Circular Bioprocess for Phosphorus Nutrient Recovery to Grow Lettuce in Lunar Space

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Supporting Information

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S1. Phosphorus content of plants

Table 1: Phosphorus content of different parts and varieties of plants.

Vegetable	Parts/Varieties	Amount of P (mg/100g)	Source
Amaranth	Grain	557	[1]
	Leaves	50	[2]
Cabbage	Chinese (pak-choi)	37	[3]
	Chinese (pe-tsai)	29	
	Common (danish, domestic and pointed)	23	
	Red	30	
	Regular	26	
	Savoy	42	
	Swamp (skunk)	39	
Common bean	N/A	450-720 (of dry matter)	[4]
		483.5 (of dry matter)	[5]
Corn	Sweet white and yellow	89	[6]
	White and yellow	210	
		450	[7]
Lentils	Pink/red	294	[8]
	Regular	281	[9]
		451	[8b]
	Sprouted	173	[10]
Pumpkin/squash	'Flesh'	44	[11]
	Flowers	49	[12, 11c]
	Leaves	104	[13, 11c]
	Seed kernels, dried	1233	[14]
Rice	Brown, long-grain	333	[15]
	Brown, medium-grain	264	[15a, 16]
	Glutinous	71	[17]
	Long-grain, white	115	[18]
	Medium-grain, white	108	[17a, 19]
	Short-grain, white	95	[17a, 20]
Soybean	Green	194	[21]
	Mature seeds	704	
	Mature seeds, sprouted	164	

S2. Statistical assessment of phosphorus extraction

In order to set the optimal working conditions, the effect of the extraction agent, extraction time and roots calcination temperature was studied using analysis of variance. For this purpose, a sequential sum of squares for two-factor interaction design (2FI) was performed using the Design-Expert software (Version-13.0.1.0). Significances were evaluated at 95 % confidence level.

Table 2 shows the ANOVA assessment carried out using results obtained at 4 levels of extraction times (1, 2, 3 and 4 hours), 3 calcination temperatures (250, 450 and 750 °C) as well as 3 extraction solvents (sulphuric acid 0.05 M, citric acid 0.1 M and oxalic acid 0.1 M). The model obtained resulted to be significant at 95 % confidence level with an R^2 of 0.81. Figure 1 shows the residuals normal plot (a) as well as the predicted vs experimental plot (b) in order to show the accuracy of the model.

Best results were obtained for oxalic 0.1 M and citric acid 0.1 M with, respectively, 117 and 96 mg P L^{-1}, whilst sulphuric acid of 0.05 M yielded a 67 mg P L^{-1} solution, with a 250 °C calcination temperature and 4 h extraction.

Table 2: ANOVA factorial assessment of the effect of calcination temperature, extraction time and solvent on dissolved P measurements (refers to significant values at 95 % confidence level, Df: degree of freedom)*

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.0232	15	0.0015	5.92	0.0001	*
A-Time	0.0016	1	0.0016	5.96	0.0236	*
B-Temp.	0.0012	1	0.0012	4.69	0.0419	*
C-Solvent	0.0172	3	0.0057	21.89	< 0.0001	*
AB	0.0003	1	0.0003	0.9920	0.3306	
AC	0.0001	2	0.0001	0.2486	0.7822	
BC	0.0015	3	0.0005	1.96	0.1503	
A^2	3.683E-06	1	3.683E-06	0.0141	0.9066	
B^2	0.0018	1	0.0018	6.92	0.0156	*
ABC	0.0004	2	0.0002	0.7903	0.4668	
Residual	0.0055	21	0.0003			
Total	0.0287	36				

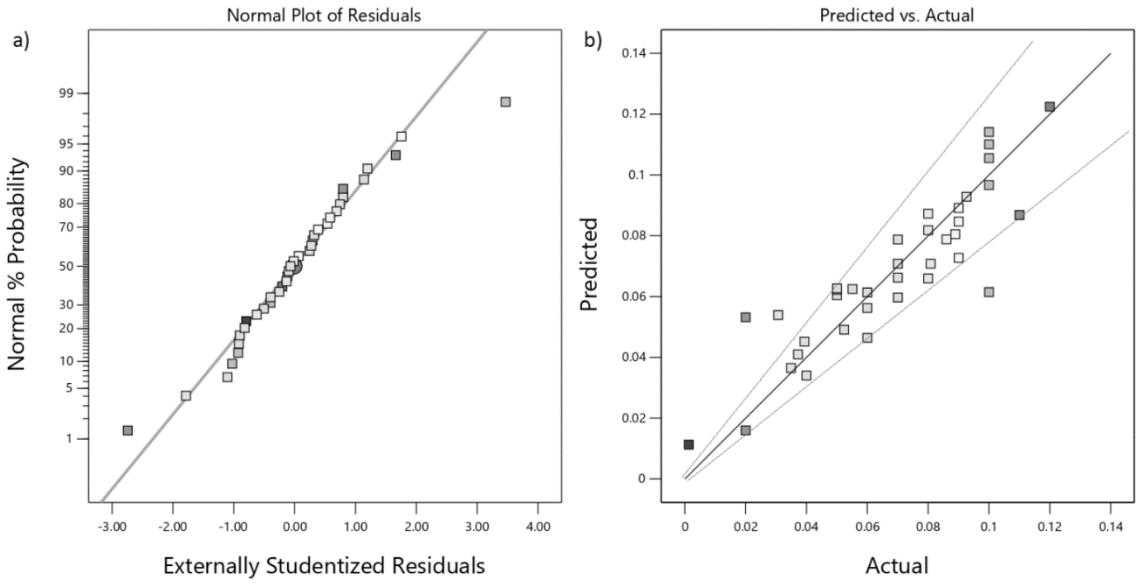

Figure 1: a) P residuals normal plot of experiments, b) predicted vs experimental results for P extraction with 20 % deviation lines.

S3. Statistical assessment of potassium extraction

The effect of the extraction agent, extraction time and roots calcination temperature were studied using two-factor interaction design (2FI) analysis of variance at 95% confidence level. Table 3 shows the ANOVA assessment for K content carried out using results obtained at 4 levels of extraction times (1, 2, 3 and 4 hours), 3 calcination temperatures (250, 450 and 750 °C) as well as 3 extraction solvents (sulphuric acid 0.05 M, citric acid 0.1 M and oxalic acid 0.1 M). The model obtained resulted to be significant at 95 % confidence level with an R^2 of 0.80. Figure 2 shows the normal (a) and experimental vs predicted (b) plots, which confirm the good correlation of the model with good prediction capacity.

The optimal conditions for K extraction were achieved when calcining lettuce roots at 250 °C and 0.5 h with 0.1 M citric acid as extracting acid. The optimal K extraction yield is 1083 mg K mL^{-1}.

Table 3: ANOVA factorial assessment of the effect of calcination temperature, extraction time and solvent on dissolved K measurements (refers to significant values at 95 % confidence level. Df: Degree of freedom)*

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.74	15	0.1161	5.44	0.0002	*
A-Time	0.0277	1	0.0277	1.30	0.2674	
B-Temp.	0.5630	1	0.5630	26.36	< 0.0001	*
C-Solvent	0.2566	3	0.0855	4.00	0.0212	*
AB	0.0422	1	0.0422	1.97	0.1745	
AC	0.0756	2	0.0378	1.77	0.1949	
BC	0.1017	3	0.0339	1.59	0.2225	
A^2	0.0000	1	0.0000	0.0014	0.9701	
B^2	0.3919	1	0.3919	18.34	0.0003	*
ABC	0.1034	2	0.0517	2.42	0.1133	
Residual	0.4486	21	0.0214			
Total	2.19	36				

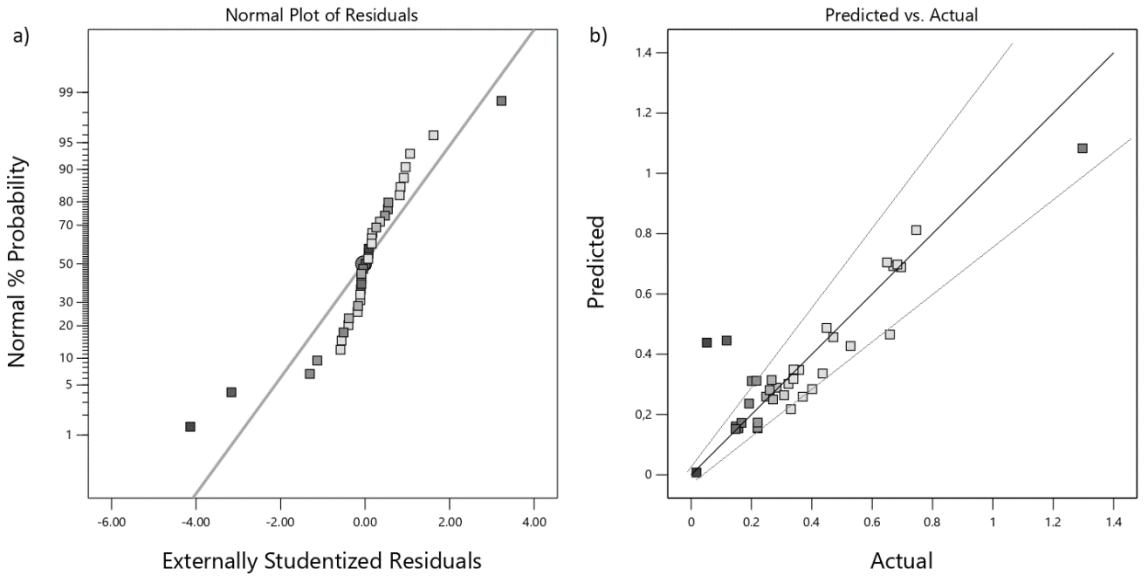

Figure 2: a) P residuals normal plot of experiments, b) predicted vs experimental results for K extraction with 20 % deviation lines.

S4. Combining P-K extraction from lettuce roots

From one side, optimal P extraction is achieved using oxalic acid 0.1 M during 4 hours using ashes calcinated at 250 °C, giving a maximum concentration of 117 mg P L^{-1}. From the other side, the optimal K extraction is achieved with citric acid 0.1 M during 0.5 h using also ashes calcinated at 250 °C. The optimal K extraction yield is 1083 mg K mL^{-1}. Based on the hypothesis of maximum simultaneous extraction, a new variable was created: P+K, meaning the sum of both nutrients in the extracted solution in the same operating conditions based on previous experiments. The scenarios under study are shown in Table 4. As a result, optimal extraction conditions of 250 °C calcination temperature using 30 min extraction time were confirmed using oxalic acid 0.1 M. In these conditions, the prediction pattern estimates a simultaneous extraction of max. 92 mg P L^{-1} and 688 mg K L^{-1} in just one run, which means 77 % P and 64 % K of the single maximum extraction capacities.

Table 4: Statistical optimization of simultaneous P and K extractions from lettuce root ash. Results are sorted according to the value of P+K variable.

Item	Time, h	T, °C	Acid	P, g L^{-1}	K, g L^{-1}	P+K, g L^{-1}
1	**0.5**	**250.0**	**Oxalic**	**0.092**	**0.688**	**0.733**
2	0.7	250.0	Oxalic	0.094	0.688	0.726
3	0.5	260.3	Oxalic	0.091	0.659	0.726
4	1.1	250.0	Oxalic	0.097	0.689	0.712
5	0.5	250.0	Citric	0.073	1.083	0.690
6	0.5	253.8	Citric	0.072	1.069	0.688
7	0.5	257.7	Citric	0.071	1.054	0.685
8	0.5	260.3	Citric	0.071	1.045	0.683
9	0.5	262.3	Citric	0.071	1.037	0.682
10	0.9	250.0	Citric	0.077	1.012	0.676
11	1.2	250.0	Citric	0.080	0.957	0.665
12	0.5	250.0	Sulphuric	0.043	0.419	0.547
13	0.5	253.9	Sulphuric	0.043	0.411	0.544
14	0.5	265.0	Sulphuric	0.042	0.389	0.537
15	0.9	250.0	Sulphuric	0.046	0.427	0.529
16	0.5	250.0	None	0.004	0.469	0.443
17	0.5	250.0	None	0.004	0.466	0.441
18	0.5	260.3	None	0.003	0.442	0.436
19	0.9	250.0	None	0.008	0.444	0.426

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