Electronic Supplementary Information (ESI) for

On the improvement of properties of composites derived from wasted cottonseed protein by rational cross-linking and natural fiber reinforcement

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Section S1 Supplementary experiments

Cottonseed protein waste powder treatment

Purification of cottonseed protein (CP) powder was carried out using the alkali dissolution and acid precipitation method. Experimental procedures are described as follows: Firstly, 30 g of CP powder was added into 500 mL NaOH solution with pH of 12, and stirred for 1 h at room temperature. After the solution was centrifuged at the rate of 10000 r/min for 10 min to obtain a supernatant, its pH was adjusted to 5 using 1 mol/L HCl, and conditioned at 4 °C for 12 h. Then, the mixture was separated using the same centrifugation process (10000 r/min for 10 min), and the sediment was washed with deionized water 3 times to remove the excess NaCl. Finally, the precipitate was freeze-dried to obtain cottonseed protein concentrate (CPC) for further uses.

Determination of amino acids

The contents of hydrolyzed amino acids in CP powder and CPC were obtained by an automatic amino acid analyzer (L-8900, Hitachi) in accordance with GB 5009. 124-2016, and the results were listed in Table S1. The specific composition of amino acids guarantees a good processibility of cottonseed protein due to its high plasticizing efficiency, calculated by the ratio of the total contents of threonine, serine, and tyrosine to cysteine[1].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Sample CPC was solubilized in 1 M Tris-HCl buffer. After heating at 100 °C for 5 min, 10 μL of the mixture was loaded into 12% polyacrylamide gel slabs. Electrophoresis pattern was determined at a constant voltage of 150 V. The gel was stained with Coomassie blue (0.1% Coomassie brilliant blue R-250, 25% isopropanol, 10% glacial acetic acid, 64.9% deionized water), and later de-stained using decolorizing solution (10% acetic acid, 5% ethanol, 85% deionized water).

Sisal fiber treatment

Natural sisal fiber bundles were cut at a length of 10 cm, immersed in 10% NaOH for 4 h, washed repeatedly with pure water to get a neutral filtrate, dried at 60 °C overnight in vacuum, and alkalized sisal fibers were obtained and stored prior to further processing.

Preparation of dialydehyde starch

A modified method based on previously reported approach[2] was used. In a typical experiment, 8 g soluble starch was added to 100 mL $NaIO_4$ solution (0.3 mol/L, pH=2.0), stirred at 38 °C for 4 h. Then the precipitate was washed thoroughly with water and ethanol absolute until a colorless and neutral filtrate was obtained. After that, the cleaned precipitate was dried at 45 °C in vacuum for 24 h, grinded and sieved to obtain DAS cross-linker.

Section S2 Supplementary tables and figures

Table S1 Amino acid composition of cottonseed protein (CP), CPC and soy protein isolate (SPI).

	CP	CPC	SPI[3]
hydrolyzed content (g/100g)	48.89	57.43	64.92
Asp	2.32	3.04	7.44
Thr	1.54	1.98	2.24
Ser	4.56	5.02	3.04
Glu	10.32	11.97	11.0
Pro	0.85	2.09	1.97
Gly	3.19	3.35	3.36
Ala	3.23	3.86	2.00
Cys	2.34	2.41	0.30
Val	0.35	0.72	10.7
Met	0.59	1.24	0.73
Ile	1.79	0.93	2.69
Leu	4.93	5.45	4.67
Tyr	3.02	3.35	2.06
Phe	2.22	2.46	3.23
Lys	1.54	1.99	3.72
His	5.14	5.68	1.49
Arg	0.96	1.89	4.28

Table S2 Chemical composition of natural and alkalized sisal fibers.

	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Natural sisal fiber	60.5±0.9	26.4±0.6	3.5±0.1
Alkalized sisal fiber	72.1±0.5	14.2±0.6	5.7±0.4

Note: Chemical composition of natural and alkalized sisal fibers was determined by the classic acid-detergent fiber method, also known as VAN SOEST method[4]. Pretreatment: sisal fiber was crushed into tiny particles and passed through a sieve (60 mesh).

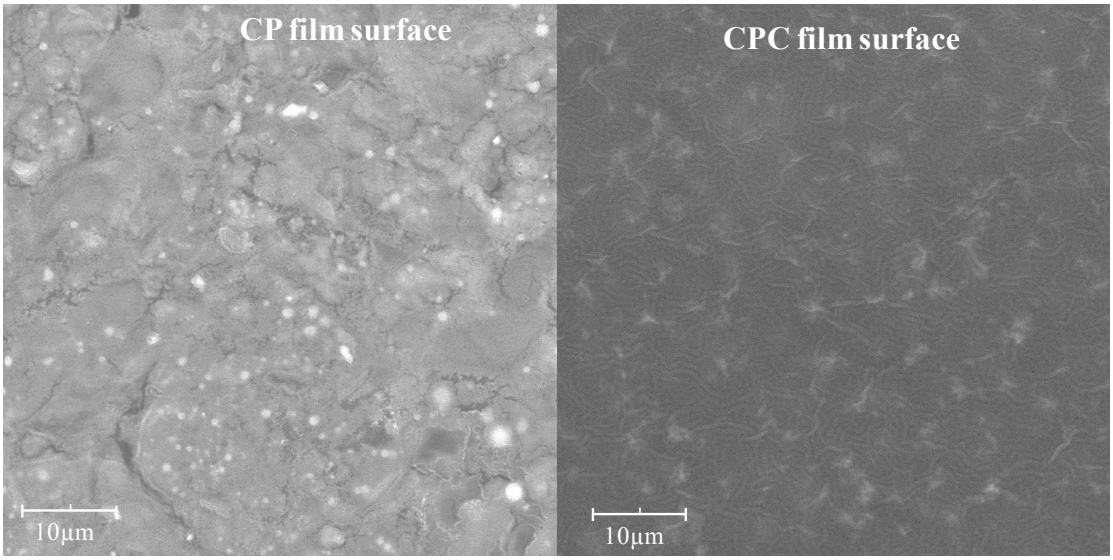

Figure S1 SEM images of cottonseed protein derived bioplastic films.

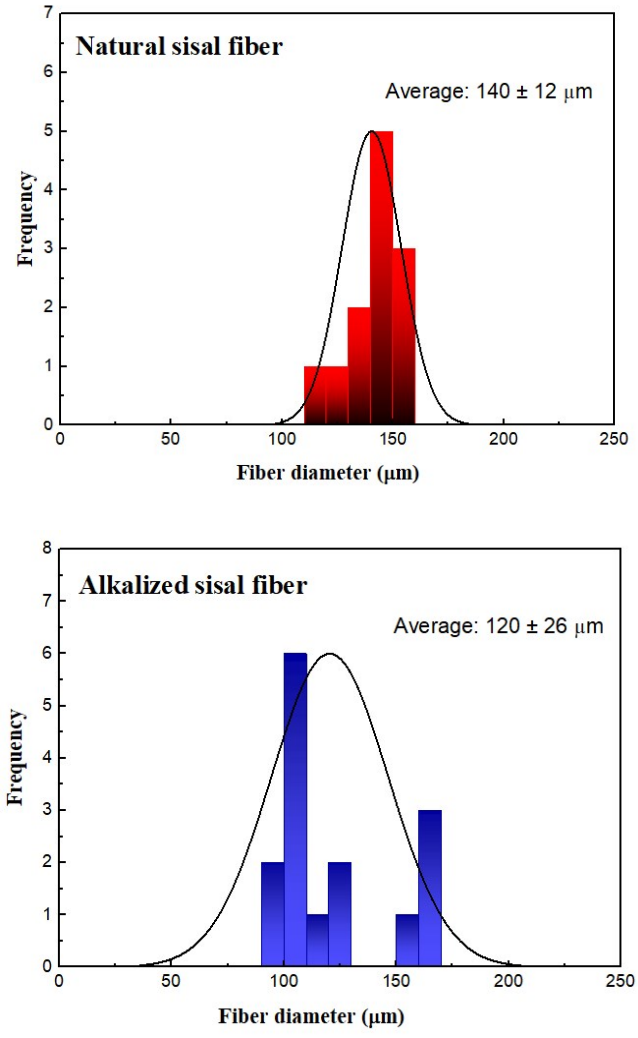

Figure S2 Histogram showing the diameter distribution of natural and alkalized sisal fibers.

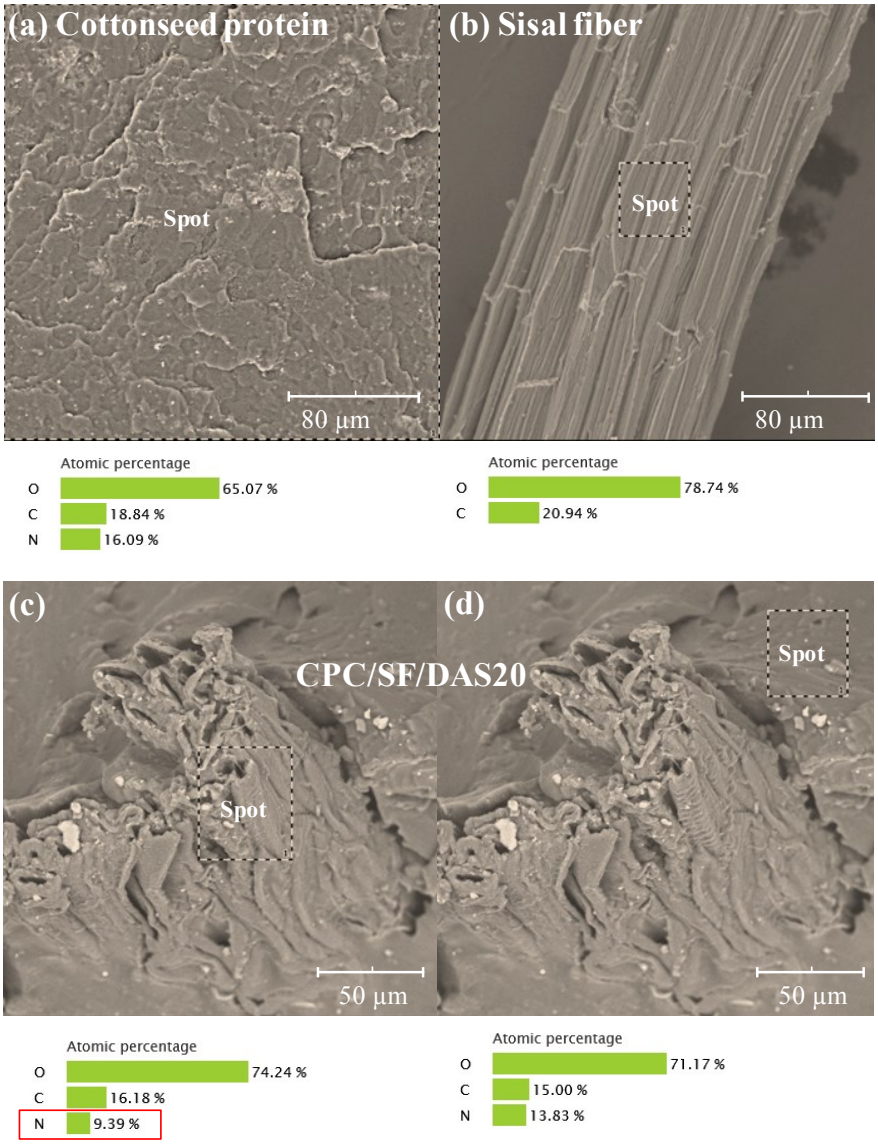

Figure S3 Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) results of cottonseed protein, sisal fiber, and CPC/SF/DAS20 composite. SEM images of (a) cottonseed protein and (b) sisal fiber were collected from sample surfaces; SEM images (c-d) were obtained from the cross-section of CPC/SF/DAS20 composite after tensile test. Atomic percentage of elements detected from each SEM image is shown below the image.

While carbon (21 at.%) and oxygen (79 at.%) atoms were detected on the surface of sisal fiber (Figure S2b), the newly found nitrogen atom (9 at.%) from the same fiber imbedded in cottonseed protein (Figure S2c) indicates the presence of some amount of protein on the fiber; therefore, good protein/fiber compatibility with strong interface adhesion is expected.

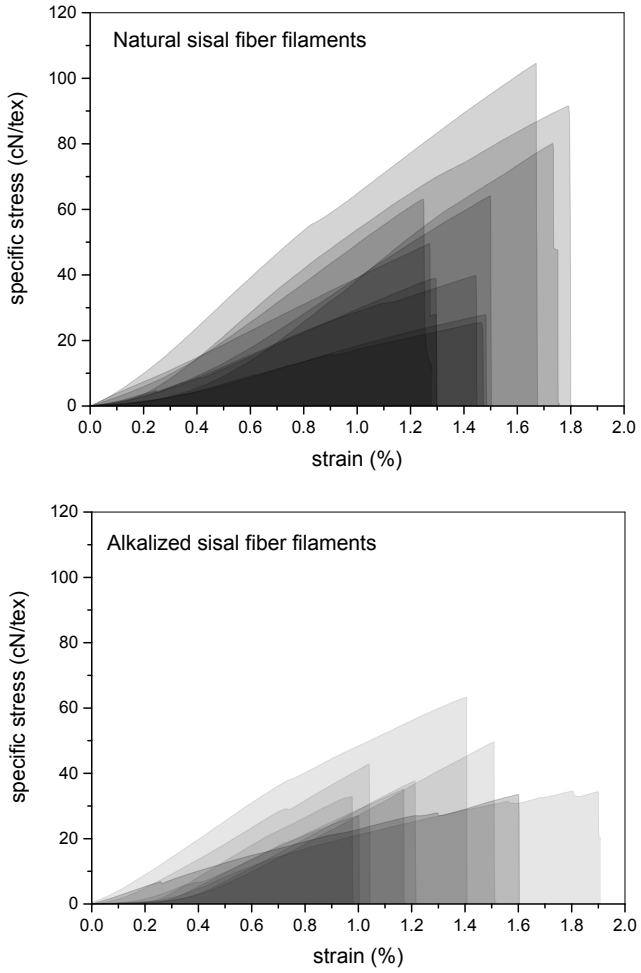

Figure S4 Tensile stress-strain curves of natural and alkalized sisal fibers. Fiber specific stress (tenacity) is expressed by force to break per tex (linear density), N/tex is equivalent to GPa/SG. Gauge length: 20 mm; Cross-head speed: 2 mm/min.

Table S3 Contact angle and surface energy components CP derived materials

Samples	Contact angle (°)		Surface energy (mJ/m^2)	Dispersion component (mJ/m^2)	Polarity component (mJ/m^2)
	pure water	ethylene glycol			
CPC/Glycerol	34.38	36.08	73.88	1.47	72.41
CPC/SF/DAS0	39.61	40.26	68.84	1.67	67.17
CPC/SF/DAS20	80.13	67.43	25.01	8.32	16.69

Note: Total surface energy was determined by the combination of dispersion component and polarity component of surface free energy, using pure water and ethylene glycol as probing

molecules[5].

Table S4 Water resistance property of protein-based biocomposites

	samples	contact angle (°)	water absorption/update (%)
this work(CPC/SF/DAS)	CPC/glycerol	34	58.1
	CPC/SF/DAS0	39	59.3
	CPC/SF/DAS5	62	49.5
	CPC/SF/DAS10	68	46.6
	CPC/SF/DAS20	80	38.3
	CPC/SF/DAS30	72	43.5
jute/soy based green composites[6]	Jute fiber	42.1	190
	SRF10	50.4	110
	WJS3	63.5	52.1
	NJS3	67.8	46.6
poly(dopamine) soy protein isolate-based film[7]	SPI	65	239
	MCC/SPI-1	56.7	187
	MCC/SPI-2	53.3	175
	MCC/SPI-3	52.7	204
	PDMCC/SPI-1	64.2	187
	PDMCC/SPI-2	74.8	164
	PDMCC/SPI-3	70.4	179
licorice residue soy protein isolate nanocomposites[8]	LNC0	60.2±0.2	-
	LNC2	61.6±1.2	-
	LNC4	68.2±0.4	-
	LNC6	72.4±0.2	-
	LNC8	73.5±0.4	-
rise protein composite[9]	RP-film	54	-
	RP-MA film	60	-
	RP-WK film	79	-
	RP-CPP film	70	-
chitin whiskers/soy protein isolate composites[10]	GSPI sheet	-	40
	SPI-5	-	35
	SPI-10	-	33
	SPI-15	-	31
	SPI-20	-	31
	SPI-25	-	29
	SPI-30	-	23
acid treated carbon nanotubes/ soy protein nanocomposites[11]	SPI/NWNTs-0	-	50
	SPI/NWNTs-1	-	48
	SPI/NWNTs-2	-	46

	SPI/NWNTs-3	-	43
	SPI/NWNTs-4	-	40
	SPI/NWNTs-5	-	38
carbon nanotubes reinforced soy protein nanocomposites[12]	SPI-S	-	35
	SC-0.25	-	29
	SC-0.75	-	35
	SC-3	-	33
	SC-0.25-Ⅲ	-	33
	SC-0.25-IV	-	42
starch nanocrystal-reinforced soy protein plastics[13]	SPI-S	-	34
	SStN-1	-	32
	SStN-2	-	31
	SStN-3	-	31
	SStN-8	-	30
	SStN-16	-	29

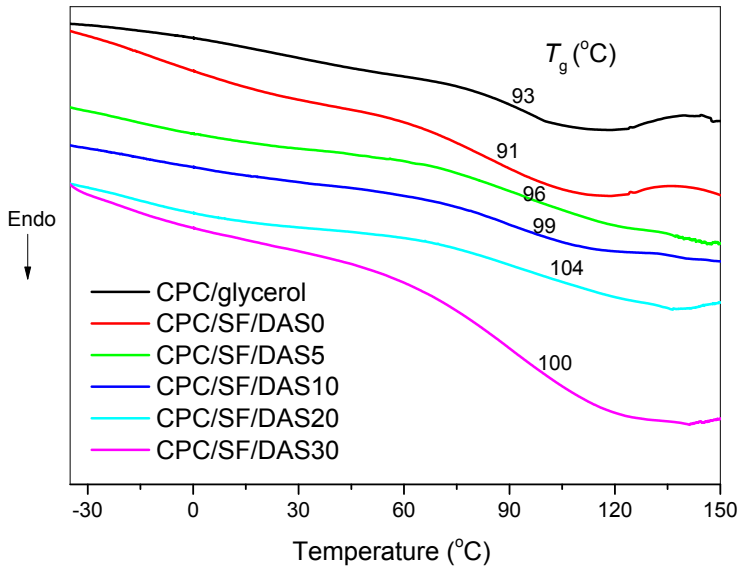

Figure S5 DSC thermograms showing the changes of grass transition temperature T_g with varying DAS content.

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