

Supplementary material

Extraction of High-Purity Native State Gutta-Percha from Enzymatic Hydrolyzed *Eucommia ulmoides* Pericarps by Ultrasound Treatment and Surfactant Aqueous Phase Dispersion

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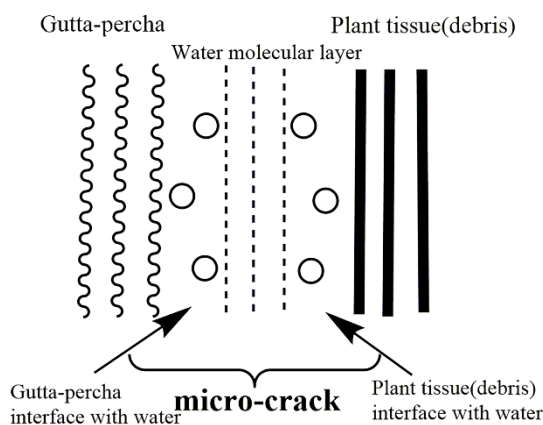
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1. Ultrasonic stripping mechanism

Gutta-percha exists in plant cells. Due to the cell walls being destroyed during the enzymatic hydrolysis pretreatment, a micro-crack is formed between gutta-percha and outside plant tissue debris (Scheme 1). When the enzymatic hydrolyzed *E. ulmoides* pericarps are put into water for ultrasonic treatment, water first penetrates into this crack, and the water molecular layer in the crack becomes an ultrasonic reflection superposition zone due to the close superposition of two interfacial layers of Gutta-percha and plant tissue debris, thus forming a concentrated generation zone of ultrasonic cavitation microbubbles (cavitation nuclei) [1]. When collapsing, the cavitation microbubbles generate a microjet with a powerful impact force at a speed of approximately 110 m/s, which can weaken the adhesive force of plant tissue debris on gutta-percha and facilitate them to strip from gutta-percha. In addition, the high-frequency vibrations of the cavitation microbubbles drives the high-frequency vibration of gutta-percha and plant debris [2]. As gutta-percha and plant debris have different vibration frequencies, their different frequency vibration also loosens the adhesion of plant debris, further driving the plant debris to be stripped from gutta-percha [3].



Scheme S1. A micro-crack layer between gutta-percha and plant tissue debris.

2. Aqueous phase dispersion mechanism of surfactant

During the ultrasonication process, the plant debris were stripped off from gutta-percha. But gutta-percha is filamentous and highly hydrophobic, it could be easily entangled with each other, and consequently, some plant debris were still wrapped in it

and could hardly be released. In order to release the wrapped debris, the intertwined gutta-percha should be fully spread in the aqueous solution, i.e., the affinity between gutta-percha and water molecules must be enhanced. The surfactant has an "amphiphilic structure", with one end being a hydrophilic polar group that can affinity with polar water molecules and the other end being a lipophilic non-polar group that can affinity with hydrophobic gutta-percha, thus reducing the interfacial tension between the gutta-percha phase and the water phase, making the two substances affinity with each other. As a result, the curled and tangled gutta-percha can be extended in an aqueous solution and then release the wrapped plant tissue debris. Afterward, the released debris precipitates to the bottom due to the greater density than that of water, while gutta-percha with a smaller density than water floats to the surface, so as to realize the effective separation plant debris from gutta-percha, as shown in Figure S1.

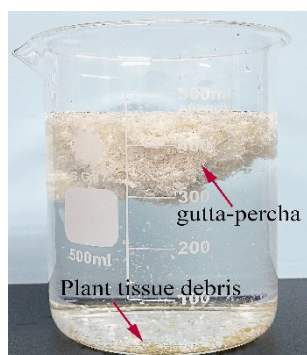


Figure S1. Gutta-percha in aqueous solution containing SDS.

Table S1 Experimental design and results of response surface analysis for ultrasonic purification processes.

Run	Factors					Purity of gum %
	X ₁	X ₂	X ₃	X ₄	X ₅	
	Frequency kHz	Power W	Time h	Temperature °C	Material-to-liquid ratio g/mL	
1	1	0	1	0	0	59.57
2	0	1	0	0	-1	56.26
3	1	-1	0	0	0	51.76
4	0	0	0	-1	-1	53.72
5	0	-1	0	0	-1	52.05
6	0	0	0	1	-1	59.40
7	0	-1	0	1	0	53.99
8	0	0	1	-1	0	57.33
9	0	1	-1	0	0	51.66
10	-1	0	1	0	0	56.22
11	-1	-1	0	0	0	51.48
12	0	0	-1	1	0	53.85
13	0	1	0	-1	0	52.44
14	0	0	0	0	0	63.1
15	0	0	1	0	-1	60.32
16	0	0	0	0	0	64.67
17	1	1	0	0	0	56.37
18	0	0	0	0	0	64.32
19	-1	1	0	0	0	53.95
20	0	1	0	0	-1	56.42
21	1	0	0	0	-1	54.78
22	0	0	-1	0	-1	54.37
23	0	0	0	0	0	65.02
24	0	-1	-1	0	0	51.14
25	0	1	1	0	0	59.11
26	1	0	0	1	0	56.35
27	-1	0	-1	0	0	52.02
28	0	0	1	1	0	60.18
29	-1	0	0	0	-1	56.88
30	1	0	0	0	-1	57.01
31	1	0	0	-1	0	55.33
32	0	0	0	0	0	63.88
33	0	-1	0	-1	0	53.62
34	0	0	-1	-1	0	49.45
35	1	0	-1	0	0	52.84
36	0	0	1	0	-1	60.73
37	-1	0	0	1	0	58.74
38	0	0	0	0	0	64.84
39	0	-1	1	0	0	54.41
40	-1	0	0	-1	0	51.01
41	0	0	-1	0	-1	50.56
42	0	0	0	1	-1	61.33
43	0	-1	0	0	-1	54.35
44	0	0	0	-1	-1	55.42
45	0	1	0	1	0	59.66
46	-1	0	0	0	-1	52.03

Table S2 Analysis of variance (ANOVA) results of the regression equation for ultrasonic purification.

Factors	Sum of squares	df	Mean square	F-value	p-value ^a
Model	808.4626	20	40.42313	34.98511	< 0.0001**
X ₁	8.5264	1	8.5264	7.379365	0.0118*
X ₂	30.7012	1	30.7012	26.57104	< 0.0001**
X ₃	128.3015	1	128.3015	111.0414	< 0.0001**
X ₄	32.58466	1	32.58466	28.20112	< 0.0001**
X ₅	6.843179	1	6.843179	5.922583	0.0224*
X ₁ X ₂	1.62517	1	1.62517	1.40654	0.2468
X ₁ X ₃	0.536033	1	0.536033	0.463922	0.5021
X ₁ X ₄	9.765625	1	9.765625	8.45188	0.0075**
X ₁ X ₅	0.179867	1	0.179867	0.15567	0.6965
X ₂ X ₃	4.3681	1	4.3681	3.78047	0.0632
X ₂ X ₄	11.73063	1	11.73063	10.15253	0.0038**
X ₂ X ₅	1.1449	1	1.1449	0.990879	0.3291
X ₃ X ₄	0.600625	1	0.600625	0.519824	0.4776
X ₃ X ₅	2.89	1	2.89	2.501216	0.1263
X ₄ X ₅	3.294225	1	3.294225	2.851061	0.1038
X ₁ ²	233.3796	1	233.3796	201.9836	< 0.0001**
X ₂ ²	290.1158	1	290.1158	251.0872	< 0.0001**
X ₃ ²	177.366	1	177.366	153.5054	< 0.0001**
X ₄ ²	128.4516	1	128.4516	111.1713	< 0.0001**
X ₅ ²	108.0704	1	108.0704	93.53197	< 0.0001**
Residual	28.88596	25	1.155438		
Lack of fit	26.32241	20	1.31612	2.566988	0.1499
Pure error	2.56355	5	0.51271		
Correlation Total	837.3486	45			
R ² =0.9655, R ² _{Adj} =0.9379, R ² _{Pred} =0.8708, C.V %=1.91					

^a $p > 0.05$ is not significant difference; $p < 0.05$ is significant difference, indicated by "**"; $p < 0.01$ is highly significant difference, indicated by "**".

Table S3 Experimental design and results of response surface analysis for surfactant purification processes.

Run	Factors					Purity of gum %
	X' ₁	X' ₂	X' ₃	X' ₄	X' ₅	
	Concentration %	Temperature °C	Time h	Stirring rpm	Material-to-liquid ratio g/mL	
1	1	0	0	0	-1	93.13
2	1	1	0	0	0	90.91
3	0	0	0	-1	1	91.50
4	0	-1	0	0	-1	92.07
5	0	0	1	1	0	89.69
6	-1	-1	0	0	0	87.14
7	0	0	1	0	1	94.19
8	-1	0	1	0	0	88.10
9	1	0	0	0	1	91.35
10	0	1	0	0	1	92.46
11	0	0	-1	-1	0	77.29
12	0	0	0	0	0	95.22
13	0	0	0	0	0	94.11
14	0	1	0	-1	0	86.07
15	-1	0	0	-1	0	84.23
16	0	-1	0	-1	0	83.89
17	0	1	0	1	0	91.14
18	0	0	0	1	1	93.98
19	0	0	0	0	0	93.95
20	0	1	1	0	0	88.66
21	1	-1	0	0	0	87.60
22	-1	0	0	0	-1	90.29
23	0	0	-1	0	-1	90.24
24	0	-1	1	0	0	94.68
25	1	0	1	0	0	92.03
26	0	0	0	0	0	95.06
27	-1	0	-1	0	0	80.94
28	1	0	0	-1	0	86.19
29	1	0	-1	0	0	84.05
30	0	0	0	0	0	95.64
31	0	0	0	0	0	94.27
32	0	0	1	-1	0	89.39
33	0	-1	0	0	1	91.77
34	0	1	0	0	-1	92.51
35	-1	1	0	0	0	85.79
36	0	-1	0	1	0	88.27
37	0	-1	-1	0	0	76.83
38	0	0	1	0	-1	92.48
39	1	0	0	1	0	90.23
40	0	0	0	-1	-1	93.55
41	0	0	-1	1	0	89.73
42	0	0	0	1	-1	94.34
43	0	1	-1	0	0	88.77
44	0	0	-1	0	1	84.11
45	-1	0	0	0	1	90.27
46	-1	0	0	1	0	85.19

Table S4. ANOVA results of the regression equation for surfactant purification.

Factors	Sum of squares	df	Mean square	F-value	p-value ^a
Model	872.4160593	20	43.62080296	25.28398189	< 0.0001**
X ₁	34.68658829	1	34.68658829	20.1054316	0.0001**
X ₂	12.37179553	1	12.37179553	7.171079688	0.0129*
X ₃	205.0272858	1	205.0272858	118.8402283	< 0.0001**
X ₄	57.95936873	1	57.95936873	33.59506313	< 0.0001**
X ₅	5.017561472	1	5.017561472	2.908335583	0.1005
X ₁ X ₂	5.422460058	1	5.422460058	3.143027469	0.0884
X ₁ X ₃	0.168289692	1	0.168289692	0.09754597	0.7574
X ₁ X ₄	2.362198496	1	2.362198496	1.36920414	0.2530
X ₁ X ₅	0.77330374	1	0.77330374	0.448231037	0.5093
X ₂ X ₃	80.58411272	1	80.58411272	46.70907247	< 0.0001**
X ₂ X ₄	0.123145682	1	0.123145682	0.07137909	0.7915
X ₂ X ₅	0.01420353	1	0.01420353	0.00823281	0.9284
X ₃ X ₄	36.84769719	1	36.84769719	21.35807792	< 0.0001**
X ₃ X ₅	15.39410241	1	15.39410241	8.922903297	0.0062**
X ₄ X ₅	0.712351346	1	0.712351346	0.412901123	0.5264
X ₁ ²	148.9050055	1	148.9050055	86.30999904	< 0.0001**
X ₂ ²	89.66120348	1	89.66120348	51.97043821	< 0.0001**
X ₃ ²	189.0450107	1	189.0450107	109.5764017	< 0.0001**
X ₄ ²	108.621798	1	108.621798	62.96059188	< 0.0001**
X ₅ ²	7.630805934	1	7.630805934	4.423053817	0.0457*
Residual	43.13086755	25	1.725234702		
Lack of fit	40.75311979	20	2.037655989	4.284844727	0.0567
Pure error	2.377747759	5	0.475549552		
Correlation Total	915.5469268	45			
R ² =0.9529, R ² _{Adj} =0.9152, R ² _{Pred} =0.8182, C.V. %=1.47					

^a $p > 0.05$ is not significant difference; $p < 0.05$ is significant difference, indicated by "**"; $p < 0.01$ is highly significant difference, indicated by "**".

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