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FACTORIAL ANALYSIS ON PROCESSING FACTORS FOR NITROGEN, PHOSPHORUS AND POTASSIUM CONTENTS IN MUSHROOM WASTE

Norazwina Zainol*, Muhammad Faiz Mohamed Ameen, Sunarti Abdul Rahman, Muhammad Adib Rinalto, Nur Syahirah Muhammad Nor
Faculty of Chemical & Natural Resources Engineering,
Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia
∗ Corresponding author: E-mail: azwina@ump.edu.my
Tel.: +60192719331 Fax: +6095492889

ABSTRACT

The agriculture industry relies heavily on the use of bio-fertilizer and the main components in bio-fertilizer are nitrogen (N), phosphorous (P), and potassium (K). Thus, a study was conducted to identify the N, P, and K contents in mushroom waste (MW). These components are numerous building blocks that plants need for healthy growth. Therefore, by increasing the N, P and K contents in MW, it can be utilized to produce high and better quality of bio-fertilizer. Five independent factors, i.e., aging of waste (fresh 0 day & aged 14 days), waste pH (7 & 8.5), composition [MW only & mixture of MW & spent medium (SM)], technique of drying (oven 50 °C & sunlight), and MW size (powder & cut) were the affecting factors on N, P, and K contents in MW. A 2^{5-1} fractional factorial design was used to investigate the effect of the independent factors as well as the interaction factors on the N, P, and K contents. The N, P, and K contents were measured using HACH spectrophotometer. The objective of this research is to identify the best combination of processing factors. Some of the independent factors were shown to have significant effects on the N, P and K contents. The results showed that the most significant factor in N content are MW size and aging of waste, while for P and K contents are technique of drying and MW size. The best condition was identified to maximize the amount of N, P, and K contents in MW. The identified conditions were the MW aged for 7 days, MW size at powder form, waste pH at 7, drying under sun light and the composition MW only. Based on the proposed best condition the N (12.08 mg/L), P (3.04 mg/L), and K (8.09 mg/L) contents were achieved. The results show that fractional factorial design was suitable in investigating the effect of a large number of factors with a minimum number of experiments.

Keywords: Bio-fertilizer; mushroom waste (MW); two level factorial analysis (TLFA); N, P and K contents

1.0 INTRODUCTION

Most mushroom waste (MW) in Malaysia is mainly from grey oyster mushroom and it has a good source of protein, vitamins, and minerals. Besides, it is also used as food and medicine (Caglarirmak, 2007). Bio-fertilizer is the substance containing living microorganisms where it increases the productivity and availability of primary nutrients when applied to soil (Chatterjee *et al.,* 2006). It has significant contribution in the growth and yield of the plants (Chandrasekar *et al.,* 2005). Furthermore, it able to fix atmospheric nitrogen in the available form for plants either by living freely in the soil or being associated symbiotically with plants (Karami *et al.*, 2011).

Apart from that, chemical fertilizers pose a health hazard and a microbial population problem in soil besides beings quite expensive and making the cost of production high. In such a situation, the bio-fertilizers play a major role (Mahdi et al., 2010). Several authors such as Fawzy *et al.* (2007) and Ahmad *et al.* (2008) concentrated on the quality and influence on the growth of plants by applying the bio-fertilizer in farming system. Since MW has high levels of nutrients such N, P, K and high amounts of organic matter (Peyvast *et al.,* 2007a; Peyvast *et al.,* 2008b and Shabani *et al.,* 2011) it be superior to the farmyard. Based on the nutrient contents in MW, it could be utilized as bio-fertilizer by maximising the N, P and K contents.

Therefore, the study conducted focus on the factors contributing on N, P and K contents in MW. The factors that been selected in this research were aging of waste, waste pH, composition, technique of drying and MW size. Two level factorial analysis (TLFA) factorial design used for factor analysis. The aims of TLFA was to study a response (output variable) that influenced by several independent factors (input variables). Besides, it identifies the factors that have the highest contribution on N, P and K contents. Two-level analysis is very important because it quickly filters out unwanted effects. Thus, the attention can then be focused on the important ones.

2.0 MATERIALS AND METHODS

Analysis method

The MW samples were analyzed to determine the N, P, and K contents using HACH spectrophotometer. The methods and chemicals used during the analyses were listed in the Table 1.

Table 1: Method and chemical for N, P and K analysis

Method	Chemical	Component analysed
Persulfate digestion method	Total nitrogen persulfate reagent powder pillow, total nitrogen hydroxide reagent vials, TN reagent A powder pillow and TN reagent B powder pillow	N
Molybdovanadate method with acid persulfate digestion	Total phosphorus test 'N' tube vial, molybdovanadate reagent and 1.54 N sodium hydroxide	P
Tetraphenylborate method	Potassium persulfate powder pillow, potassium 1 reagent pillow, potassium 2 reagent pillow and potassium 3 reagent pillow	K

Mushroom Waste Sample collection and preparation

In this study, the MW samples from grey oyster mushrooms (*Pleurotus sajor-caju* spp) were collected from Rozeriya Enterprise in Kuala Krai, Kelantan, Malaysia. The samples were collected after the mushroom harvesting time.

Experimental design setup

Five factors were chosen and divided to categorical and numerical factors. There were three categorical factors, namely composition of MW, technique of drying, and MW size. The other two factors were numerical factors which were aging of waste and waste pH. The effects of these factors were explored by using a 2^{5-1} fractional factorial design. The experimental design for factorial analysis was performed by using Design Expert software V.7. The two-level factorial design was used in this study. This method was used to analyze five factors that were found to affect the N, P and K contents. Table 2 lists the design factors and levels coded as −1 (low level) and +1 (high level). The low level indicates the lowest range of the factors and high level indicates the highest range of the factors. The 16 runs of experiments were performed in this study. The responses of the experimental design were N, P and K contents measured using HACH spectrophotometer. The experimental data were analyzed using analysis of variance (ANOVA) performed using Design Expert software V7.

Table 2: 2^{5-1} Fractional Factor Design Matrix

Factor	**Level**	
	−1	+1
Aging of MW	0 day - fresh	7 days
MW size	Cut	Powder
pH	7	8
Technique of drying	Sunlight	Oven 50 °C
Composition	MW	Mix MW and SM (spent media)

3.0 RESULTS AND DISCUSSION

Screening of factors affecting on the N, P and K contents

The screening of the factors that contributed on N, P and K content was carried out through 2^{5-1} fractional designs as shown in Table 3. This was to categorize the degree of the effectiveness of the factors on the N, P and K contents. It can also determine the effect for the main factors and the interactions effect between the factors (Golshani *et al.,* 2013). The independent factors were taken into consideration during study the effect on the N, P and K content. The N ranged from 0.14 to 8.68 mg/L, while P content ranged from 0.33 to 1.86 mg/L and the K content ranged from 1.39 to 7.19 mg/L.

Table 3: 2^{5-1} Fractional Factor Design analysis using Design Expert software with its respective responses

Run	Factor 1: Aging	Factor 2: MW size	Factor 3: pH	Factor 4: Drying	Factor 5: Composition	Response 1 N (mg/L)	Response 2 P (mg/L)	Response 3 K (mg/L)
1	Fresh 0	Cut	7	Sunlight	Mix MW & SM	1.85	0.73	2.31
2	7	Cut	7	Sunlight	MW	4.02	1.46	2.97
3	Fresh 0	Powder	7	Sunlight	MW	6.02	1.55	3.41
4	7	Powder	7	Sunlight	Mix MW & SM	6.77	2.40	7.17
5	Fresh 0	Cut	8.5	Sunlight	MW	2.18	0.48	2.61
6	7	Cut	8.5	Sunlight	Mix MW & SM	2.85	1.40	3.98
7	Fresh 0	Powder	8.5	Sunlight	Mix MW & SM	5.09	2.02	5.29
8	7	Powder	8.5	Sunlight	MW	7.68	1.97	5.33
9	Fresh 0	Cut	7	Oven 50 °C	MW	4.23	2.47	7.88
10	7	Cut	7	Oven 50 °C	Mix MW & SM	2.03	2.02	2.07
11	Fresh 0	Powder	7	Oven 50 °C	Mix MW & SM	4.91	2.68	6.02
12	7	Powder	7	Oven 50 °C	MW	12.25	4.05	13.32
13	Fresh 0	Cut	8.5	Oven50 °C	Mix MW & SM	4.26	2.74	7.86
14	7	Cut	8.5	Oven 50 °C	MW	2.06	1.19	4.81
15	Fresh 0	Powder	8.5	Oven 50 °C	MW	2.15	3.53	12.55
16	7	Powder	8.5	Oven 50 °C	Mix MW & SM	6.30	3.20	6.09

Analysis of variance (ANOVA) for N, P and K contents

The analysis of variance (ANOVA) was done to determine the significance of the model. Table 4 shows the ANOVA analysis summary of N, P and K contents to estimate the coefficient of the model, the significance of each parameter, and indicate the interaction strength of each parameter. The significance of a regression equation was checked using F-values while the p-values were used to check the significance of each coefficient (Wang *et al.,* 2013). The p-value tests the data from the experiment with the identical means. If the p-value was less than 0.05, the model terms are significant. Meanwhile, the p-value greater than 0.1 indicate the model terms are not significant.

For the ANOVA analysis, the F-value was 37.31 for N, 35.33 for P and 24.43 for K. For the N content, the significant model terms were B (MW size), A (aging), AB (aging and MW size) and CE (pH and composition). For the P content, it was observed that the significant model terms were B (MW size), D (technique of drying), AD (aging and technique of drying) and CE (pH and composition). For the K content, the B (MW size), D (technique of drying), AC (aging and pH), AD (aging and technique of drying) and DE (technique of drying and composition) were significant model terms.

The R-squared (R^2) from the ANOVA was used to indicate how close the data to the fitted regression line. Ölmez (2009) suggested that a good fit of a model R^2 should more than 80% (0.80) for a good fitting model. The coefficient of determination (R^2) and adjusted coefficient of determination (R^2 adj) for the N, P and K were 0.99 and >0.94, respectively. It specified that the model was a good fit and regression models explained the mechanism well since the R^2 for these response variables are higher than 0.80. To express the N, P and K contents as a function of independent variables, the final empirical model in terms of actual factors were determined as the regression Equations 1-3.

$$\text{N content} = 4.66 + 0.83A + 1.73B - 0.59C + 0.11D - 0.41E + 1.03AB - 0.60AE - 0.50BC - 0.49CD + 0.96CE \quad (1)$$

$$\text{P content} = 2.12 + 0.093A + 0.56B - 0.051C + 0.62D + 0.030E + 0.14AB - 0.22AC - 0.21AD - 0.13BE + 0.24CE - 0.11DE \quad (2)$$

$$\text{K content} = 5.85 - 0.14A + 1.54B + 0.21C + 1.72D - 0.76E + 0.72AB - 0.88AC - 0.86AD + 0.38BD - 0.50BE + 0.50CE - 1.31DE \quad (3)$$

where A is aging, B is MW size, C is waste pH, D is technique of drying and E is composition.

Pareto Chart

Main and interaction effects between factors based on Pareto chart for nitrogen (N), phosphorus (P) and potassium (K) contents. The Pareto chart in Figure 1 demonstrates the main and interaction effects of the factors for the N. The MW size (B) gave the highest contribution for N content. It followed by aging (A), pH (C) and composition (E). There were five interaction effects that contributed on N content. There were two positive interaction effects between AB (aging and MW size) and between CE (pH and composition). Positive effects referred to increase of N content as the value of factors increased. The interaction between AE (aging and composition), BC (MW size and pH) and CD (pH and technique of drying) represented negative effects. Negative effects referred to N content decreased as the value of the factors increased.

Figure 2 demonstrates the main effects and interaction effects of the factors for P. The technique of drying (D) has the highest contribution on P content and followed by MW size (B). For interaction effects, it shows to have three interaction effects that contributed on P content. There was one positive effects interaction between CE (pH and composition). The interaction between AC (aging and pH) and AD (aging and technique of drying) represented negative effects.

Figure 3 demonstrates the main effects and interaction effects of the factors for K. The technique of drying (D) gave the highest contribution on K content followed by MW size (B) and composition (E). It has four interaction effects that contributed to the K content. There was one positive effect interaction between AB (aging and MW size). The interaction between AC (aging and pH), AD (aging and technique of drying) and DE (technique of drying and composition) represented negative effects.

Table 4: ANOVA analysis for N, P and K

N				P			
Source	Sum of Squares	F-value	p-value Prob > F	Source	Sum of Squares	F-value	p-value Prob > F
Model	1.13E+08	37.31	0.0005	Model	1.40E+07	35.33	0.0018
A: Aging	1.10E+07	36.57	0.0018	A: Aging	137289	3.70	0.1269
B: MW size	4.80E+07	158.70	< 0.0001	B: MW size	4974796	133.95	0.0003
C: pH	5652506	18.73	0.0075	C: pH	42199.4	1.14	0.3465
D: Technique of Drying	189443	0.63	0.4641	D: Technique of Drying	6073637	163.54	0.0002
E: Composition	2666689	8.84	0.0311	E: Composition	14768.3	0.40	0.5625
AB	1.70E+07	55.71	0.0007	AB	299674	8.07	0.0468
AE	5750404	19.06	0.0073	AC	772509	20.80	0.0103
BC	3939233	13.05	0.0153	AD	733635	19.75	0.0113
CD	3801525	12,60	0.0164	BE	270998	7.30	0.054
CE	1.50E+07	49.18	0.0009	CE	936201	25.21	0.0074
Residual	1508853			DE	176421	4.75	0.0948
Cor Total	1.14E+08			Residual	148552		
R-Squared	0.99			Cor Total	1.50E+07		
Adj R-Squared	0.96			R-Squared	0.99		
				Adj R-Squared	0.96		

P			
Source	Sum of Squares	F-value	p-value Prob > F
Model	1.66E+08	24.43	0.0116
A: Aging	300907	0.53	0.5184
B: MW size	3.80E+07	67.45	0.0038
C: pH	714194	1.26	0.3427
D: Technique of Drying	4.70E+07	83.72	0.0028
E: Composition	9139738	16.17	0.0276
AB	8214243	14.54	0.0317
AC	1.20E+07	21.70	0.0187
AD	1.20E+07	21.17	0.0193
BD	2276930	4.01	0.1383
BE	3984415	7.05	0.0766
CE	3926739	6.95	0.0779
DE	2.70E+07	48.59	0.0061
Residual	1695175		
Cor Total	1.67E+08		
R-Squared	0.99		
Adj R-Squared	0.95		

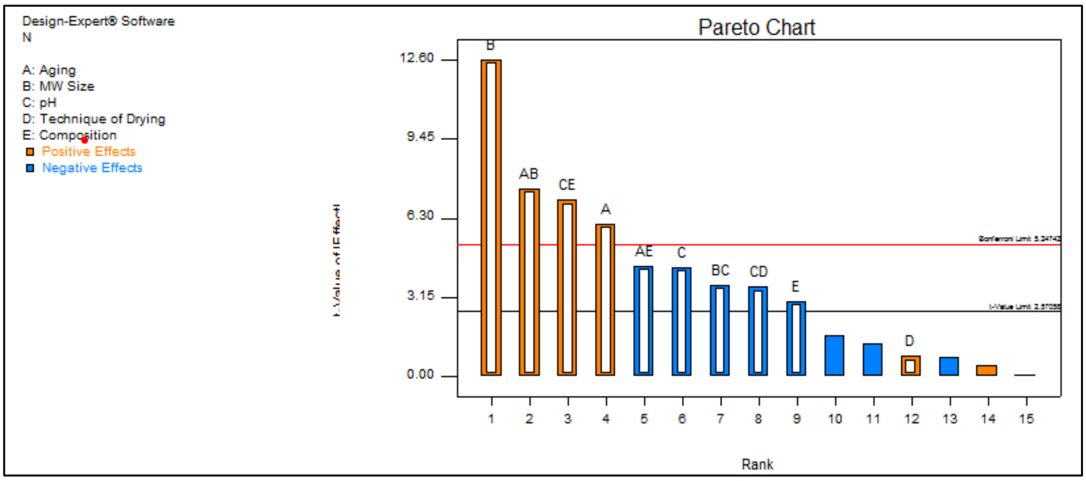


Figure 1: Pareto chart for nitrogen (N) main and interaction effect between factors

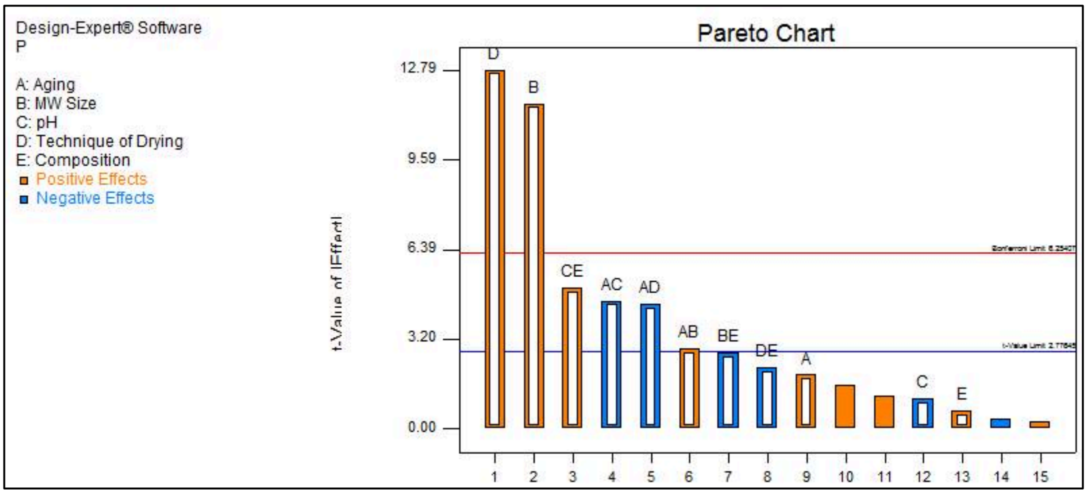


Figure 2: Pareto chart for phosphorus (P) main and interaction effect between factors

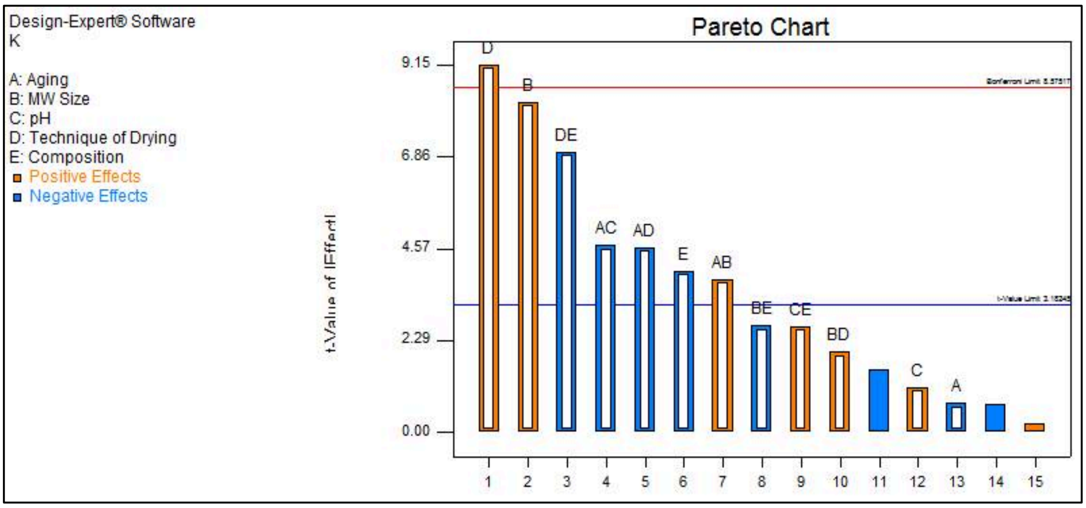
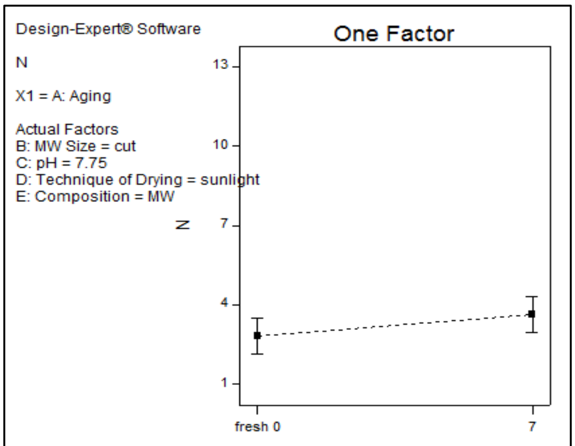


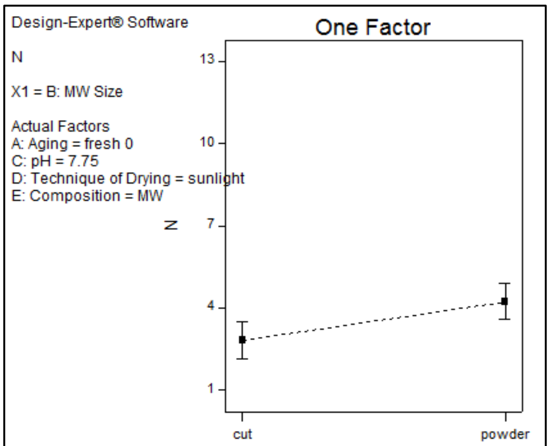
Figure 3: Pareto chart for potassium (K) main and interaction effect between factors

Effect of independent processing factors on N content

The effect of two independent factors on the N content is shown in Figure 4. The N content increases with the increasing of aging days as shown in Figure 4a. The N content was lower at 0-day aging than at 7-day aging. N content achieved 2.83 mg/L at 0-day aging and 3.64 mg/L at 7-day aging. Thus, it has significant difference with 22.27 % increment of N between 0-day aging and 7-day aging. The difference was due to increase of freely available N after aging (Hubbe *et al.,* 2010). While, Figure 4b demonstrates, the N content increases with the decreasing of MW size. The N content achieved was 4.24 mg/L for powder size factor, while for cut size factor, only 2.83 mg/L was achieved. It indicates that higher N content achieved using the powder size form. It also showed that there was a significant difference of N content in both MW size. As the MW size decreases, the availability N increases as it dissolves more in water. Thus, it increases N content in MW. Sangamithirai *et al.* (2015) stated that the smallest particles have much more surface area for a given volume and more rapidly broken down by microbes. Several authors such as Xu *et al.* (2014) and Jing *et al.* (2007) concentrated on size as important factor controlling mobility of nutrients in soil by changing nutrients speciation. Therefore, MW size gave the highest contribution in N content.



(a) Aging factor

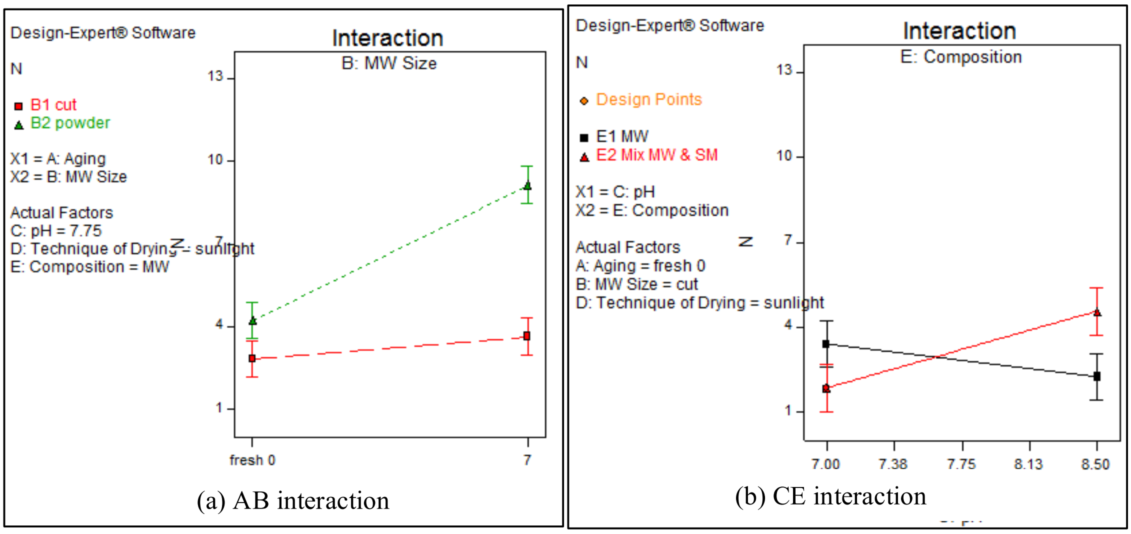


(b) MW size

Figure 4: Most effective independent factors in N content. (a) Aging factor; (b) MW size

Interaction effects between factors on N content

Figure 5a demonstrates the interaction between A and B (aging and MW size). It gave the highest positive contribution effect on N content based on the Pareto chart (Figure 1). Higher N content achieved as MW aging 7-day and MW in powder form size. At the 0-day aging N content higher in powder form than cut size. Dardanelli *et al.,* (2010) found that the use of long composting and small particle size increase the N content and avoid loss as NH_3 gas during composting. Saleh *et al.,* (2013) reported there N increases as dry matter losses increases when composting. For the interaction between C and E (pH and composition) in Figure 5b, it shows two behaviors. First, the higher N content was achieved in MW only at pH 7. Second, higher N content achieved in composition of mix MW and SM at pH 8.5. However, the highest N content at pH 7 (3.40 mg/L) was almost similar with pH 8.5 (4.40 mg/L). According to Wang *et al.,* (2013) and Charisiou (2014), the N content decreases when composting sewage sludge enriched with lime (pH <7). Thus, the pH need to keep in the neutral range (7-8.5) as mention by Chandrasekar (2005).

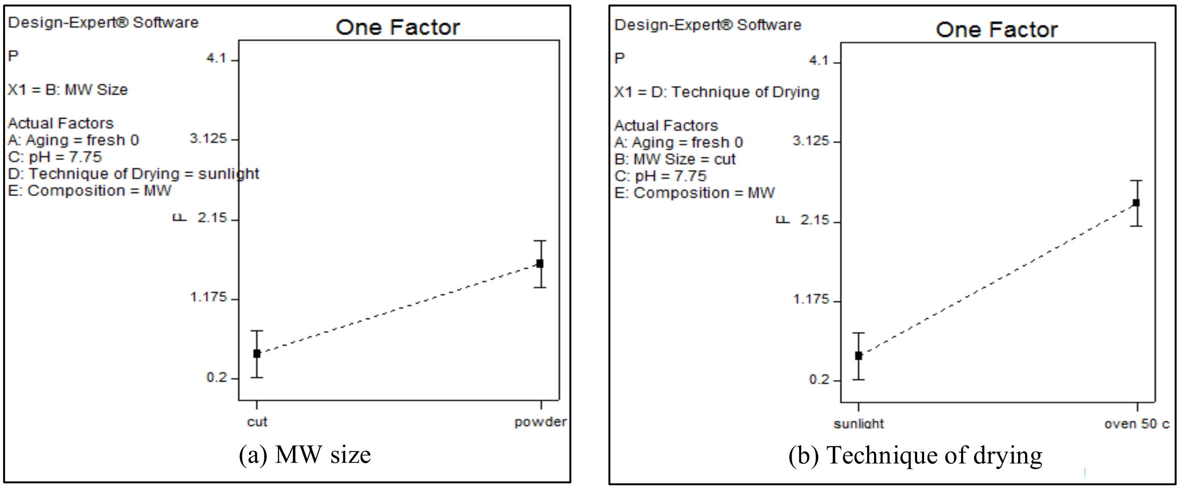


(a) AB interaction (b) CE interaction

Figure 5: Interactions effect between factors on N content; (a) AB; (b) CE

Effect of independent processing factors on P content

There were two independent variables affected the P content as shown in Figure 6. In Figure 6a, MW size affects the P content in MW. The P content achieved was about 1.61 mg/L at powder size compare to cut size which is 0.51 mg/L only. It shows there were significant differences at both MW size. Kaye *et al.,* (2003) state size of organic waste highly influence the nutrients mineralization. P content present higher in powder size compare in cut size. Big size of samples has a smaller total surface area and less accessible to microbes than the small size samples (Duong *et al.,* 2013). Duong *et al.* (2013) stated that the powder composts release more P than granular compost. As the MW size smaller, the availability P increases as it dissolves more in water. Figure 6b shows that P content in MW affected by the technique of drying. P content was 0.51 mg/L at sunlight drying and 2.38 mg/L at oven drying. It also showed that there were significant differences at both technique of drying. This may be due to the rapid increase in microorganism activities and interaction that occurred. The P content in MW was lower at sunlight drying compare to oven drying. The trend of the results in this study generally indicates that oven drying retains higher P content in MW than sun-drying.

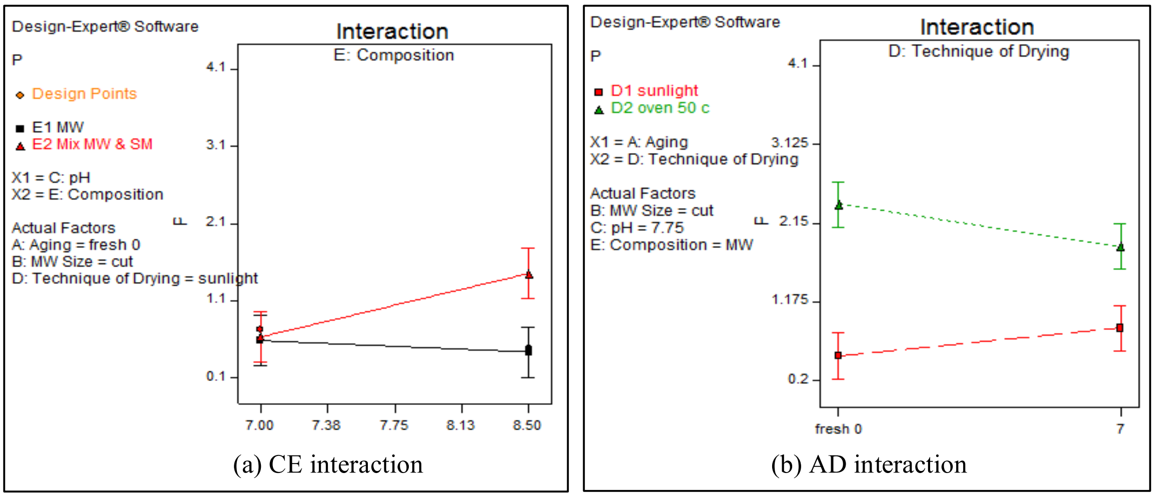


(a) MW size (b) Technique of drying

Figure 6: Most effective independent factors in P content; (a) MW size; (b) Technique of drying

Interaction effects between factors on P content

Figure 7a shows the interaction between C and E (composition and pH) has high significant effect to the P content. It gave the highest positive contribution effect on P content based on Pareto chart (Figure 2). It can be observed that at pH 7, the P content almost similar either in the presence of MW only or Mix MW and SM. But at pH 8.5 with the addition of spent medium (SM), the P content was high. The pH influences the rate of mineralization which affects the P content. The addition of inorganic substance at high pH increases the rate of mineralization due to the different micro-organisms activity (Lou *et al.,* 2015). Besides, the SM contains mix culture tends to increase the P content (Kwak *et al.,* 2015). The interaction between A and D (aging and technique of drying) is shown in Figure 7b. At 0-day of aging, the P content higher under oven drying than sunlight drying. However at 7-day aging, the P content decreases under oven drying because it has been leached away (Ige *et al.,* 2007).

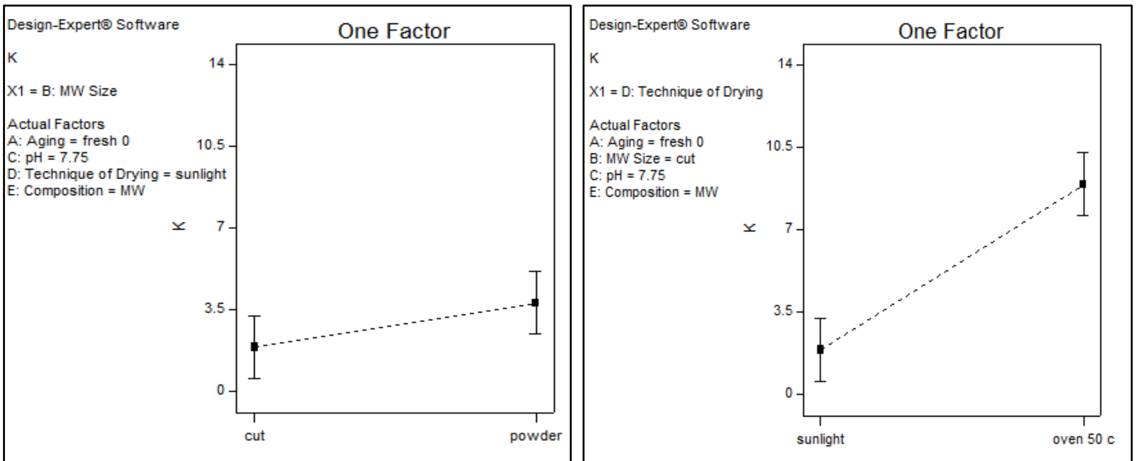


(a) CE interaction (b) AD interaction

Figure 7: Most effective Interaction effect between factors on P; (a) CE; (b) AD

Effect of independent processing factors on K content

The effects of two independent variables on the K content in MW are shown in Figure 8. In Figure 8a, MW size affects the K content. For powder size factor, K content was 3.08 mg/L. However, it was only 1.90 mg/L for cut size factor. It shows that higher K content was achieved by using powder size. It also shows that there was significant difference of K at both MW sizes. K content is higher in powder size compare in cut size. Small size have a larger total surface area and more accessible to microbes than the big size (Tahir *et al.,* 2016). Figure 8b shows the effects of technique of drying to K content. The K content observed was 1.90 mg/L at sunlight drying while 8.94 mg/L at oven drying. It shows that there was significant difference on K content.

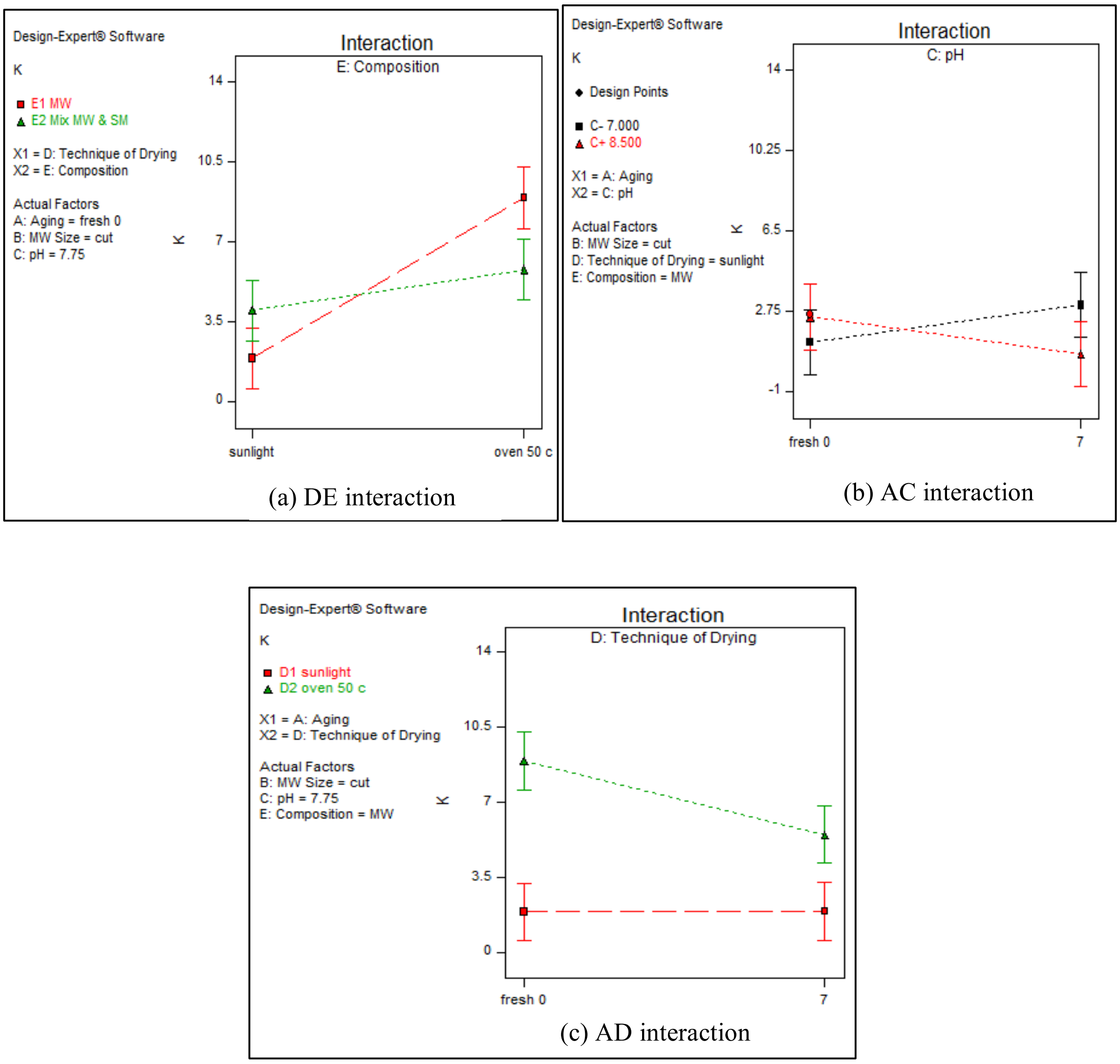


(a) MW size (b) Technique of drying

Figure 8: Most effective independent factors in K content; (a) MW size; (b) Technique of drying

Interaction effects between factors on K content

Interaction between D and E (technique of drying and composition) have the highest significant to the K content in MW based on Pareto chart (Figure 3). At the sunlight drying, the K content was higher in the present of SM (4.00 mg/L) compared to MW only (1.90 mg/L) (Figure 9a). While at oven drying, the K content was higher in MW only (8.90 mg/L) compare to Mix MW and SM (5.80 mg/L). Banlangsawan *et al.* (2016) observed that the addition of organic matter and constant drying technique increase the nutrients content. Figure 9b shows the interaction between A and C (aging and pH). At 0-day aging, the K content was higher at pH 8.5 (2.50 mg/L) compared to pH 7 (1.30 mg/L). While at 7-day aging, the K content was higher at pH 7 (3.10 mg/L) compared to pH 8.5 (0.77 mg/L). At 0-day aging, there was no significant difference between the K content at both pH level. But at 7-day aging, there was a significant difference between the K content. Furthermore, the K content in the MW dried for long period increased more than twice compared to the fresh MW. The K in MW mostly preferable at pH 7 since it provides better degradation of organic matter and increases nutrients. Sundberg *et al.* (2013) agreed that the rate of organic waste decomposition was high at pH 7. Figure 9c shows the interaction effect between A and D (aging and technique of drying). The K content decreases as it was dried for 7 days under oven drying. While for sunlight drying, the K content remains similar even it was dried for 7 days. During the drying, the K content dissolves in water and leached away. Kargar *et al.* (2015) also stated that K would be dissolved and leached by water as it was leachable nutrient.



(a) DE interaction

(b) AC interaction

(c) AD interaction

Figure 9: Interaction effect between factors on K; (a) CE; (b) AC; (c) AD

Validation experiment

Equations 1-3 predict the N, P and K contents by using the best processing conditions. The criteria setup to select the best processing conditions is given in Table 5.

Table 5: Criteria for the best processing conditions

Criteria	Goal	Value
Aging	In range	0 day–7 days
MW Size	In range	Cut or powder
pH	In range	7–8.5
Technique of Drying	In range	sunlight or oven 50 °C
Composition	In range	MW only or Mix MW & SM
N	Maximize	-
P	Maximize	-
K	Maximize	-

The suggested best processing conditions by Design Expert V.7 and their predicted N, P and K contents are shown in Table 6. The experiments were carried out to verify this suggested best condition and the results of the experiments are shown in Table 7. The error from this experiment was calculated by Equation 4. Run 3 was chosen as the best processing condition. This was due to their error of 8.69% for N, 1% for P and 3.06% for K which were acceptable values since the error does not exceed 10%.

$$\text{Error} = 100\% \times \left| \frac{\text{actual} - \text{predicted}}{\text{actual}} \right| \tag{4}$$

Table 6: Best condition for N, P and K contents

Run	Factor 1: Aging	Factor 2: MW Size	Factor 3: pH	Factor 4: Technique of drying	Factor 5: Composition	Predicted N (mg/L)	Predicted P (mg/L)	Predicted K (mg/L)
1	7	Powder	7	Oven 50 °C	MW	11.91	4.02	12.93
2	Fresh 0	Powder	7	Oven 50 °C	MW	7.00	3.55	11.75
3	7	Powder	7	Sunlight	MW	13.23	3.01	7.85

Table 7: Data from validation experiment

Run	Exp.N (mg/L)	Predicted N (mg/L)	Error %	Exp. P (mg/L)	Predicted P (mg/L)	Error %	Exp.K (mg/L)	Predicted K (mg/L)	Error %
1	10.53	11.91	11.59	3.24	4.02	19.40	14.58	12.93	12.76
2	5.32	7.00	24.00	4.16	3.55	17.18	12.01	11.75	2.21
3	**12.08**	**13.23**	**8.69**	**3.04**	**3.01**	**0.99**	**8.09**	**7.85**	**3.06**

4.0 CONCLUSION

It can be concluded that the MW contains high N, P and K contents. To obtain the maximum amount of N, P and K contents, the effects of various processing factors were analyzed using Design Expert software V7. The results showed that the most significant factors in N content were MW size and aging of waste. Meanwhile for P and K contents, technique of drying and MW size were the most significant factors. The best condition for the processing factors of MW was identified to achieve a maximum amount of N, P and K contents, which were aging of 7 days, MW size in powder form, waste pH at 7, sun light drying and the composition of MW only. The predicted results were compared with the experimental results with the error does not exceed 10% for N, P and K. Based on the best conditions proposed, the N (12.08 mg/L), P (3.04 mg/L) and K (8.09 mg/L) contents were achieved.

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