Protein Extraction, Precipitation, and Recovery from *Chlorella sorokiniana* Using Mechanochemical Methods

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SUPPLEMENTAL INFORMATION

Table S1. Forage analysis characterization of C. sorokiniana biomass. Samples were freeze dried prior to analysis. Values represent the average of two samples

Component	Value (% dry matter)
Crude Protein	58.3 ± 1.84
ADF	3.70 ± 0.141
NDF	6.70 ± 2.55
Lignin	2.45 ± 0.0707
Starch	2.60 ± 1.98
ESC	4.95 ± 0.636
TFA	9.42 ± 0.184
Ash	9.52 ± 1.05
TDN	82.5 ± 0.707

Table S2. Results of the factorial ANOVA for optimization of mechanochemical cell disruption. More than one variable indicates the presence of a significant interaction between variables.

Variable	df	Sum Squared	Mean Squared	Statistic	P Value
Milling media	1	3355	3355	162.7	0.0000
Ball to biomass ratio	1	4.402	4.402	0.2135	0.6461
Milling speed	2	39.36	19.68	0.9546	0.3923
Milling time	1	422.4	422.4	20.49	0.0000
KOH to biomass ratio	1	14545	14545	705.5	0.0000
Milling media x Ball to biomass ratio	1	20.93	20.93	1.015	0.3189
Milling media x Milling speed	2	170.9	85.47	4.146	0.0220
Ball to biomass ratio x Milling speed	2	232.0	116.0	5.627	0.0064
Milling media x Milling time	1	2.991	2.991	0.1451	0.7050

Ball to biomass ratio x Milling time	1	0.1044	0.1044	0.0051	0.9436
Milling speed x Milling time	2	34.28	17.14	0.8312	0.4418
Milling media x KOH to biomass ratio	1	578.1	578.1	28.04	0.0000
Ball to biomass ratio x KOH to biomass ratio	1	121.9	121.9	5.912	0.0189
Milling speed x KOH to biomass ratio	2	66.51	33.26	1.613	0.2101
Milling time x KOH to biomass ratio	1	212.5	212.5	10.31	0.0024
Milling media x Ball to biomass ratio x Milling speed	2	18.88	9.440	0.4578	0.6354
Milling media x Ball to biomass ratio x Milling time	1	34.34	34.34	1.666	0.2032
Milling media x Milling speed x Milling time	2	44.38	22.19	1.076	0.3491
Ball to biomass ratio x Milling speed x Milling time	2	309.4	154.7	7.504	0.0015
Milling media x Ball to biomass ratio x KOH to biomass ratio	1	20.77	20.77	1.008	0.3206
Milling media x Milling speed x KOH to biomass ratio	2	217.8	108.9	5.281	0.0085
Ball to biomass ratio x Milling speed x KOH to biomass ratio	2	193.9	96.95	4.702	0.0138
Milling media x Milling time x KOH to biomass ratio	1	8.764	8.764	0.4251	0.5176
Ball to biomass ratio x Milling time x KOH to biomass ratio	1	114.5	114.5	5.552	0.0227
Milling speed x Milling time x KOH to biomass ratio	2	38.35	19.17	0.9300	0.4017
Milling media x Ball to biomass ratio x Milling speed x Milling time	2	16.63	8.313	0.4032	0.6705
Milling media x Ball to biomass ratio x Milling speed x KOH to biomass ratio	2	4.721	2.361	0.1145	0.8921
Milling media x Ball to biomass ratio x Milling time x KOH to biomass ratio	1	7.546	7.546	0.3660	0.5481
Milling media x Milling speed x Milling time x KOH to biomass ratio	2	32.38	16.19	0.7853	0.4619
Ball to biomass ratio x Milling speed x Milling time x KOH to biomass ratio	2	283.4	141.7	6.873	0.0024
Milling media x Ball to biomass ratio x Milling speed x Milling time x KOH to biomass ratio	2	6.848	3.424	0.1661	0.8475