

Supplementary Information

Impact of canola seed dehulling and pressing temperature on the microstructure of canola meals, protein structure within the meals, and protein isolate extraction and properties

Abouzar Karimi^a, Tos Phuangmarayat^a, Pankaj Bhowmik^b, Anusha Samaranayaka^b, Lingyun Chen^{a*}

^a *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, T6G 2P5, AB, Canada*

^b *Aquatic and Crop Resource Development, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, S7N0W9, SK, Canada*

* Corresponding author, email address: lingyun.chen@ualberta.ca

Treatments	Seed + hull weight before dehulling (g)	Hull weight (18% hull content) (g)	Seed + hull weight after dehulling (g)	Removed hulls (g)	Dehulling efficiency (%)
Dehulled meal 1	502.12	90.3816	429.3	72.82	80.56949645
Dehulled meal 2	500.59	90.1062	426.12	74.47	82.64692108
Dehulled meal 3	504.33	90.7794	428.55	75.78	83.47708841

Table S1. Canola meal dehulling efficiency

Table S2. Protein content of canola meals

	Nitrogen content (%)	Protein content (%) (Conversion Factor: 5.7)
ND401	6.447	36.7479
ND402	6.408	36.5256
ND403	6.474	36.9018
ND801	6.957	39.6549
ND802	6.753	38.4921
ND803	6.602	37.6314
ND1201	6.964	39.6948
ND1202	6.955	39.6435
ND1203	6.967	39.7119
D401	7.688	43.8216
D402	7.774	44.3118
D403	7.871	44.8647
D801	7.951	45.3207
D802	7.8	44.46
D803	7.781	44.3517
D1201	7.974	45.4518
D1202	7.904	45.0528
D1203	7.723	44.0211

Abbreviations: ND40, ND80, and ND120, non-dehulled meals treated at 40, 80, and 120 °C, respectively; D40, D80, and D120, dehulled meals treated at 40, 80, and 120 °C, respectively.

Notes
 Input Data
 Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
ND40	3	0	36.7251	0.18913	0.1092
ND80	3	0	38.5928	1.0155	0.5863
ND120	3	0	39.6834	0.0356	0.02055
D40	3	0	44.3327	0.52186	0.3013
D80	3	0	44.7108	0.53096	0.30655
D120	3	0	44.8419	0.7383	0.42626

ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	192.1533	38.43066	106.37634	<0.0001
Error	12	4.33525	0.36127		
Total	17	196.48855			

Null Hypothesis: The means of all levels are equal.
 Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.97794	0.01449	0.60106	41.48112

Means Comparisons

Tukey Test

Grouping Letters Table

Tukey Test

	Mean	Groups
D120	44.8419	A
D80	44.7108	A
D40	44.3327	A
ND120	39.6834	B
ND80	38.5928	B
ND40	36.7251	C

Means that do not share a letter are significantly different.

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
 Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Figure S1. ANOVA and Tukey's test results of non-dehulled (ND) and dehulled (D) canola meals treated at 40 °C (ND40 and D40), 80 °C (ND80 and D80) and 120 °C (ND120 and D120). Different letters in the Tukey's test (a-c) indicate significant differences ($p < 0.05$) between the protein contents of the meals.

Table S3. Protein content and extraction efficiency of isolates.

	Extract Weight (g)	Nitrogen content (%)	Protein content (%) (Conversion Factor: 5.7)	Extraction efficiency (%)
ND40A1	3.2	13.9	79.23	69.03616328
ND40A2	3.05	13.76	78.432	65.13735837
ND40A3	3.1	13.47	76.779	64.80987118
ND80A1	3.04	14.28	81.396	64.11658133
ND80A2	3	14.29	81.453	63.31725089
ND80A3	3.02	14.31	81.567	63.82857424
ND120A1	2.27	14.96	85.272	48.77793737
ND120A2	2.13	15.02	85.614	45.95317438
ND120A3	2.37	14.85	84.645	50.55228383
D40A1	3.95	14.83	84.531	75.31629023
D40A2	3.89	14.66	83.562	73.32199031
D40A3	3.92	14.74	84.018	74.2906613
D80A1	3.78	14.78	84.246	71.22437532
D80A2	3.8	14.8	84.36	71.69811321
D80A3	3.79	14.79	84.303	71.46111678
D120A1	3.43	15.14	86.298	66.01016906
D120A2	3.27	15.08	85.956	62.68158129
D120A3	3.38	14.85	84.645	63.80195754
ND40S1	2.12	15.4	87.78	50.67204718
ND40S2	2.13	15.32	87.324	50.6465932
ND40S3	2	15.39	87.723	47.77277666
ND80S1	2.03	15.92	90.744	47.73178417

ND80S2	2.16	15.6	88.92	49.76762505
ND80S3	2.03	15.76	89.832	47.25206774
ND120S1	1.82	15.75	89.775	41.17351336
ND120S2	1.94	15.58	88.806	43.41453605
ND120S3	1.84	15.7	89.49	41.49382361
D40S1	2.8	15.68	89.376	56.44880641
D40S2	2.83	15.62	89.034	56.83529765
D40S3	2.75	15.81	90.117	55.90044143
D80S1	2.89	15.69	89.433	57.80736869
D80S2	2.65	15.97	91.029	53.9527027
D80S3	2.71	16.02	91.314	55.34701683
D120S1	2.66	15.64	89.148	52.88216601
D120S2	2.6	15.76	89.832	52.08592856
D120S3	2.6	15.67	89.319	51.78848354

Abbreviations: ND40A, ND80A, ND120A, D40A, D80A, and D120A, protein isolates extracted from non-dehulled and dehulled meals via alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S, protein isolates extracted from non-dehulled and dehulled meals via salt extraction.

Notes

Input Data

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
ND40A	3	0	66.3278	2.35122	1.35748
ND80A	3	0	63.75414	0.40483	0.23373
ND120A	3	0	48.4278	2.31946	1.33914
D40A	3	0	74.30965	0.99729	0.57578
D80A	3	0	71.4612	0.23687	0.13676
D120A	3	0	64.16457	1.69366	0.97784

ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	1219.9943	243.99886	97.65678	<0.0001
Error	12	29.98242	2.49853		
Total	17	1249.97671			

Null Hypothesis: The means of all levels are equal.
 Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.97601	0.02442	1.58068	64.74086

Means Comparisons

Tukey Test

Grouping Letters Table

Tukey Test

	Mean	Groups
D40A	74.30965	A
D80A	71.4612	A
ND40A	66.3278	B
D120A	64.16457	B
ND80A	63.75414	B
ND120A	48.4278	C

Means that do not share a letter are significantly different.

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
 Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Notes

Input Data

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
ND40S	3	0	49.69714	1.6666	0.96221
ND80S	3	0	48.25049	1.33559	0.7711
ND120S	3	0	42.02729	1.21202	0.69976
D40S	3	0	56.39485	0.46976	0.27121
D80S	3	0	55.70236	1.95175	1.12684
D120S	3	0	52.25219	0.56548	0.32648

ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	426.25007	85.25001	49.27707	<0.0001
Error	12	20.76017	1.73001		
Total	17	447.01024			

Null Hypothesis: The means of all levels are equal.
 Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.95356	0.02593	1.3153	50.72072

Means Comparisons

Tukey Test

Grouping Letters Table

Tukey Test

	Mean	Groups
D40S	56.39485	A
D80S	55.70236	A B
D120S	52.25219	B C
ND40S	49.69714	C D
ND80S	48.25049	D
ND120S	42.02729	E

Means that do not share a letter are significantly different.

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
 Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Figure S2. ANOVA and Tukey's test results of the extraction efficiency of the isolates extracted from non-dehulled and dehulled meals through alkaline (ND40A, ND80A, ND120A, D40A, D80A and D120A) and salt (ND40S, ND80S, ND120S, D40S, D80S, D120S) extraction. Different letters (a-e) in the Tukey's test indicate significant differences ($p < 0.05$) between the extraction efficiency of the isolates.

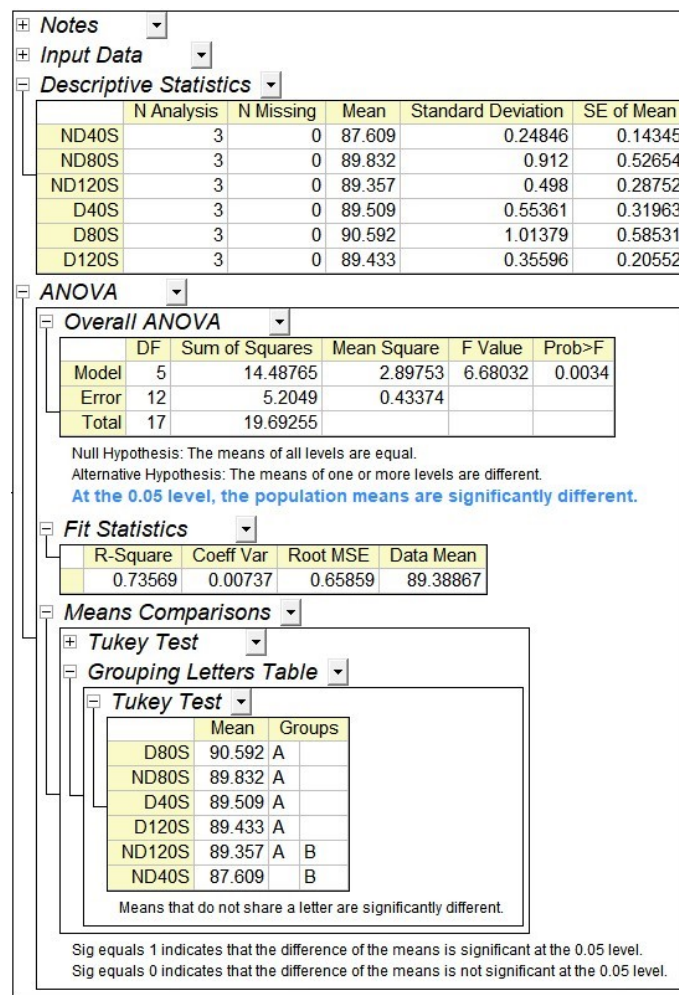
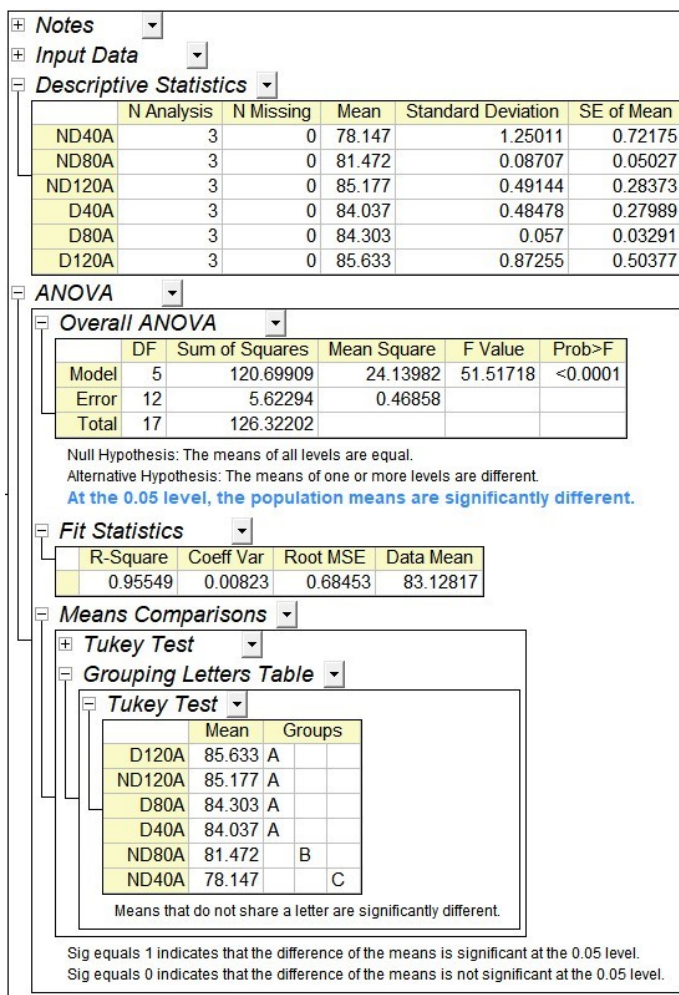


Figure S3. ANOVA and Tukey's test results of the protein content of the isolates extracted from non-dehulled and dehulled meals through alkaline (ND40A, ND80A, ND120A, D40A, D80A and D120A) and salt (ND40S, ND80S, ND120S, D40S, D80S, D120S) extraction. Different letters (a-c) in the Tukey's test indicate significant differences ($p < 0.05$) between the protein content of the isolates.

Table S3: CIELAB color space parameters of canola meals and protein isolates

	L*	a*	b*
ND401	74.1	-2.82	23.26
ND402	74	-2.8	23.27
ND403	73.56	-2.73	23.21
ND801	74.38	-2.91	23.96
ND802	74.96	-2.98	24.24
ND803	75.4	-3.01	24.32
ND1201	60.07	-0.44	22.22
ND1202	60.02	-0.44	22.26
ND1203	59.9	-0.46	22.13
D401	80.91	-2.83	29.23
D402	80.71	-2.84	29.65
D403	80.46	-2.76	29.83
D801	81	-2.8	29.73
D802	81.06	-2.8	29.44
D803	81.64	-2.94	29.69
D1201	77.52	-0.69	32.49
D1202	77.85	-0.86	32.2
D1203	78.28	-0.91	32.04
ND40A1	41.66	2.08	17.94
ND40A2	41.58	2.15	18.18
ND40A3	41.42	2.15	17.9

ND80A1	42.89	2.15	18.57
ND80A2	43.08	2.27	18.88
ND80A3	42.97	2.26	18.89
ND120A1	42.61	2.8	17.51
ND120A2	42.19	2.83	17.55
ND120A3	42.16	2.85	17.49
D40A1	41.97	-0.47	15.32
D40A2	41.72	-0.43	15.3
D40A3	41.83	-0.45	15.27
D80A1	43.44	-0.35	14.81
D80A2	43.31	-0.35	15.14
D80A3	43.57	-0.33	14.97
D120A1	43.67	-0.52	15.12
D120A2	43.02	-0.52	15.15
D120A3	43.47	-0.51	15.42
ND40S1	62.86	2.11	23.76
ND40S2	62.71	2.12	23.8
ND40S3	62.94	2.13	24.05
ND80S1	65.97	1.71	22.28
ND80S2	66.12	1.7	22.43
ND80S3	66.16	1.72	22.46
ND120S1	63.75	-1.05	24.96
ND120S2	63.81	-1.05	24.97
ND120S3	63.93	-1.03	25.32
D40S1	65.81	1.28	26.58
D40S2	65.53	1.26	26.37
D40S3	66.17	1.26	26.93
D80S1	66.26	1.01	24.72
D80S2	67.05	0.99	25.46
D80S3	66.79	1.08	25.52
D120S1	65.59	-1.75	24.21

D120S2	65.5	-1.67	24.3
D120S3	65.6	-1.72	24.51

Abbreviations: ND40, ND80, and ND120, non-dehulled meals treated at 40, 80, and 120 °C, respectively; D40, D80, and D120, dehulled meals treated at 40, 80, and 120 °C, respectively; ND40A, ND80A, ND120A, D40A, D80A, and D120A, protein isolates extracted from non-dehulled and dehulled meals via alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S, protein isolates extracted from non-dehulled and dehulled meals via salt extraction.

Table S4: Percentages of protein fractions in canola protein isolates

	Agg. And unfolded Cruciferins (%)	Cruciferin (%)	alpha chain (%)	beta chain (%)	napin (%)	napin long chain (%)	napin short chain (%)
ND40A1	30.9472	24.86541	6.16318	10.03284	21.03184	5.00975	1.11794
ND40A2	31.5672	25.48541	5.8885	10.17063	19.78574	5.19113	1.14682
ND40A3	30.3272	26.10541	6.4379	9.89505	22.27794	5.37251	1.1757
ND80A1	30.31269	25.90936	6.2068	10.09345	21.50167	4.56196	1.08906
ND80A2	29.2717	26.7553	5.9408	10.16816	20.88486	5.20988	1.16585
ND80A3	31.3537	25.0634	6.4728	10.01874	22.11848	4.88592	1.01227
ND120A1	15.12703	27.01478	7.958	11.66055	30.37054	6.25267	1.18383
ND120A2	16.1501	26.1601	8.3888	11.40139	29.93716	6.10201	1.15715
ND120A3	14.104	27.8695	8.8196	11.91971	30.80392	6.40333	1.21051
D40A1	31.08469	26.07676	6.34887	9.87647	20.13926	4.60086	0.95256
D40A2	31.7914	25.4146	6.0262	10.44994	21.05855	4.51629	0.9848
D40A3	30.378	26.7389	6.6715	9.303	21.97784	4.68543	0.92032
D80A1	31.57124	26.1362	6.36882	9.96252	20.95162	4.49125	0.90833
D80A2	31.0565	26.3555	6.8202	9.42565	20.56083	4.40268	0.85236
D80A3	32.086	24.3555	6.9175	10.49939	21.34241	4.57982	0.9643
D120A1	25.98175	26.72855	8.01222	11.30362	22.32985	4.81859	0.89262
D120A2	26.6612	25.9357	7.5473	11.97244	22.24234	4.7274	0.91568
D120A3	25.3023	27.5214	8.4771	10.6348	22.41736	4.63621	0.93874
ND40S1	6.8585	30.2058	7.92232	6.45944	39.41418	7.20311	1.5774
ND40S2	7.0371	29.5907	7.7341	6.63515	40.48503	7.045	1.55066
ND40S3	7.2158	30.8209	8.1105	6.81086	39.08503	7.36122	1.60414
ND80S1	6.6816	30.73967	7.99743	6.67196	38.84333	7.71684	1.80553

ND80S2	6.485	31.5547	7.9181	6.29111	37.52597	7.43989	1.77396
ND80S3	6.8782	29.9247	8.0768	7.05281	39.30441	7.99379	1.74239
ND120S1	3.9716	28.4085	7.34419	6.49326	43.81744	8.09782	1.31843
ND120S2	4.3087	29.0111	7.2798	6.63398	43.88264	7.1866	1.15779
ND120S3	4.6459	29.6136	7.4086	6.7747	41.74784	9.00904	1.47907
D40S1	6.67421	31.74363	7.091	6.38696	36.64961	8.78123	1.90159
D40S2	6.5799	31.1787	7.2854	6.64332	37.22354	8.45027	1.86461
D40S3	6.7685	32.3085	7.4798	6.1306	37.79747	9.11219	1.93857
D80S1	6.358	32.7122	8.2185	7.02485	35.29868	9.15009	2.01768
D80S2	7.0595	31.7188	7.7275	6.93886	34.72685	9.02067	1.96431
D80S3	7.761	30.7254	7.2365	7.11084	35.87051	9.27951	2.07105
D120S1	7.45271	32.34443	8.492	7.12112	34.21524	9.1977	1.85182
D120S2	7.2765	32.2014	7.9102	7.25984	33.69769	8.84077	1.8226
D120S3	7.6289	32.4874	8.2011	6.9824	34.73279	8.48384	1.79338

Abbreviations: ND40A, ND80A, ND120A, D40A, D80A, and D120A, protein isolates extracted from non-dehulled and dehulled meals via alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S, protein isolates extracted from non-dehulled and dehulled meals via salt extraction.

Table S5: Aqueous solubility of canola protein isolates

	Weight of the solubilized fraction after freeze drying (mg)	Protein content of the solubilized fraction (%)	Aqueous solubility (%)
ND40A1	70.21	82.3	28.891415
ND40A2	70.94	81.4	28.87258
ND40A3	70.52	81.85	28.86031
ND80A1	72.01	83.31	29.9957655
ND80A2	72.44	83.74	30.330628
ND80A3	72.25	83.53	30.1752125
ND120A1	94.12	82.77	38.951562
ND120A2	96.32	83.13	40.035408
ND120A3	95.26	82.95	39.509085
D40A1	70.9	87.08	30.86986
D40A2	70.01	87.89	30.7658945
D40A3	69.57	87.49	30.4333965
D80A1	72.12	87.41	31.520046
D80A2	72.56	87.41	31.712348
D80A3	72.25	87.41	31.5768625
D120A1	78.81	88.39	34.8300795
D120A2	75.52	87.64	33.092864
D120A3	76.75	88.02	33.777675
ND40S1	132.22	89.49	59.161839
ND40S2	132.04	89.33	58.975666
ND40S3	132.68	89.41	59.314594
ND80S1	119.23	88.88	52.985812

ND80S2	119.66	88.41	52.895703
ND80S3	119.56	88.65	52.99497
ND120S1	115.23	89.25	51.4213875
ND120S2	120.51	88.84	53.530542
ND120S3	117.44	89.05	52.29016
D40S1	137.5	89.02	61.20125
D40S2	131	90.45	59.24475
D40S3	134.21	89.74	60.220027
D80S1	131.87	89.88	59.262378
D80S2	128.55	90.71	58.3038525
D80S3	129.51	90.3	58.473765
D120S1	115.33	88.53	51.0508245
D120S2	120.12	89.29	53.627574
D120S3	117.58	88.91	52.270189

Abbreviations: ND40A, ND80A, ND120A, D40A, D80A, and D120A, protein isolates extracted from non-dehulled and dehulled meals via alkaline extraction; ND40S, ND80S, ND120S, D40S, D80S, and D120S, protein isolates extracted from non-dehulled and dehulled meals via salt extraction.

Notes

Input Data

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
ND40A	3	0	28.95542	0.41882	0.24181
ND80A	3	0	30.16792	0.58509	0.3378
ND120A	3	0	39.49869	0.542	0.31292
D40A	3	0	30.68903	0.65803	0.37991
D80A	3	0	31.60309	0.47498	0.27423
D120A	3	0	33.90021	0.87507	0.50522

ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	219.06943	43.81389	117.57611	<0.0001
Error	12	4.47171	0.37264		
Total	17	223.54114			

Null Hypothesis: The means of all levels are equal.
 Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.98	0.0188	0.61044	32.46906

Means Comparisons

Tukey Test

Grouping Letters Table

	Mean	Groups
ND120A	39.49869	A
D120A	33.90021	B
D80A	31.60309	C
D40A	30.68903	C
ND80A	30.16792	C D
ND40A	28.95542	D

Means that do not share a letter are significantly different.

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
 Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Notes

Input Data

Descriptive Statistics

	N Analysis	N Missing	Mean	Standard Deviation	SE of Mean
ND40S	3	0	59.1507	0.16974	0.098
ND80S	3	0	52.95883	0.05486	0.03167
ND120S	3	0	52.41403	1.06002	0.612
D40S	3	0	60.22201	0.97825	0.56479
D80S	3	0	58.68	0.51146	0.29529
D120S	3	0	52.3162	1.28899	0.7442

ANOVA

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	211.8056	42.36112	62.98229	<0.0001
Error	12	8.07105	0.67259		
Total	17	219.87665			

Null Hypothesis: The means of all levels are equal.
 Alternative Hypothesis: The means of one or more levels are different.
At the 0.05 level, the population means are significantly different.

Fit Statistics

R-Square	Coeff Var	Root MSE	Data Mean
0.96329	0.01466	0.82011	55.95696

Means Comparisons

Tukey Test

Grouping Letters Table

	Mean	Groups
D40S	60.22201	A
ND40S	59.1507	A
D80S	58.68	A
ND80S	52.95883	B
ND120S	52.41403	B
D120S	52.3162	B

Means that do not share a letter are significantly different.

Sig equals 1 indicates that the difference of the means is significant at the 0.05 level.
 Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Figure S4. ANOVA and Tukey's test results of the aqueous solubility of the isolates extracted from non-dehulled and dehulled meals through alkaline (ND40A, ND80A, ND120A, D40A, D80A and D120A) and salt (ND40S, ND80S, ND120S, D40S, D80S, D120S) extraction. Different letters (a-d) in the Tukey's test indicate significant differences ($p < 0.05$) between the aqueous solubility of the isolates