SUPPLEMENTARY MATERIAL

From waste to function: valorization of collagen-based wastes with Natural Deep Eutectic Solvents for bioadhesive applications

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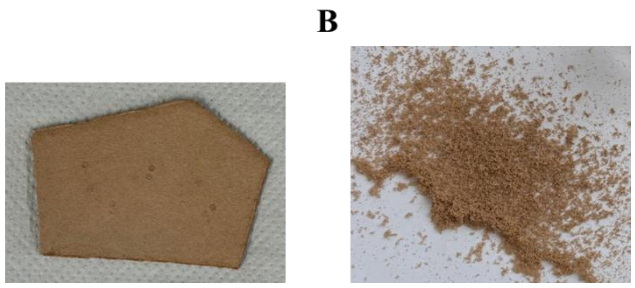

Figure S 1: Vegetable-tanned leather as supplied by the tannery (A) and after grating into small pieces (B).

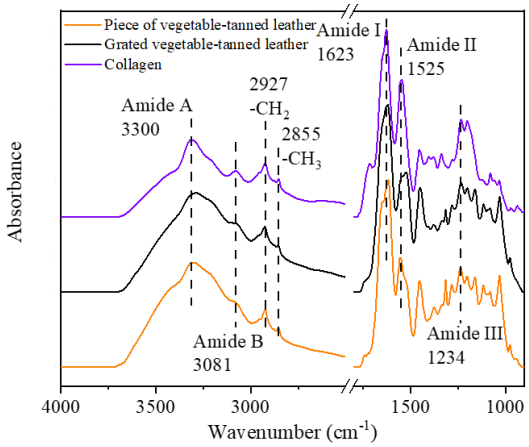

Figure S 2: ATR-FTIR spectrum of the collagen (purple line), vegetable-tanned leather sample as provided by the supplier (orange line) and grated vegetable-tanned leather (black line).
Characteristic bands associated with collagen structure are highlighted.

Table S 4: TGA analysis of the samples: collagen, as-received tanned leather and grated tanned leather.

						Mass loss (wt%)		
Sample	**T_onset (°C)**	**T (1° Peak) (°C)**	**T (2° Peak) (°C)**	**T max (°C)**	**Residue Mass % (T=900°C)**	**Stage I (25-200°C)**	**Stage II (200-600°C)**	**Stage III (600-900°C)**
Collagen	299.3	38.3	169.7	326.1	13	32	52	3
Leather as recived	268.8	46.2	/	309.6	26	15	54	5
Grated leather	263.7	39.1	/	310.3	23	11	59	7

*Error (expressed as ± standard deviation) was evaluated by performing in triplicate representative samples. More in detail, error in the temperatures was ± 0.3°C, and in the mass losses was ± 4%.

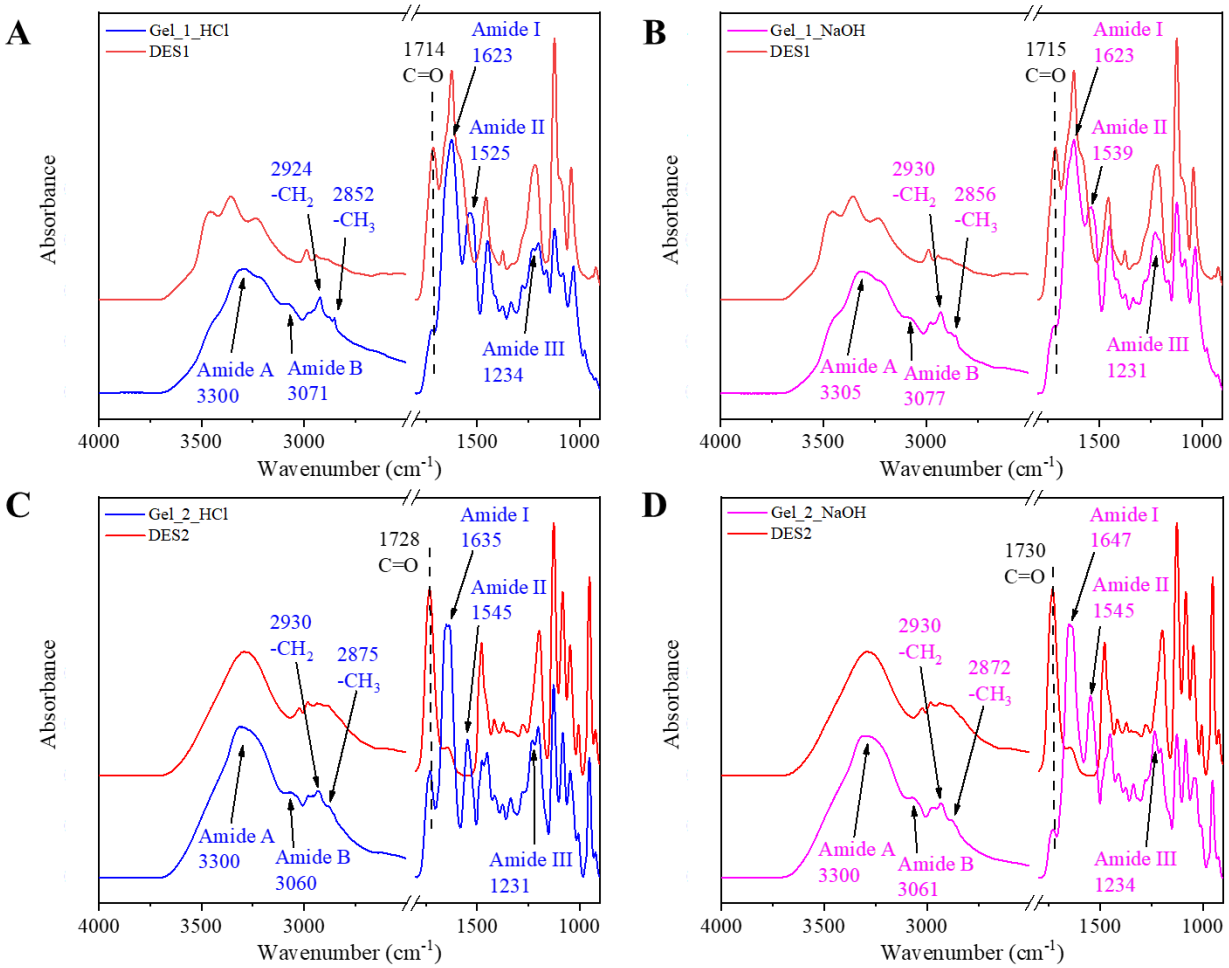

Figure S 3: ATR-FTIR spectra of samples analyzed.

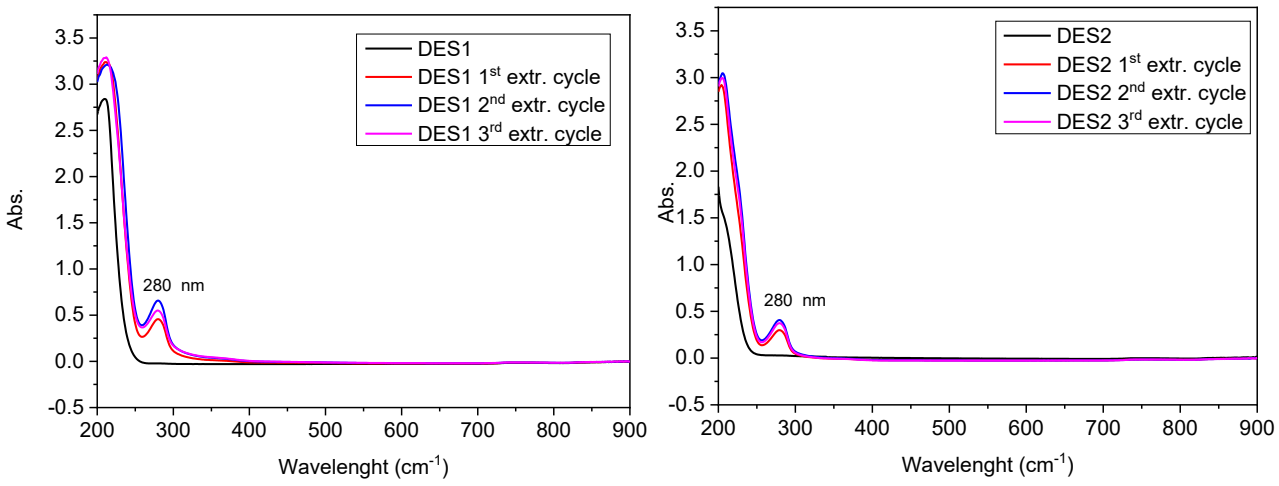

Figure S 4: UV-Vis of DES 1 (on the left) and DES 2 (on the right) used in subsequent cycles.

Samples are diluted 1:300 in water. The signal at 280 nm of mimosa and quebracho extracts containing tannins are visible (DOI: http://dx.doi.org/10.1016/j.indcrop.2016.07.022) while the signal of chestnuts extracts and the other signals of previous extracts are covered by the signal of DES.

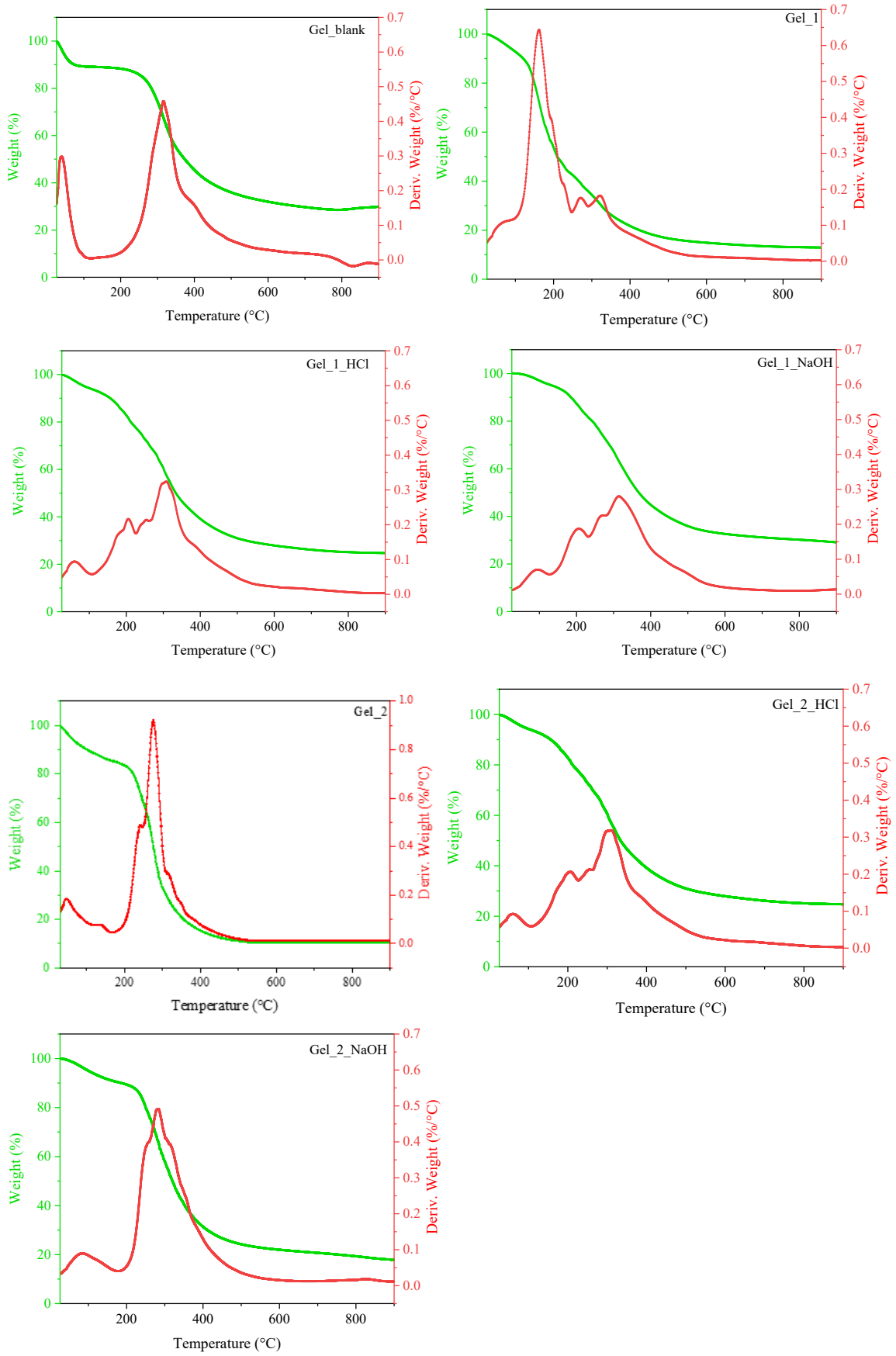

Figure S 5: TGA of the samples Gel_blank, Gel_1, Gel_1 HCl, Gel_1_NaOH, Gel_2, Gel_2 HCl, Gel_2_NaOH.

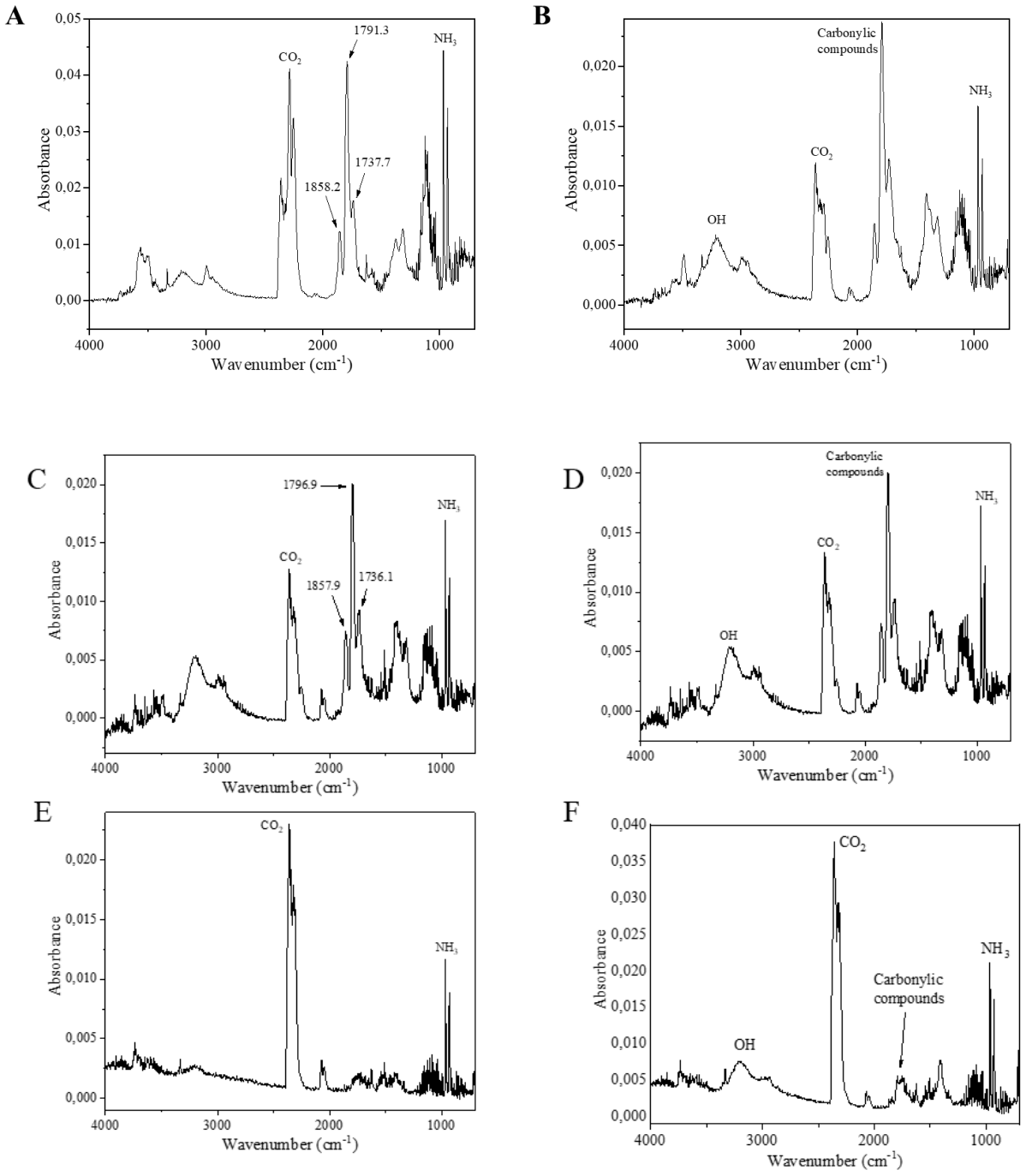

Figure S 6: FTIR spectrum of the gases evolved during thermal degradation of Gel_1 at 163°C (A) and at 323°C (B), Gel_1_HCl at 210°C (C) and at 310°C (D) and Gel_1_NaOH at 210°C (E) and at 315°C (F), under nitrogen flow at a heating rate of 20 °C/min.

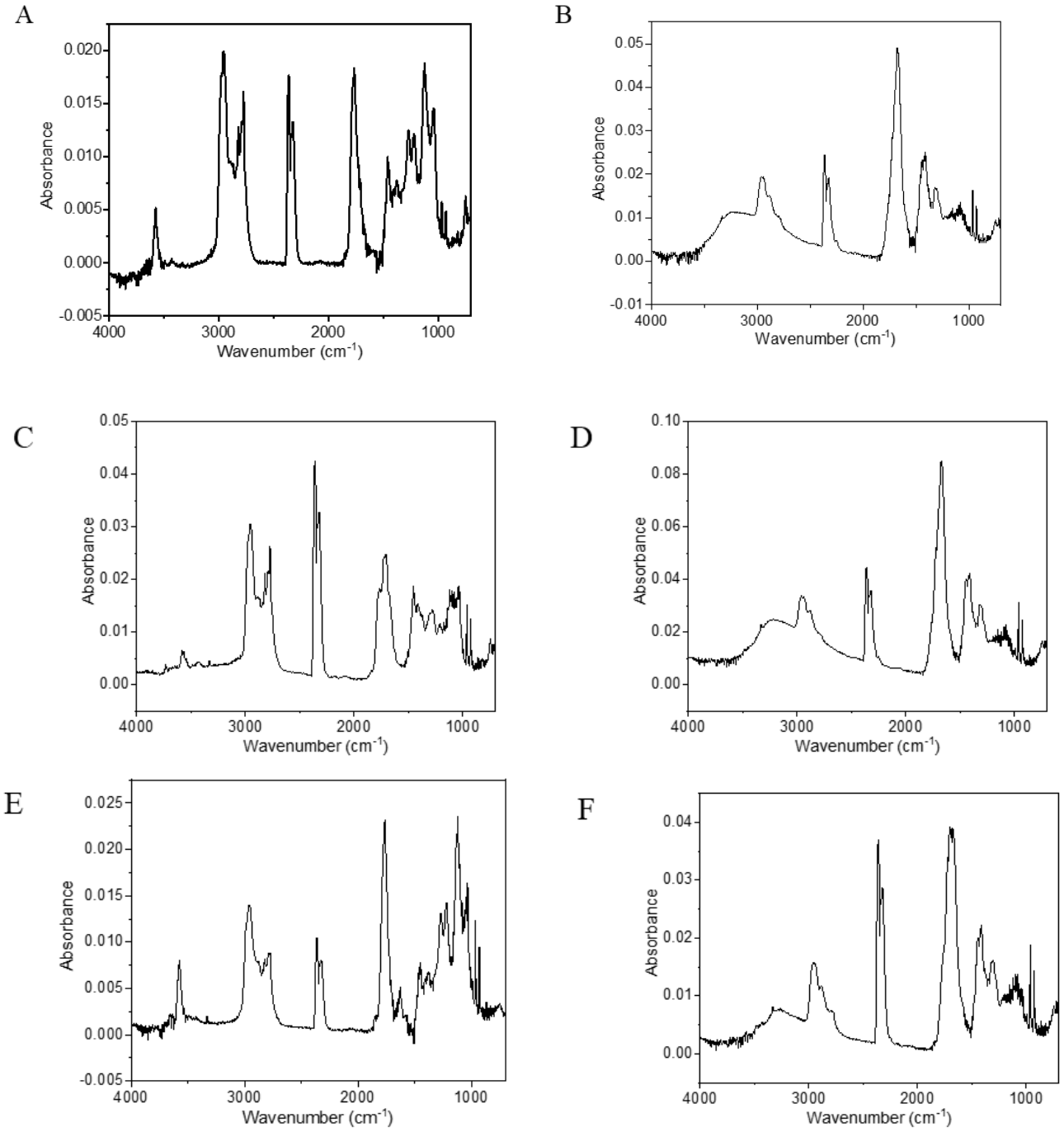

Figure S 7: FTIR spectrum of the gases evolved during thermal degradation of Gel_2 at 220°C (A) and at 280°C (B), Gel_2_HCl at 220°C (C), and at 280°C (D) and Gel_2_NaOH at 220°C (E) and at 280°C (F), under nitrogen flow at a heating rate of 20 °C/min.

Table S 5: Maximum temperature in DTG associated with collagen degradation.

Sample	**Collagen**	**Leather as recived**	**Grated leather**	**Gel-blank**	**Gel_1**	**Gel_1_HCl**	**Gel_1_NaOH**	**Gel_2**	**Gel_2_HCl**	**Gel_2_NaOH**
T max DTG (°C)	326	310	310	317	302	301	314	275	283	282

*Error (< ±2°C, expressed as ± standard deviation) was evaluated by performing in triplicate representative samples.

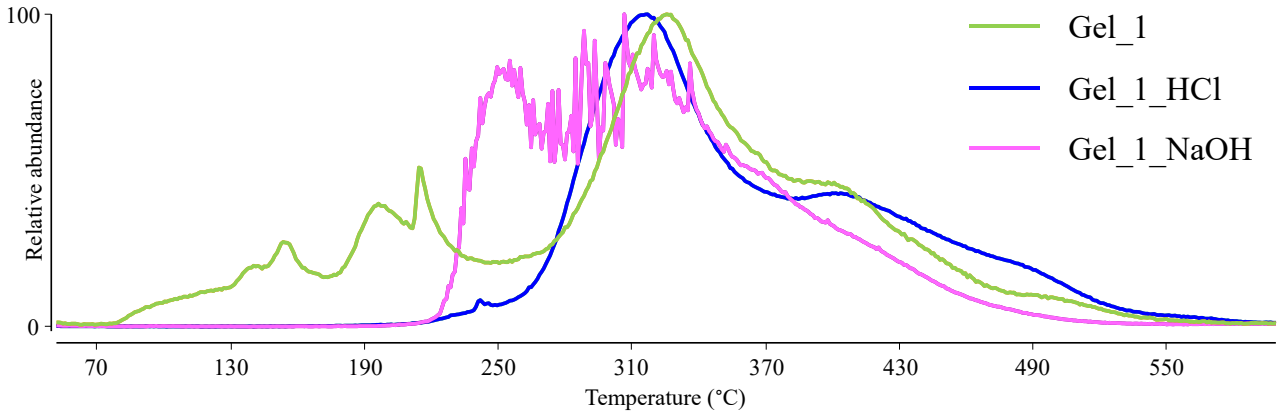

Figure S 8: Total Ion Thermograms (TITs) of Gel_1 (green), Gel_1_HCl (blue) and Gel_1_NaOH (pink).

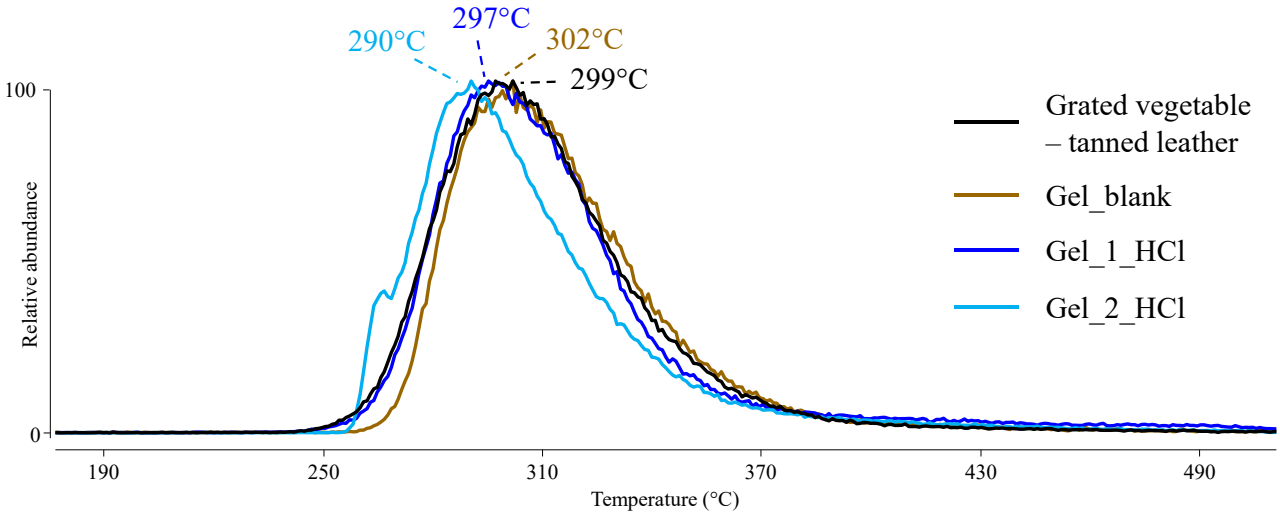

Figure S 9: Extracted Ion Thermograms (EITs) of fragment ion m/z 154 of grated vegetable – tanned leather (black), Gel_blank (brown), Gel_1_HCl (blue) and Gel_2_HCl (light blue).

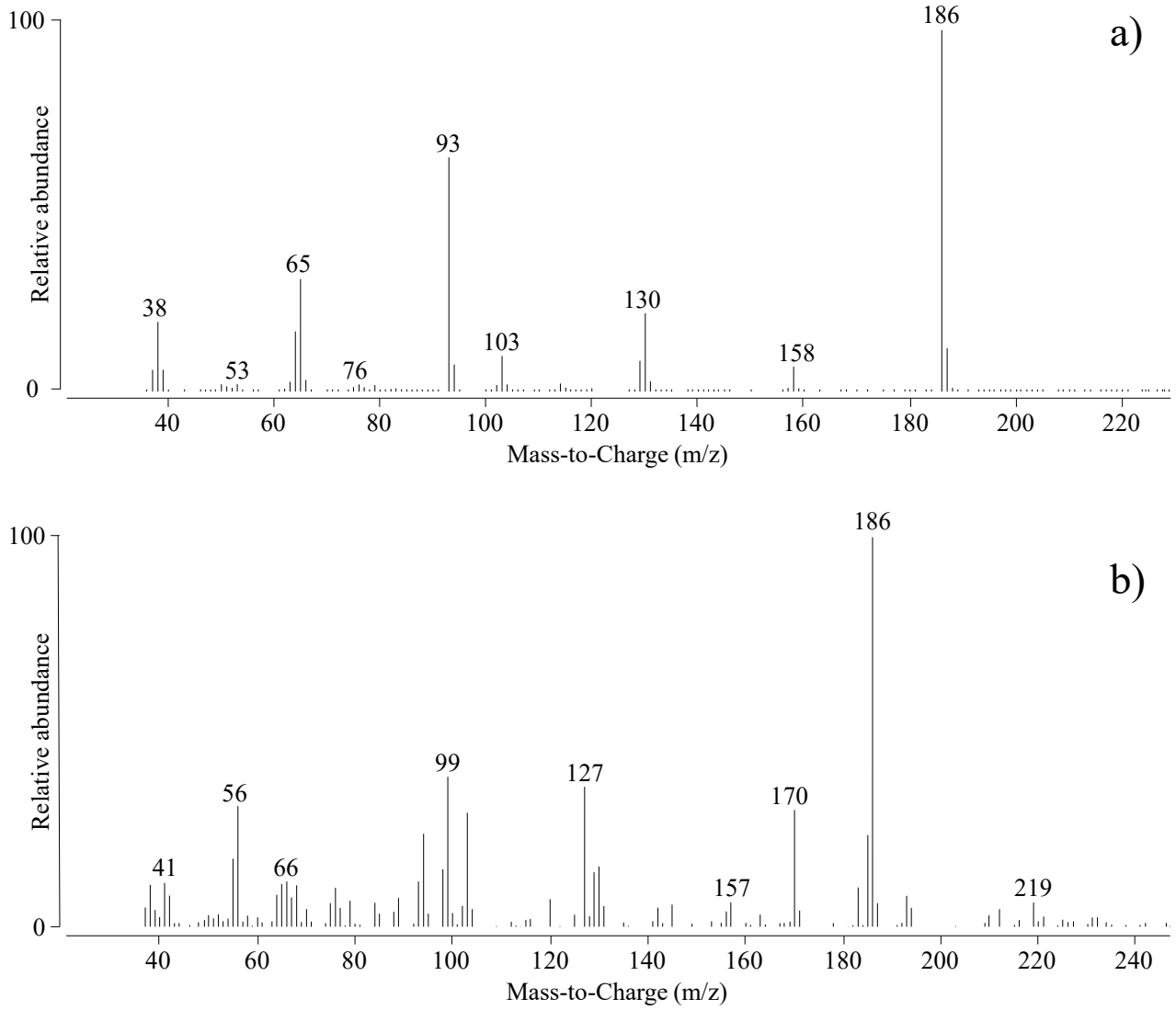

Figure S 10: Mass spectra of heteroaromatic compounds from EICs in Fig 4 at a) 19.1 min and b) 22.9 min.

Table S 6: Summary of protein identification results.

For each protein, data shows number of identified peptides, percentage of sequence coverage, number of peptide isoforms, and counts of unmodified and modified peptides. Modified peptide counts represent the number of peptide isoforms containing the specific modification used for calculating modification extent.

Sample	Protein name (Uniprot ID)	Identified peptides	Sequence coverage(%)	Peptide isoforms	Unmodified Peptides	Oxidized M	Deamidated N	Deamidated Q
Gel_1_REP1	Collagen alpha-1(I) chain (P02453)	77	58.8	255	31	14	24	33
Gel_1_REP2		46	42.9	145	11	16	16	15
Gel_1_HCl_REP1		72	59.3	289	30	20	37	53
Gel_1_HCl_REP2		57	53.4	297	25	14	89	49
Gel_1_NaOH_REP1		74	59.1	297	57	14	33	33
Gel_1_NaOH_REP2		68	57.3	363	47	14	55	41
Gel_2_REP1		74	57.4	300	42	20	23	35
Gel_2_REP2		65	54.0	227	16	21	17	25
Gel_2_HCl_REP1		78	61.0	310	50	19	29	48
Gel_2_HCl_REP2		80	59.3	346	55	18	33	44
Gel_2_NaOH_REP1		76	61.2	316	47	20	21	37
Gel_2_NaOH_REP2		75	60.4	326	53	21	28	38
Gel_blank_REP1		63	56.2	256	28	17	26	30
Gel_blank_REP2		46	44.4	105	14	14	9	15
Grated_leather_REP1		52	49.9	137	16	17	9	19
Grated_leather_REP2		30	31.6	52	10	11	6	4
Gel_1_REP1	Collagen alpha-2(I) chain (P02465)	53	54.9	108	8	5	28	7
Gel_1_REP2		28	33.6	56	4	0	13	4
Gel_1_HCl_REP1		62	66.9	165	9	8	43	14
Gel_1_HCl_REP2		51	56.0	140	6	7	43	14
Gel_1_NaOH_REP1		55	59.1	166	12	9	47	14
Gel_1_NaOH_REP2		54	56.6	182	17	8	49	12
Gel_2_REP1		55	58.3	132	10	10	36	7
Gel_2_REP2		48	51.8	109	10	8	33	6
Gel_2_HCl_REP1		62	64.9	171	14	9	42	13
Gel_2_HCl_REP2		64	68.9	210	11	12	52	13
Gel_2_NaOH_REP1		66	67.8	213	14	8	44	10
Gel_2_NaOH_REP2		63	66.7	203	11	12	41	8
Gel_blank_REP1		51	57.0	144	9	13	45	6
Gel_blank_REP2		33	36.6	63	4	5	19	5
Grated_leather_REP1		43	50.6	75	6	5	28	3
Grated_leather_REP2		22	26.6	33	2	1	7	2
Gel_1_REP1	Collagen alpha-1(III) chain (P04258)	55	70.1	168	7	7	42	21
Gel_1_REP2		33	50.5	70	4	8	21	8
Gel_1_HCl_REP1		56	66.9	168	6	16	48	20
Gel_1_HCl_REP2		53	65.9	195	8	13	68	29
Gel_1_NaOH_REP1		56	66.9	177	7	17	47	18
Gel_1_NaOH_REP2		57	65.7	173	6	14	51	21
Gel_2_REP1		59	70.9	188	3	18	48	25
Gel_2_REP2		46	61.2	125	4	13	37	12
Gel_2_HCl_REP1		64	70.2	197	7	20	56	18
Gel_2_HCl_REP2		59	68.6	192	8	24	47	20
Gel_2_NaOH_REP1		56	67.2	167	6	19	42	12
Gel_2_NaOH_REP2		59	68.9	185	8	17	45	21

Gel_blank_REP1	*48*	*62.8*	*150*	*5*	*10*	*43*	*16*
Gel_blank_REP2	*36*	*44.7*	*70*	*3*	*8*	*22*	*6*
Grated_leather_REP1	*37*	*50.1*	*87*	*3*	*11*	*27*	*10*
Grated_leather_REP2	*20*	*26.9*	*35*	*1*	*5*	*9*	*3*

Table S 4: Overall extent of backbone cleavage.
Data shows the mean ratio of semitryptic to total peptides across samples along with its standard error. Semitryptic/TOT ratios indicate the extent of backbone cleavage in samples, where higher ratios reflect increased protein degradation. Total counts of identified tryptic and semitryptic peptides backbone cleavage data is based on are reported for each sample replicate.

Sample	***Experiment***	***Total Tryptic***	***Total Semi-tryptic***	***Semitryptic/TOT (Mean)***	***Std Error***
Gel_1	*Gel 1 REP1*	*199*	*25*	*0.11*	*0.00*
	Gel_1_REP2	*120*	*16*		
Gel_1_HCl	*Gel 1 HCl REP1*	*454*	*146*	*0.25*	*0.01*
	Gel 1 HCl REP2	*466*	*164*		
Gel_1_NaOH	*Gel 1 NaOH REP1*	*480*	*68*	*0.14*	*0.01*
	Gel_1_NaOH_REP2	*470*	*81*		
Gel_2	*Gel 2 REP1*	*317*	*33*	*0.11*	*0.02*
	Gel 2 REP2	*305*	*45*		
Gel_2_HCl	*Gel_2_HCl_REP1*	*477*	*83*	*0.15*	*0.00*
	Gel_2_HCl_REP2	*517*	*86*		
Gel_2_NaOH	*Gel_2_NaOH_REP1*	*486*	*102*	*0.15*	*0.02*
	Gel 2 NaOH REP2	*515*	*81*		
Gel_blank	*Gel blank REP1*	*282*	*22*	*0.07*	*0.00*
	Gel_blank_REP2	*99*	*7*		
Grated_leather	*Grated_leather_REP1*	*62*	*5*	*0.08*	*0.01*
	Grated_leather_REP2	38	4		

Table S 5: Analysis of protein modification extent.
Mean values and standard errors for the extent of methionine (M) oxidation and deamidation (N|Q) are presented by sample, along with the number of detected and modified amino acid sites the data is based on. N|Q represents combined deamidation at both asparagine (N) and glutamine (Q) residues.

	Oxidation (M)				***Deamidation (N|Q)***			
Sample	***Detected M***	***Modified M***	***Fraction of modified/TOT***	***Std Error***	***Detected N|Q***	***Modified N|Q***	***Fraction of modified/TOT***	***Std Error***
Gel_1	*77*	*67*	*0.86*	*0.01*	*1094*	*485*	*0.33*	*0.02*
Gel_1_HCl	*76*	*65*	*0.86*	*0.01*	*1092*	*394*	*0.44*	*0.06*
Gel_1_NaOH	*49*	*42*	*0.86*	*0.03*	*624*	*198*	*0.36*	*0.00*
Gel_2	*99*	*90*	*0.89*	*0.04*	*1168*	*403*	*0.31*	*0.00*
Gel_2_HCl	*106*	*92*	*0.90*	*0.00*	*1085*	*334*	*0.34*	*0.00*
Gel_2_NaOH	*70*	*63*	*0.87*	*0.03*	*747*	*229*	*0.31*	*0.02*
Gel_blank	*58*	*48*	*0.85*	*0.10*	*509*	*177*	*0.37*	*0.05*
Grated_leather	*35*	*31*	*0.90*	*0.00*	*236*	*96*	*0.40*	*0.00*

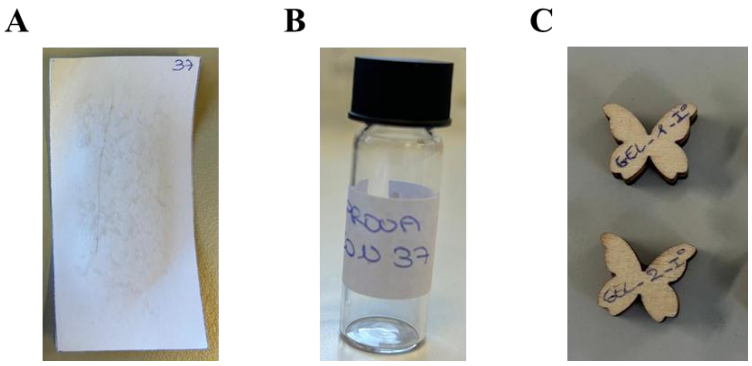

Figure S 11: Adhesion tests with Gel_1 as bioadhesive. A) paper–paper, B) paper–glass, and C) wood–wood.

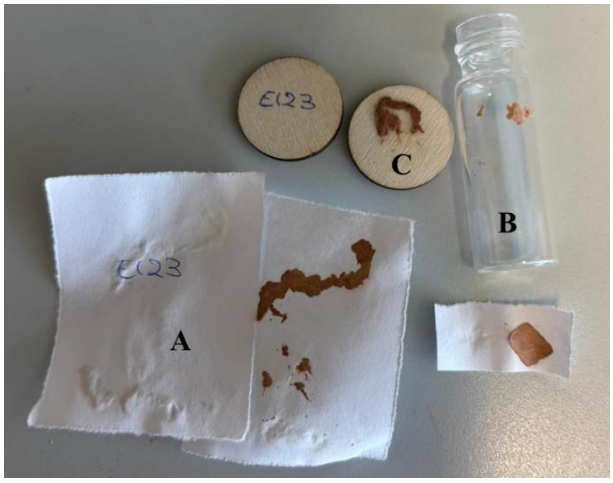

Figure S 12: Adhesion test with control experiment (Gel-blank). A) paper–paper, B) paper–glass, and C) wood–wood.

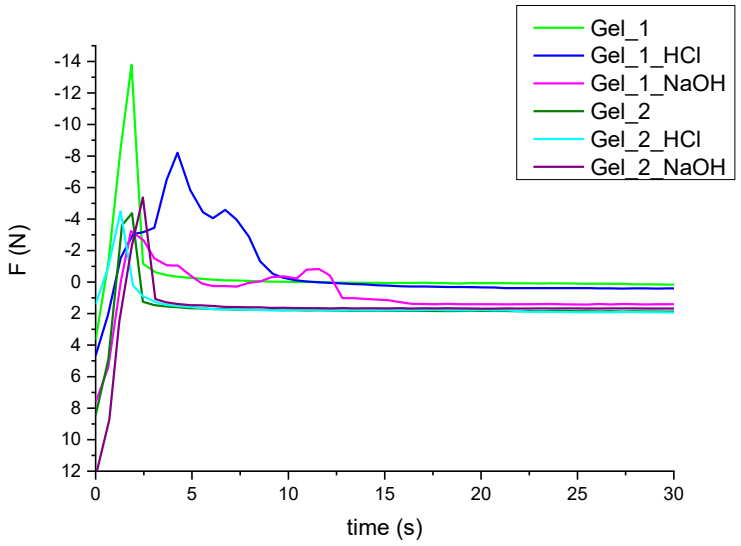

Figure S 13: tack test made on the samples to test the adhesive strength on steel-wood surfaces.

Table S 6: Scores attributed to each principle to evaluate the process greenness, made according to Path2Green application.

Principles		Score*	Motivation	Weight**
1	**Biomass**	+1	Waste biomasses	6
2	**Transport**	+0.5	40 km distance	5
3	**Pre-treatment**	-0.2	Physical pretreatment	2.5
4	**Solvent**	+1	Recommended solvents (DESs)	6
5	**Scaling**	-1	In batches	3
6	**Purification**	-0.5	Water	2.5
7	**Yield**	+1	Complete valorization of the biomass	4
8	**Post-treat.**	+1	Ready-to-use extract	2.5
9	**Energy**	+0.5	Low energy dependence	5
10	**Application**	+0.66	To be applied in four domains	4.5
11	**Repurposing**	+1	Non-Virgin raw materials are used	6
12	**Waste**	-1	Waste generated: 100% m/m	6

**Scores and weights were chosen following the recommendations of (DOI: 10.1039/d4gc02512a).*