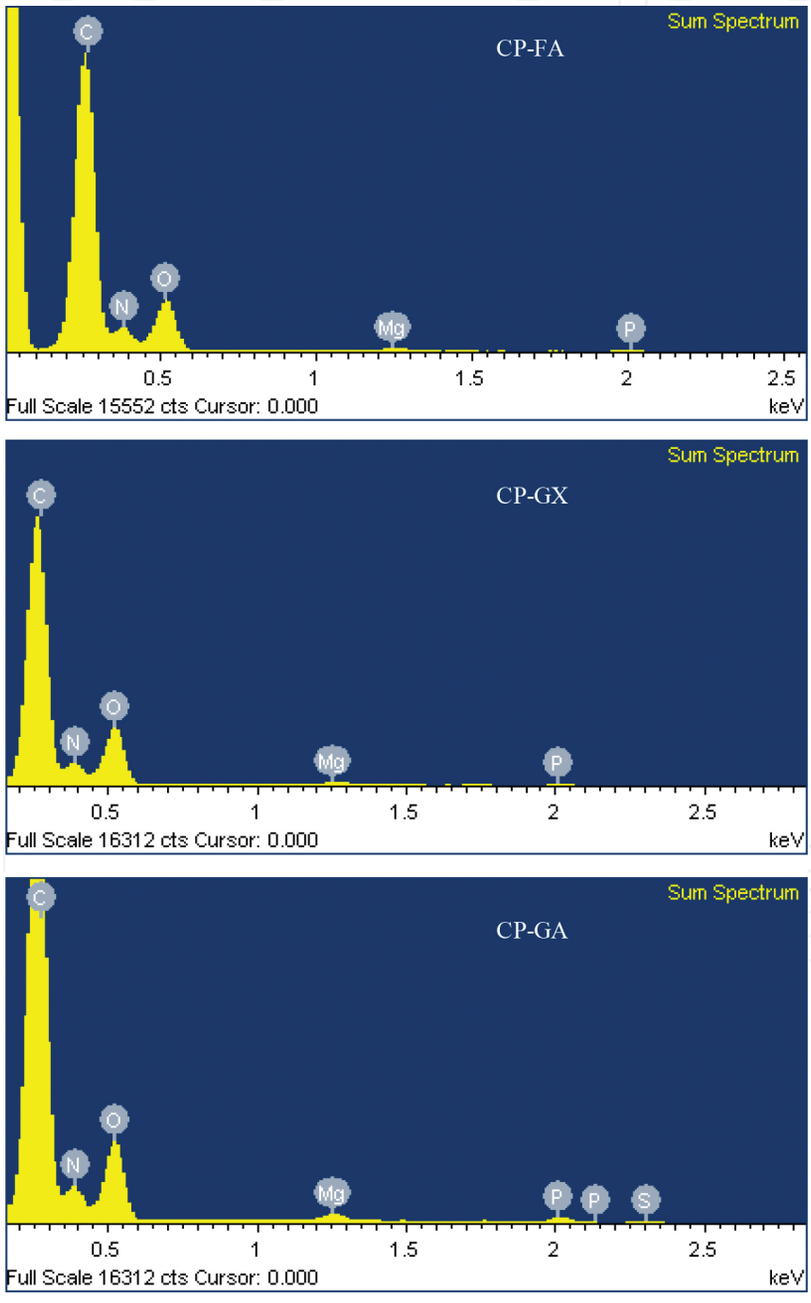

Figure 5. EDS-SEM analysis of the bioplastics, showing all the elements detected.

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a characteristic of heterogeneity of the bioplastics. This characteristic is further confirmed by its infrared spectra collected from different locations across the sample surface at micro-scale resolution.

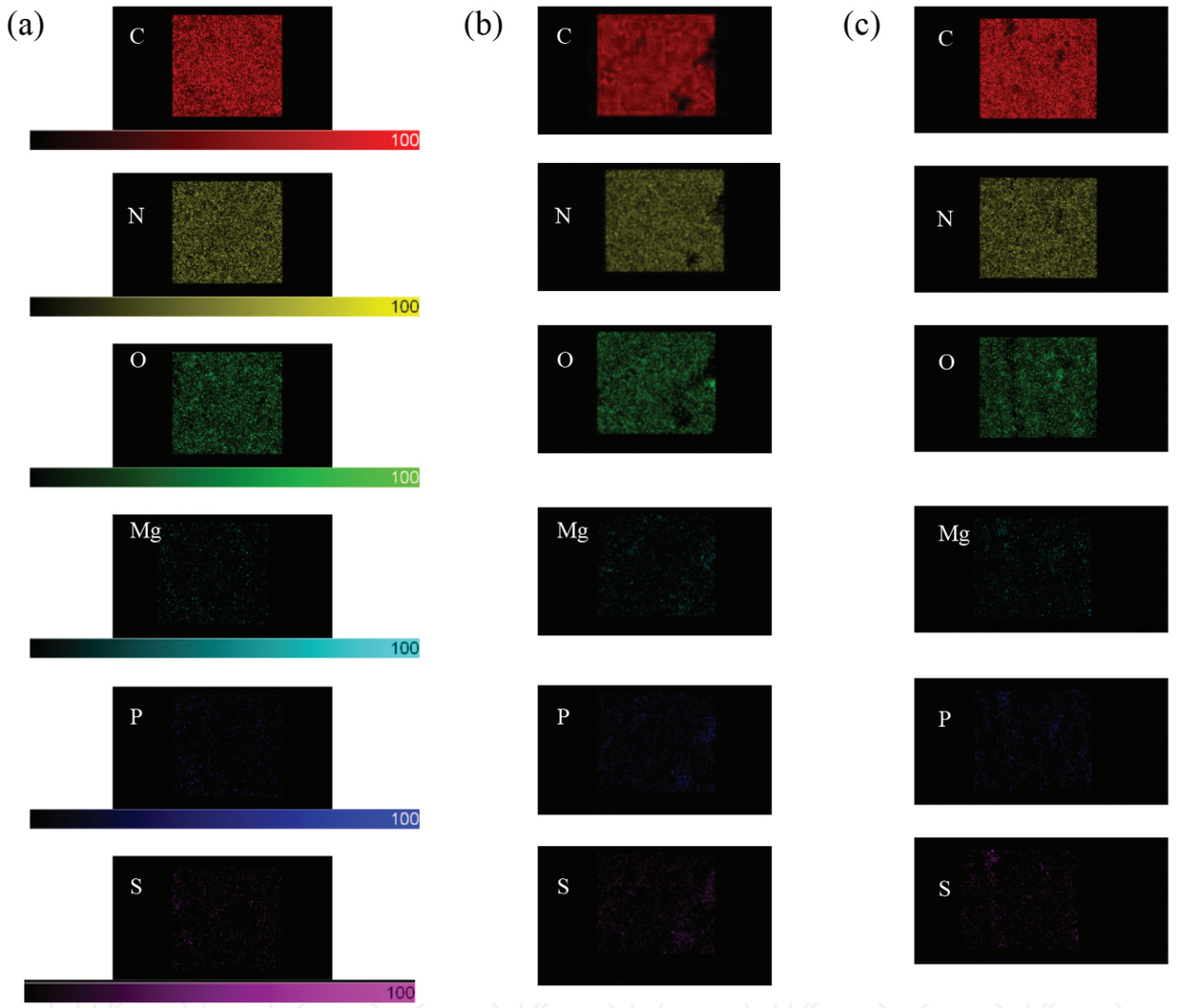

Figure 6. EDS-SEM analysis using element mapping of (a) CP-FA, (b) CP-GX and (c) CP-GA.

Figure 7 shows infrared spectra recorded from various positions on the different samples using an attenuated total reflection (ATR) objective with a Ge crystal with a contact surface of 100-μm diameter. It can be observed that for each cross-linked sample, heterogeneity is observed, notably reflected in the relative intensity of the band appearing around 1697 cm^{-1} associated with carbonyl-stretching vibration (marked) of unreacted or partially reacted aldehyde distributed with variable concentration in the CPB networks.

To develop environmentally friendly protein-derived bioplastics with desired optimized properties, it is expected that a homogeneous distribution of all elements within the sample network and a balanced formula with respect to ideal chemical reaction (in stoichiometry)

involved in the synthesis process are initially important. Future studies addressing these two issues are currently under investigation.

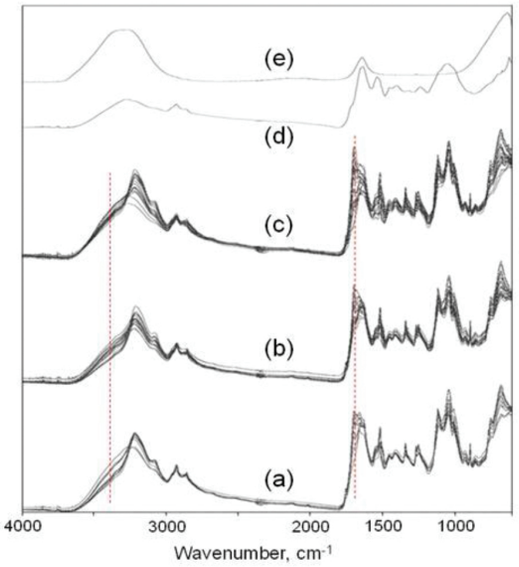

Figure 7. Infrared spectra recorded from different positions of samples (a) CP-FA, (b) CP-GX and (c) CP-GA. Spectrum of (d) water and (e) CPL-0CL is shown for comparison.

3.2. Biodegradability under natural-storage and soil-burial conditions

Biodegradability of the synthesized bioplastics was verified under both natural-storage and soil-burial conditions. Fungi growth on the surface of the CPBs after 6 months under ambient condition is pictured in **Figure 8a** at two different magnifications. It can be seen that filamentous fungi, commonly known as moulds growing as multicellular colonies, lives by absorbing nutrients from the contacting organic matter (the bioplastic). In fact, the natural-storage condition—room temperature and 40% relative humidity—is an unfavourable environment for mould growth for the reason that, on one hand, only a limited number of filamentous fungi such as *Bacillus subtilis* and *Aspergillus niger* are actually available in air; on the other hand, the sample surface starts to unevenly dehydrate and dry due to the evaporation of moisture in the desiccators storing the CPBs, which is unpleasant for moulds to live thus covering only part of the sample surface. However, in spite of its small quantities, the microbial biomass can live on with the carbon source stored in the sample, indicating that the cottonseed protein-derived bioplastics is biodegradable to a certain extent. Still, it is necessary to use a more standard test of biodegradability for the synthesized bioplastics—for example, the measurements in a nutrient agar culture medium, which contain a large number of microbial fungi (e.g. *B. subtilis*, *Staphylococcus aureus*, *Bacillus*, *Escherichia coli*, *Aspergillus flavus*, *A. oryzae* and *A. niger*) to degrade almost all organic matter under ideal conditions.

Figure 8b illustrates how the biodegradability of the synthesized bioplastics was measured in soil environment. Specifically, soil was firstly collected at the campus of Zhongkai University of Agriculture and Engineering, crushed, sieved and transferred to a wide-mouth bottle.

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Secondly, the samples were carefully added in the bottle in the way that they were buried in parallel in the soil. Then, the bottle was kept in an incubator and thirdly the samples were taken out after a certain period of time, rinsed with 75% ethanol solution as well as deionized water, dried and weighed. The ability of soil to degrade organic matters is often quantified by the weight loss (%) of the sample after the soil-burial test, shown as

$$\text{Weight loss} = \frac{W_a - W_b}{W_b} \times 100\% \quad (1)$$

where W_b and W_a are the weight of the bioplastic sample before and after the soil-burial test, respectively. Background experiments for comparison, the same bioplastics, were stored in an empty wide-mouth bottle under the same condition, and their weight loss measured using Eq. (1) following exactly the same procedure as described above. **Figure 8c** shows the weight loss of a typical CPB bioplastic after the soil-burial measurement as a function of the recorded degrading time. It can be seen that the bioplastic degrades fast in the first 30 days, losing its weight up to 40%, followed by no obvious changes of its weight in the following month. The experimental data are fitted with multiple regression fitting, written as Weight loss = 0.1192

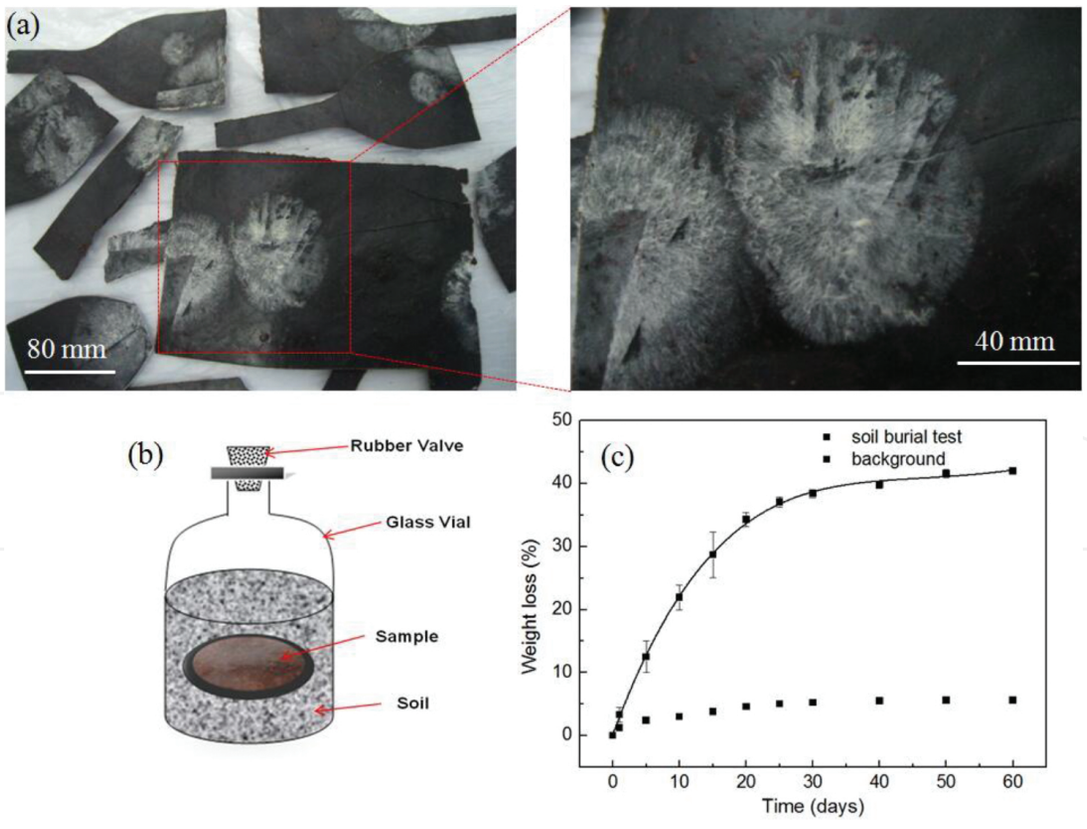

Figure 8. Biodegradability test of the CPB bioplastics. (a) Microbial fungi growth after 6 months under natural-storage condition (at room temperature and 40 RH%); (b) schematic of soil-burial test and (c) results of weight loss (%) of the bioplastics measured by soil-burial test referenced with a background.

+ 2.8908t − 0.0772t^2 + 0.0001t^3, where t is the number of days of degradation. The weight loss of the cross-linked (CP-GA) and non-cross-linked (CP-0CL) bioplastics, after 30 days of being buried in the soil, are 28 and 45%, respectively, indicating that the cross-linked treatment can effectively decrease the degree of biodegradation. It should be pointed out that the cottonseed protein reacts with the aldehyde under certain circumstances (Section 2.2.2), creating a high-density cross-linked network, in which the cross-linked/entangled macromolecules and their chains contribute to resisting the invasion of the microorganisms.

4. Thermal stability and thermomechanical relaxation

4.1. Thermogravimetry analysis of thermal stability

Thermal stability of glandless cottonseed protein bioplastics was evaluated by thermogravimetry analysis (TGA), which was carried out on a TG 209 under nitrogen atmosphere (protective gas flow was 15 ml min^{-1}) at a heating rate of 30°C min^{-1} from 25 to 500°C. Overall, the cross-linked bioplastics (CP-FA, CP-GX and CP-GA) have less mass loss compared with CP-0CL over the tested temperature range, implying an improvement to thermal stability. The formation of strong imine covalent bonds for the cross-linked samples after the hot press should account for the improved thermal stability, similar to the cross-linked soy protein bioplastics [16].

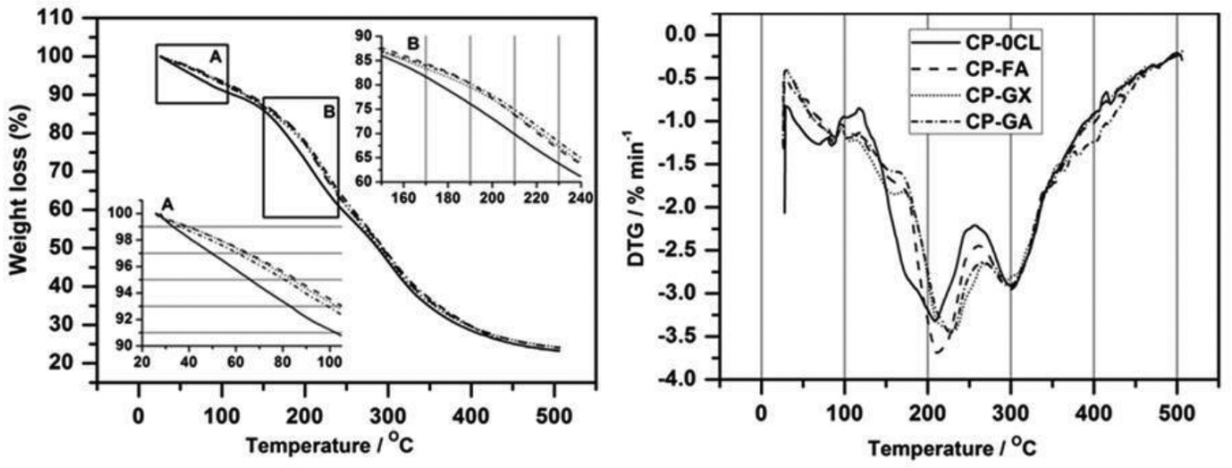

Figure 9. Weight loss and DTG curves of the CPBs as a function of temperature. Inset graphs denote the first stage (A) and second stage (B) of the CPB weight loss [15]. Reproduced with permission from The Royal Society of Chemistry (2012).

Specifically, three distinct stages of mass loss for the CPBs are observed from the TGA curves in **Figure 9**. In the first one (Inset graph A, **Figure 9**), less than 10 wt% of sample mass losses between room temperature and 100°C, mainly because of the evaporation of moisture previously absorbed by samples. In the next stage from 160 to 230°C (Inset graph B, **Figure 9**), the CPBs decomposed rapidly with a mass loss of 20–40 wt%, which is due to the decompo-

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sition of small molecules (glycerol and urea residues), as verified by Zhang et al. [17]. In the last one, decomposition of CP occurs at temperature of >260°C, in which the volatile molecules such as CO_2, CO and NH_3 are released as a consequence of degradation. Unsaturated compounds with carbonyl groups may also present in this stage, according to the study carried out by Schmidt et al. [18] using FTIR spectra.

It is also interesting to see that the CP-FA sample showed the least mass loss at temperature below 190°C, suggesting the best thermal stability among all the CPBs, while CP-GA was the best at temperature above 190°C. This might suggest that the interactions between CP and GA are greater than that between CP and FA with increasing temperature, probably due to the higher cross-linking efficiency of GA compared to FA at elevated temperatures [19].

4.2. Differential scanning calorimetry and dynamic mechanical analysis of thermal relaxation behaviours

To investigate the thermal relaxation behaviours of glandless cottonseed protein bioplastics, both differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA) were carried out; see the detailed experimental testing conditions in the published papers [15, 20]. **Figure 10a** shows a baseline increase from the minima at ~150°C, suggesting a complete denaturation of the pristine CP within the bioplastic matrix, in contrast to other proteins in particular soy protein, which exhibited two similar temperature characteristic of gossypin and congossypin globulin fractions [21]. In addition, all the modified CPBs regardless of the type of aldehyde cross-linking agent used showed such trend at similar denaturation temperature (T_d). Since protein macromolecules comprise a large number of amino acid species, multiple movements simultaneously occur during the CP denaturation including hydrogen bonding, dipole-dipole, charge-charge and hydrophobic interactions. As a consequence of these

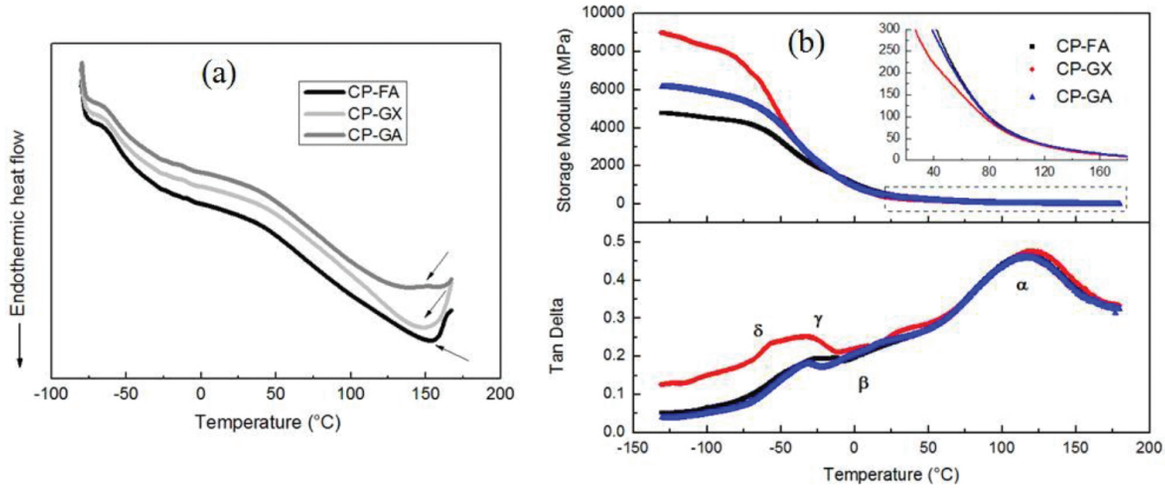

Figure 10. DSC (a) and DMA (b) thermograms of the aldehyde cross-linked CP-FA, CP-GX and CP-GA CPBs. The temperatures marked in DSC curves indicate the T_d. Inset is the enlarged plots marked by dashed rectangle [20]. Reproduced with permission from The Royal Society of Chemistry (2014).

interactions, insoluble protein aggregation is often formed, which is, however, accompanied by a low transitional energy [22], thus making it difficult to detect using DSC.

Dynamic mechanical analysis has better sensitivity to detect thermal events such as thermo-mechanical relaxation of macromolecule chains, similar to the sub-T_g transitions. Denaturation temperature of the cross-linked CPBs can be clearly identified from DMA curves (**Figure 10b**) at T_d about 120°C, corresponding to the α-transition temperature. In line with DSC results, DMA signals suggest that cross-linking leads to little change in T_d, meaning that the three aldehydes—formaldehyde, glyoxal and glutaraldehyde—cross-linked network have a similar density, which is nonetheless dependent on the type of plasticizer [23]. A small β-transition peak at ~0°C, shown in the Tan δ graph, **Figure 10b**, is probably associated with the absorption of moisture from air. A similar behaviour occurring at a relative humidity of >35% for soy protein-glycerol-water ternary systems was observed by Zhang's group [17].

The large decrease/increase in E' from DMA graphs usually suggests the occurrence of glass transitions of macromolecules. Storage modulus, E' profile shown in **Figure 10b**, indicates a broad glass transition irrespective of the type of cross-linking agent used, specifically showing two transitions below 0°C from the tan delta curves—γ and δ observed at approximately -25 and -55°C, respectively.

It is well acknowledged that protein-based bioplastics have more than one T_g [17, 24] due to its heterogeneous feature. A sub-T_g transitional behaviour is apparent for the CP-GX sample at the start of the E' decrement at approximately -100°C, but is less obvious in the other samples. Changes in storage modulus at low temperatures are more severe: the highest E' value (~8500 MPa) was found for CP-GX and the lowest for CP-FA (~4500 MPa) at -140°C. However, after the glass transition, a slightly lower value of E' for CP-GX may suggest a lower cross-linking density, implying longer molecular chain length between cross-links. This may lead to the increase of chain mobility favouring the formation of multiple intra- and intermolecular interactions such as hydrogen bonding, which would contribute to the mechanical reinforcement in the glassy state. Indeed, glyoxal is more easy to form hydrogen bonds with electronegative elements in the protein (O, S, N, etc.) than formaldehyde would, according to hydrogen-bonding theories [25].

5. Interaction between bioplastics and water

5.1. Water absorption and transportation kinetics

Water absorption of the CPB (CP-0CL, CP-FA, CP-GX and CP-GA) films was carried out according to ASTM D570-98 standards. Briefly, the pre-dried and weighed CPBs were firstly immersed in distilled water at room temperature, removed from the water at regular time intervals, dabbed with filter paper to remove excess water on the sample surface and the weight of the sample registered.

Water absorption (WA) is calculated using Eq. (2)

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$$\mathrm{WA}(\%) = \frac{W_t - W_0}{W_0} \times 100 \tag{2}$$

where W_0 and W_t are the initial weight of the bioplastics and the weight of the sample after being immerged in water for t min, respectively. It is found that water absorption of all bioplastics increased markedly over the first 2 h of immersion, followed by a gradual decrease in absorption rate in the subsequent 8 h. After this stage, water absorption reaches an equilibrium state, ensuring no further mass increase. It is found that cross-linking helps reducing water absorption, as the bioplastics (CP-FA, CP-GX and CP-GA) absorbed less water than the control sample (CP-0CL) over the course of testing period. These bioplastics have a more compact and tightly bound network, which limits the distance water molecules can diffuse within, and hence lowers the total capacity of water that can be absorbed; so a lower amount of water absorption is obtained, compared with the control sample.

Water absorption is a big concern, in general, for practical applications of protein-based plastics due to the fact that most proteins contain a big number of carboxyl and hydroxyl groups that are typically characteristic of hydrophilicity. For example, soy protein-derived plastics increase in mass by 78.66% after being immersed in distilled water for 2 h at room temperature [26]. Strikingly, the mass increase of the cottonseed protein-derived plastics (CP-0CL, CP-FA, CP-GX and CP-GA CBPs) studied in this work, under the same experimental conditions, was much lower (19.39, 16.35, 14.15 and 13.51%, respectively), suggesting a significant improvement in water resistance properties.

The results of CPB water absorption test were further analysed using theories of Fickian diffusivity, Liquid transport and Liquid permeability, to evaluate the liquid transit properties of the polymer network [20]. As rate indicators between polymer chain relaxation and water diffusion, values of the kinetic exponent n and characteristic constant of water absorption k vary from different bioplastics, with the n value increasing, whereas the k value decreases after addition of the cross-linking agent. Interestingly, a high diffusion coefficient D value for the cross-linked bioplastics indicates that water diffuses easily in the cross-linked networks according to the theory of Liquid transport. Furthermore, the findings based on the theory of Liquid permeability suggest that the presence of cross-linked structure promotes the physical transport process, including water diffusion and permeability, while that in the absence of cross-linking, it is chemical interaction such as hydrogen bonding or van der Waals interactions that facilitate water transport within the protein.

5.2. Different water states

Differential scanning calorimetry measurements were performed on a TA Instruments Q200 DSC under nitrogen atmosphere, in order to find out possible water states within the CPBs after their water absorption. In a typical experiment, a specimen (weighed ~5–6 mg) was placed into an Al pan and a known amount of water added by a micro-syringe. The pan was then hermetically sealed by an Al lid and conditioned for 24 h at room temperature to enable the water absorption states to equilibrate. The conditioned samples were first equilibrated at

-80°C for 10 min, and then heated up to 50°C at a rate of 10°C min^{-1}, with the same programme repeated three times to verify reproducibility. All the hermetic Al pans were weighed after the measurements to confirm no loss of sample weight during the experiment.

Water content ω_{H_2O} of the bioplastics was calculated as

$$\omega_{H_2O}(\%) = \left(W_{H_2O}^{add} / W_{CPBs}\right) \times 100 \tag{3}$$

where $W_{H_2O}^{add}$ and W_{CPBs} stand for weight of added water and that of the CPBs tested, respectively. W_{CPBs} is the total mass of the sample, namely the sum of the dried sample and the water added. Free water contains both freezable bulk water and that weakly bound to the polymer, and whose weight percentage $\omega_{H_2O}^{free}$ is expressed as

$$\omega_{H_2O}^{free}(\%) = \left(W_{H_2O}^{free} / W_{H_2O}^{add}\right) \times 100 \tag{4}$$

$$W_{H_2O}^{free} = \left(\Delta H_{W_{CPBs}} / \Delta H_{pure\ H_2O}\right) \times W_{CPBs} \tag{5}$$

where $W_{H_2O}^{free}$ is the weight of the free water component within the bulk sample, obtained by multiplying the weight fraction of water (determined as $\Delta H_{W_{CPBs}}/\Delta H_{pure\ H_2O}$) with the total mass of the tested sample (W_{CPBs}). Here, $\Delta H_{W_{CPBs}}$ is calculated by integrating the endothermic ice-melting peak, related to the total mass of the tested sample (sum of the dried sample and water added). $\Delta H_{pure\ H_2O}$ is the melting enthalpy of pure water; it equals to 365 J g^{-1} using distilled water as a reference for all samples in this study. The weight percentage of non-free water $\omega_{H_2O}^{non\text{-}free}$ is estimated from the difference between the weight of water added and that of free water:

$$\omega_{H_2O}^{non\text{-}free}(\%) = \left(W_{H_2O}^{non\text{-}free} / W_{H_2O}^{add}\right) \times 100 \tag{6}$$

$$W_{H_2O}^{non\text{-}free} = W_{H_2O}^{add} - W_{H_2O}^{free} \tag{7}$$

If no endothermic peak can be detected by adding very small amount of water, it is considered to be strongly bound to the polymer and thus non-freezable.

Figure 11 shows the change in the DSC fusion of water within the CPBs as a function of water concentration. The heat of fusion changes significantly for each sample by increasing the water

http://dx.doi.org/10.5772/64045

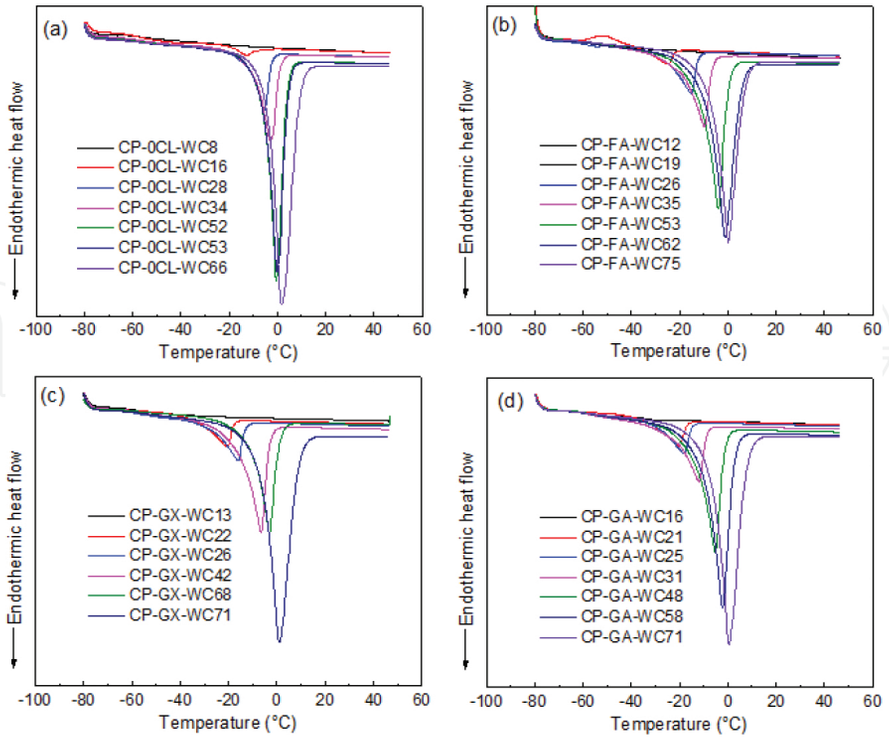

Figure 11. DSC thermograms showing the change in enthalpy and peak fusion temperature of the CPBs with respect to water content (WC, %). (a) CP-0CL, (b) CP-FA, (c) CP-GX and (d) CP-GA series [20]. Reproduced with permission from The Royal Society of Chemistry (2014).

content, from almost no endothermic signal at a water content below 15% to a strong response at water contents above 70%, causing a broad peak to appear centred around 0°C. Furthermore, increases in water content can bring about increases in the peak fusion temperature. For example, the heat fusion maximum temperature of the CP-FA sample changes by 25°C (from -24.9 to 0.1°C) as a result of the increase of the amount of water added, from 19 to 75%. We believe that the formation of water layers on/within the biomass surface largely depends on water content added into the bioplastics—being strongly bound to CPBs at a lower content, both strongly and weakly bound at a slightly higher concentration, and ultimately a ternary strongly to weakly bound to free system at an even higher water content (i.e. saturation).

The trend of the heat of fusion shown in **Figure 11** indicates that different states of water contained within the CPB structures exist, namely non-freezable water, free bulk-like water and freezable bound water, as reported for other hydrophilic polymers [27]. **Figure 12** schematically demonstrates the bonding ability of CPB polymer networks with the water molecules, correspondingly showing the presence of a fraction of strongly-bound-to-polymer water that could not freeze upon cooling, freezable weakly-bound-to-polymer water whose mobility is partially retarded and a certain amount of freezable bulk-like water.

With increasing water content within the CPB network, it is believed that water adopts at least three different states. At lower concentrations, water is present in strongly-bond-to-polymer state where it is strongly connected to protein polymer chains, and thus cannot freeze. When

the CPB system contains a content of water above a certain threshold, it appears as weakly-bond-to-polymer water, the intermediate state, suggesting a boundary where water molecules whose mobility is partially retarded could not sufficiently interact with the polymer chains to prevent the state of fusion, yet are able to crystallize. Lastly, excess water or bulk-like water is free of interactions with the protein chains, and is a predominant fraction at higher water contents.

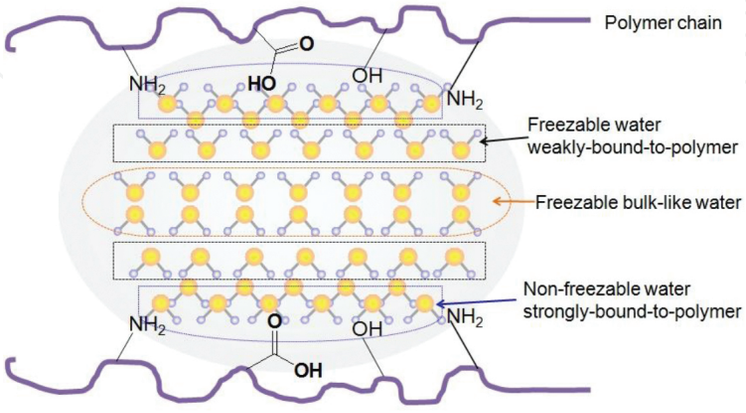

Figure 12. Schematic of CPB polymer chains affinity to water molecules, illustrating the presence of different states of water: freezable bulk-like water, freezable and non-freezable bound-to-polymer water [20]. Reproduced with permission from The Royal Society of Chemistry (2014).

6. Concluding remarks

Environmentally friendly bioplastics were successfully synthesized utilizing glandless cottonseed protein that was subjected to the processes of protein denaturation, plasticizing, cross-linking and hot compression; they are readily biodegradable, fairly thermal stable and resistive of water absorption while showing a fraction of the structural heterogeneity. The results presented in this chapter could be valuable for further development of protein-derived bioplastics with demanding properties as well as extending the value of cottonseed protein in non-feed industries. However, both the intrinsic heterogeneity feature and unsatisfied mechanical performance of protein-derived bioplastics (in general) impose large challenges to their large-scale industrial production. Future directions in this field may be taken as follows: (i) controllable design of protein-derived bioplastics with balanced properties—biodegradable, mechanical performance and the extension of service life—is needed; (ii) desired functions of the bioplastics are required to meet the needs in value-added applications where extreme environments, for example, high temperature and/or high moisture content, might be involved; and (iii) exploring new protein resources will play an important role in the development of biodegradable plastic industry. Genetically modified crops for instance can be used in the future to provide specific amino acid composition of protein as known building blocks for the resulting bioplastics, in order to optimize their structure and properties.

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Author details

Hangbo Yue[1*], Guoqiang Yin[2*] and Yingde Cui[1,3]

*Address all correspondence to: yuehangbo@163.com and yingq007@163.com

1 School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou, China

2 Institute of Green Chemical Engineering, Zhongkai University of Agriculture and Engineering, Guangzhou, China

3 Guangzhou Vocational College of Science and Technology, Guangzhou, China

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