

Supplementary material

Impact of a novel two-phase natural deep eutectic solvent-assisted extraction on the structural, functional, and flavor properties of hemp protein

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Table 1S. Protein extraction purity (%) and yield for hemp protein isolate (HPI) samples and pellet fractions at different extraction pHs (7 to 11) and ash content (%) of HPI.

Solubilization pH	HPI			Discarded Pellet ¹		Discarded Supernatant ²	
	Protein Purity ³ (%)	Protein Yield ⁴ (%)	Ash (%)	Protein Purity ³ (%)	Protein Residue ⁵ (%)	Protein Purity ³ (%)	Protein Lost ⁶ (%)
7	73.4 ^d	3.91 ^e	2.55 ^{bc}	76.8 ^a	86.3 ^a	27.8 ^{bc}	6.85 ^a
8	76.3 ^c	8.10 ^d	2.60 ^{ab}	75.0 ^b	82.4 ^b	28.5 ^b	6.22 ^{ab}
9	84.4 ^b	23.4 ^c	2.63 ^{ab}	71.1 ^c	67.7 ^c	30.5 ^a	7.10 ^a
10	90.5 ^a	63.8 ^b	2.37 ^c	49.7 ^d	26.6 ^d	28.8 ^b	6.46 ^{ab}
11	91.1 ^a	88.6 ^a	2.78 ^a	2.99 ^e	0.780 ^e	26.4 ^c	5.76 ^b

¹Pellet discarded after alkaline solubilization;

²Supernatant discarded after isoelectric point precipitation;

³Protein purity (%) represents the amount of protein in the freeze-dried sample determined by the Dumas method;

⁴Protein yield (%) represents the amount of protein extracted relative to the total amount of protein in the starting defatted hemp meal (DHM);

⁵Protein residue (%) represents the amount of protein left in the discarded pellet relative to the total amount of protein in the starting DHM;

⁶Protein lost (%) represents the amount of protein lost to the discarded supernatant relative to the total amount of protein in the starting DHM;

^{a-c} Means (n = 3) in each column with different lowercase letters are significantly different, according to the Tukey-Kramer multiple means comparison test (P < 0.05).

Table 2S. Protein solubility of pH-extracted (pH 7-11) hemp protein isolate (HPI) samples measured at 5% protein concentration at both pH 7.0 and 3.4 with and without heating.

pH of protein extraction	% Solubility			
	pH 7.0		pH 3.4	
	Non-heated	Heated at 80 °C	Non-heated	Heated at 80 °C
11	4.23 ^c	4.90 ^d	53.5 ^c	56.1 ^b
10	6.01 ^d	6.12 ^d	67.9 ^a	67.7 ^a
9	13.9 ^c	12.5 ^c	65.5 ^a	65.2 ^a
8	23.9 ^b	23.4 ^b	52.2 ^c	53.8 ^b
7	48.7 ^a	46.1 ^a	57.6 ^b	55.0 ^b

^{a-c} Means in each column with different lowercase letters are significantly different, according to the Tukey-Kramer multiple means comparison test (P < 0.05).

Table 3S. Fatty acid profile (%) in hemp flour (HF), reference pH-extracted, and NADES-extracted hemp protein isolate samples (R-HPI, N-HPI).

Fatty Acid		HF	R-HPI	N-HPI
Palmitic acid	C16:0	5.12 ^c	16.3 ^a	8.27 ^b
Stearic acid	C18:0	2.33 ^c	5.41 ^a	3.99 ^b
Oleic acid	C18:1 cis	13.3 ^a	9.70 ^b	13.8 ^a
Linoleic acid	C18:2 n-6	56.6 ^a	51.1 ^b	52.3 ^b
Gamma-linolenic acid	C18:3 n-6	5.83 ^a	3.33 ^c	5.41 ^b
Linolenic acid	C18:3 n-3	15.3 ^a	12.5 ^c	14.0 ^b
Eicosanoic acid	C20:0	0.840 ^c	0.930 ^a	0.890 ^{ab}
Gondoic Acid	C20:1 n-9	0.340 ^b	0.310 ^b	0.510 ^a
11,14-Eicosadienoic acid	C20:2 n-6	ND ¹	ND	0.550 ^a
Behenic acid	C22:0	0.310 ^b	0.360 ^b	0.330 ^{ab}

¹ND: non detected;

^{a-c} Means (n ≥ 3) in each raw, within each fatty acid and across the different samples, with different lowercase letters are significantly different, according to the Tukey-Kramer multiple means comparison test (P <0.05).

Table 4S. Concentration of fatty acid (ug/g of sample) in hemp flour (HF), reference pH-extracted, and NADES-extracted hemp protein isolate samples (R-HPI, N-HPI).

Fatty Acid		HF	R-HPI	N-HPI
Palmitic acid	C16:0	13,410 ^a	7,835 ^b	261.9 ^c
Stearic acid	C18:0	6,107 ^a	2,593 ^b	126.3 ^c
Oleic acid	C18:1 cis	34,860 ^a	4,645 ^b	435.9 ^c
Linoleic acid	C18:2 n-6	148,100 ^a	24,470 ^b	1653 ^c
Gamma-linolenic acid	C18:3 n-6	15,270 ^a	1,596 ^b	170.5 ^c
Linolenic acid	C18:3 n-3	40,120 ^a	5982 ^b	442.80 ^c
Eicosanoic acid	C20:0	2192 ^a	446.3 ^b	28.12 ^c
Gondoic Acid	C20:1 n-9	883.6 ^a	150.5 ^b	15.74 ^c
11,14-Eicosadienoic acid	C20:2 n-6	ND ¹	ND	17.39 ^a
Behenic acid	C22:0	800.3 ^a	170.5 ^b	10.34 ^b
Total		261,740	47,890	3,162

¹ND: non detected;

^{a-c} Means (n ≥ 3) in each raw, within each fatty acid and across the different samples, with different lowercase letters are significantly different, according to the Tukey-Kramer multiple means comparison test (P <0.05).

Table 5S. Volatiles in hemp flour (HF), reference pH-extracted, and NADES-extracted hemp protein isolate samples (R-HPI, N-HPI).

N°	RT	Compound	Match (%)	RICal	RITab	Δ	Concentration (ppb)		
							HF	R-HPI	N-HPI
1	2.14	Acetic acid	94.8	685	660	25	874.5 ^a	ND ¹	1.563 ^b
2	2.27	3-methyl-Butanal	84.6	693	669	24	265.9 ^b	572.1 ^a	ND
3	2.32	1-butanol	89.3	696	669	27	57.74 ^a	15.91 ^b	ND
4	2.52	2-pentanone	98.1	709	715	-6	ND	7639 ^a	ND
5	3.12	3-methyl-1-butanol	94.8	747	741	6	26.87 ^b	250.90 ^a	1.072 ^b
6	3.16	2-methyl-1-butanol	80.6	750	736	14	15.79 ^b	72.76 ^a	6.001 ^b
7	3.16	Methyl isobutyl ketone	94.2	750	733	17	11.00 ^b	965.20 ^a	ND
8	3.41	2-pentenal	82.4	766	755	11	180.0 ^a	197.7 ^a	13.02 ^b
9	3.61	1-pentanol	92.1	778	780	-2	319.4 ^a	120.0 ^b	5.144 ^c
10	4.23	Hexanal	97.5	817	817	0	8460 ^a	9452 ^a	930.80 ^b
11	5.26	3-methyl-butanoic acid	82.1	858	834	24	10.86 ^b	3220 ^a	ND
12	5.44	2-hexenal	88.1	865	854	11	668.0 ^a	397.3 ^b	20.01 ^c
13	5.88	1-hexanol	98.8	882	880	2	38770 ^a	10730 ^b	39.69 ^c
14	6.41	2-heptanone	84.3	903	898	5	23.36 ^b	9767 ^a	83.52 ^b
15	6.76	2-heptanol	83.3	917	901	16	132.2 ^a	43.60 ^b	ND
16	7.75	α - pinene	97.2	947	939	8	2057 ^a	96.17 ^b	ND
17	8.51	2-heptenal	85	970	978	-8	104.3 ^b	209.80 ^a	55.04 ^c
18	8.65	Benzaldehyde	86.5	974	965	9	802.8 ^a	248.9 ^b	103.5 ^c
19	8.96	1-heptanol	85.4	984	975	9	11.94 ^a	5.904 ^b	ND
20	9.31	1-octen-3-ol	82.2	994	986	8	12.76 ^c	612.80 ^a	103.20 ^b
21	9.61	β -pinene	92.7	1003	984	19	5031.0 ^a	149.40 ^b	18.97 ^c
22	10.83	p-cymene	93.2	1038	1030	8	985.60 ^a	209.80 ^b	21.260 ^c
23	10.98	2-ethyl-1-Hexanol	95.6	1042	1029	13	ND	154.04 ^a	173.06 ^a
24	10.99	D-limonene	96	1043	1032	11	1551.0 ^a	316.70 ^b	26.70 ^c
25	11.09	Eucalyptol	81.4	1045	1036	9	11.50 ^b	ND	47.82 ^a

Table 5S. Continued.

N°	RT	Compound	Match (%)	RICal	RITab	Δ	HF	R-HPI	N-HPI
26	11.31	3-octen-2-one	89.1	1052	1046	6	13.08 ^b	171.73 ^a	0.7179 ^c
27	11.75	γ-Hexalactone	88.1	1064	1055	9	19.33 ^a	ND	ND
28	12.04	2-octenal	90.8	1072	1050	22	81.20 ^b	197.60 ^a	22.02 ^c
29	12.36	2-octen-1-ol	95.8	1081	1067	14	ND	37.41 ^a	ND
30	12.42	3,5-octadien-2-one	83.9	1083	1093	-10	14.20 ^b	42.61 ^a	0.376 ^c
31	12.84	8-nonen-2-one	86.3	1094	1080	14	ND	205.80 ^a	ND
32	12.99	α-terpinolene	88	1099	1089	10	118.50 ^a	59.77 ^b	ND
33	13.12	Fenchone	83	1102	1088	14	9.24 ^b	20.70 ^a	ND
34	13.73	Nonanal	96.4	1119	1108	11	82.12 ^c	1289 ^a	266.9 ^b
35	15.70	2-nonenal	96.6	1173	1171	2	110.1 ^b	659.1 ^a	ND
36	16.09	Octanoic acid	85.5	1184	1182	2	ND	363.4 ^a	ND
37	16.14	1-nonanol	85.3	1185	1172	13	ND	11.22 ^a	ND
38	16.33	p-menthan-3-ol	98.7	1191	1173	18	ND	35.44 ^b	23440 ^a
39	16.53	p-Menthan-3-ol isomer	99.6	1196	1170	26	1.301 ^b	2.201 ^b	10101 ^a
40	16.76	Levomenthol	95.6	1202	1188	14	ND	ND	109.0 ^a
41	16.85	2-dodecene	86.5	1205	1205	0	38.98 ^a	50.36 ^a	ND
42	17.70	8,9-dehydrothymol	95	1229	1221	8	ND	ND	371.93 ^a
43	19.44	Nonanoic acid	81.1	1279	1280	-1	114.3 ^b	203.0 ^a	ND
44	21.45	4-sec-Butylphenol	91.2	1338	1315	23	ND	ND	29780 ^a
45	22.51	γ-Nonanolactone	85.4	1369	1368	1	21.78 ^a	13.96 ^b	ND
46	24.46	Caryophyllene	97.7	1429	1419	10	554.94 ^a	ND	ND
47	24.91	β-bergamotene	81.4	1443	1436	7	110.3 ^a	2.351 ^b	ND
48	25.59	Humulene	92.2	1465	1457	8	336.10 ^a	ND	ND
49	27.20	2,4-di-tert-butylphenol	97.9	1516	1512	4	ND	32.60 ^b	193.4 ^a

N°: Compound number; RT: Retention time; RICal: retention index calculated; RITab: Retention index reported in National Institute of Standards and Technology; Δ: variation of Ris.

¹ ND: non detected;

^{a-c} Means (n ≥ 3) in each raw, within each fatty acid and across the different samples, with different lowercase letters are significantly different, according to the Tukey-Kramer multiple means comparison test (P < 0.05).