Antioxidant Properties of Onion (*Allium cepa L.)* and Potato (*Solanum Tuberosum L.)* Dried Skin

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Abstract*— *Allium cepa L.* and *Solanum Tuberosum L.* are very good vegetables as source of vitamins and other health benefits content most likely came from the antioxidant substances in them. However, most of people didn't realize the importance of these vegetables dried skin which has leaded them to removes the skin as a waste especially the onions. Also, the use of synthetic antioxidants can be harmful to human body compared to natural antioxidants as they contain substances which may cause adverse effect to human organs and have no nutritional qualities. Natural antioxidants involved higher production costs but provide multiple health benefits and readily accepted by human body. In this research, the antioxidant properties of onion and potato skins are to be determined. The antioxidants were extracted using solvent extraction method using methanol as the medium at the extraction temperatures and concentrations ranging from 50°C to 80°C and 30% to 60%. The antioxidant activities were obtained using the DPPH radical solution in which 80% methanol is used as the blank for determining its absorbance at a wavelength of 517 nm. The total phenolic content of the potato and onion skins were determined using the Folin–Ciocalteu method and the absorbance values were determined at 765 nm using the same blank. From the results obtained, the optimum conditions of the onion skin herb for the determination of total phenolic content and antioxidant activity were at the extraction temperature of 80.00°C with 35.52% methanol concentration. The amount of total phenolic content and antioxidant activity yielded at this condition for the onion skin were 89.85 mg/g and 65.39% in which, achieved the targeted amounts of 88.08 mg/g for total phenolic content and 65.39% for antioxidant activity. For the potato skin, the optimum condition for was at the extraction temperature of 60.38°C with 30.00% methanol concentration. The amount of total phenolic content and antioxidant activity yielded at this condition were 69.05 mg/g and 66.89%, in which, achieved the targeted amount of 65.39% for antioxidant activity. In conclusion, both onion and potato dried skin contain several kind of antioxidants such as gallic acid and quercetin which gave a significant impact to this study and the research was successful.

I. INTRODUCTION

Onions (*Allium cepa L.*) are the second most imperative agricultural yield around the world, after tomatoes, with current yearly generation around 66 million tons. Potato (*Solanum tuberosum L.*) is an essential food for people and the fourth substantial crop that is developed worldwide after rice, wheat, and maize [1]. Antioxidants are natural substances that are important on preventing damage on the cells. These substances are found in foods, mostly in fruits and vegetables. Examples of antioxidants in fruits and vegetables are beta-carotene, selenium and vitamins such as vitamin A, vitamin C and vitamin E. This is why having a diet involving large amount of vegetables and fruits intake is necessary to reduce the risk of dangerous diseases. The factors maybe because of another contents of the foods but it is confirmed that antioxidants plays the major role based on studies from researchers and scientists around the world. However, there is a limitation of everything which is in this case, too much consumption dose of antioxidants may lead to another diseases such as taking too much beta-carotene from smoking may increase the chances of getting lung cancers, too much vitamin E may leads to prostate cancer and stroke [2],[3]. It is believed that the antioxidant in the onion dried skin is such as quercetin can be extracted through several methods such as solvent extraction by researchers. These antioxidants have been associated with a lower incidence of heart disease, cancers, degeneration and severity of cataracts [4].

Total phenolic compounds can be found both potato and onion skins, which is a good antioxidants for avoiding oxidation of food containing high measures of lipid. Phenolics are concentrated in the peel and sticky tissues and observed to be the least in the tuber [5]. Phenolic compounds are available in both free and bound structures.

They are for the most part substituted subsidiaries of hydroxycinnamic acid which is free frame phenolics and hydroxybenzoic acid which is in bound shape phenolics [6]. The most well-known hydroxycinnamic acid subsidiaries in potato peel are chlorogenic acid (CGA), caffeic acid (CFA), and ferulic acid (FRA), while gallic acid (GAC), protocatechuic acid (PCA), vanillic acid (VNA), and p-hydroxyl benzoic acid (PBA) are from hydroxybenzoic acid. However, gallic acid is the most widely used standard for phenolic compound in potato peel.

There are several kind of methods to extract antioxidants, but solvent extraction is the most widely used in separation of chemicals. Solvent also known as liquid-liquid extraction depends on the rule that solute can divide itself in a specific proportion between two non-forming homogenous mixture solvents. This method is a good method on extracting antioxidants and has already been tested on several onion and potato antioxidants determination. This method commonly used alcohols such as methanol as the working solvent. Methanol is a simple structure alcohol which comprised of one methyl group combined with to a hydroxyl group. It is otherwise called methyl liquor, carbinol, or wood alcohol. It has no color, volatile, and combustible fluid at room temperature. In its unrefined shape, methanol has a horrendous smell, yet in its pure form, the compound shows a slight alcoholic scent. Methanol is miscible with ethanol, ether, benzene, ketones, and most natural solvents.

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical chemical used in a method to determine the antioxidant activity of biological substrates. It is the basis of general antioxidant assay and has been generally used to determine the antioxidant properties of biological products [7]. DPPH was handled as the free radical source, since it recreates reactive oxygen and nitrogen species influencing organic systems. Furthermore, free radical scavenging

is by and large the acknowledged component for antioxidants restraining lipid oxidation [8].

For the mathematical modelling, response surface methodology (RSM) is an accumulation of scientific and empirical methods for observation of model building. RSM is a method which is determined statistical and mathematical to evaluate different variables from the specific variable and to determine the maximum production of the process prepared under a specific constrained condition [9].

II. METHODOLOGY

A. Preparation of samples

The potato skin and onion skin obtained were stored in the oven at 30°C for 24 hours. The samples were then grinded using conventional blender for 10 minutes. Then, the skins were stored in a freezer at temperature lower than 20°C for moisture removal in the samples.

B. Sodium Carbonate Na_2CO_3 preparation

The sodium carbonate, Na_2CO_3 solution was prepared by adding 7.5 g of Na_2CO_3 into 100 ml distilled water and the solution was stirred and heated up at low temperature until Na_2CO_3 was fully dissolved.

C. Calibration curve preparation:

Gallic acid equivalents (GAE) was used as a standard and the total phenolic was expressed as mg/g gallic acid equivalents (GAE) and prepared by dissolving 0.03 g of gallic acid in 100 ml of 20%, 30%, 45%, 60% and 70% methanol solutions. The solution was then diluted into different concentrations to plot the graph of standard curve. Then, 1 ml of the gallic acid from each concentration were mixed with 0.5 ml of Folin-Ciocalteu solution and 1 ml of sodium carbonate, Na_2CO_3 solution. Water was added into each of the tube containing different concentrations until the volume reached 10 ml. The solutions were left for 30 minutes. The absorbance values were obtained through UV-Vis spectrophotometry method at 765 nm against 80% methanol as blank. Hence, the graph on each the methanol concentration were plotted [10].

D. Extraction method

For the extraction method, solvent extraction was used with the aid of methanol as the solvent. First, 10 g of each samples were mixed with 100 ml methanol and then stirred using magnetic stirrer for 45 minutes using different extraction temperatures in the range of 50°C to 80°C (generated from design expert software). Finally, the mixture were centrifuged at temperature of 5°C at the speed of 3000 rpm for 10 minutes and store in freezer [11].

E. Antioxidant activity assay

The DPPH method used was proposed by (Thaipong et. al, 2006) [12]. First, the stock solution was prepared by dissolving 10 mg of DPPH radical solution in 100 ml of 80% methanol. The working solution was obtained by dilution of stock solution of DPPH radical with methanol producing an absorbance of 517 nm. Next, 1 ml of the extracted sample were mixed with 4 ml of 80% methanol and 1 ml the DPPH solution. Then, the mixture were left for 30 minutes in a dark place. The mixture containing 1 ml of DPPH solution and 4 ml of 80% methanol was prepared and measured as control. The antioxidant activity was obtained through UV-Visible spectrophotometry method and the absorbance was obtained using UV-Vis at the wavelength of 517 nm.

The steps were finalized through the calculation of antioxidant radical scavenging activity using equation below:

Antioxidant activity (%) = [(A blank/A sample)/A blank] x 100%
A = Absorbance (517nm)

F. Total phenolic content (TPC) assay

For the determination of phenolic content, 1 ml of the sample (onion or potato skin) extract were mixed with 0.5 ml of diluted Folin-Ciocalteu solution and 1 ml of sodium carbonate solution. Finally, the absorbance was measured using UV-Visible spectrophotometry method at the wavelength of 765 nm against blank of 80% methanol. The steps were finalized by the calculation of the total phenolic content yielded on each sample with the aid of standard calibration curve prepared using gallic acid. The equation was as followed:
TPC= C X V/M
TPC =total phenolic content
C=concentration of gallic acid obtained from standard calibration curve (ml)
V= volume of extracted solution (ml)
M= weight of sample used (g)

III. RESULTS AND DISCUSSION

A. The analysis software

Design Expert software was used to perform an analysis for the responses which involved the evaluation of models for response surface model (RSM), analysis of variance (ANOVA), the significant effects, diagnostics and the model graphs in quadratic process order. All the 52 experimental ran values of total phenolic content and antioxidant activity calculated are tabulated and analyzed in this software. In the analysis process, there are several steps are followed. The first step is transformation in which the response node is selected and transformation is chosen. In the fit summary, the models are evaluated for response surface methodology and mixture and also the significant effects is selected from the graph for the factorials. For the model step, the model order and desired terms were selected. On the next step which is the analysis of variance (ANOVA) the chosen model was analyzed and the result is viewed. The model fit and transformation were evaluated using the model graph which is also interpreted the model.

B. Total Phenolic Content (TPC)

The values total phenolic content are calculated the tabulated into Design Expert software. The smallest amount obtained is 18.05 at the extraction temperature of 80°C and 60% methanol concentration. In contrast, the highest yield is 158.11 mg/g at the temperature of 65°C and 20% methanol concentration. However, this is the not optimal condition to TPC. The condition will be further discussed in the optimization section.

Next, based on the total phenolic content yielded, the regression equations of the experimental design are constructed as follows:

$$\text{TPC for potato skin} = -253.468 - 10.191X_1 - 0.098\,X_2 - 0.069X_1^2 - 5.81167\times10^{-3}X_2^2 - 0.023X_1^2\,X_2^2$$

$$\text{TPC for onion skin} = -208.846 - 10.370X_1 - 0.788X_2 - 0.069X_1^2 + 5.81167\times10^{-3}X_2^2 - 0.024X_1^2\,X_2^2$$

X_1= Extraction temperature
X_2= Methanol concentration

These equations are the alternative ways to calculate the TPC yielded with the parameters used throughout the 52 experiments

The post ANOVA statistic for total phenolic content is obtained to show whether the model is good or not based on the R-squared values as shown in the table below:

Table 1.3: Post ANOVA statistics for total phenolic content

Standard Deviation	18.44	R-squared	0.6849
Mean	58.88	Adj R-squared	0.6263
C.V.	31.32	Pred – squared	0.4739
Press	24411.15	Adeq Precision	12.970

The "Pred R-Squared" of 0.4739 is in reasonable agreement with the "Adj R-Squared" of 0.6263. Same goes to this response, the R-squared value is 0.6849 which mean lower than the optimum value of 0.8 and considered not really good. There are several errors that maybe occurred during the experiments.

C. *Antioxidant Activity*

The antioxidant activity for the onion skin and potato skin extracts are calculated from the absorbance values obtained as stated in the methodology. The highest amount of antioxidant activity or percentage of inhibition obtained is 88.98% at the extraction temperature of 80°C and 60% methanol concentration and the lowest is at 41.80% at the extraction temperature of 60°C and 45% methanol concentration. However, this is the not optimal condition to yield antioxidant activity. The condition will be further discussed in the optimization section.

Therefore, based on the antioxidant activity percentages obtained, the regression equations of the experimental design for antioxidant activity are constructed as follows:

Antioxidant for potato skin = $58.553 - 0.24X_1 + 0.141X_2 + 0.012X_1^2 + 0.021X_2^2 – 0.024\ X_1^2\ X_2^2$

Antioxidant for onion skin = $86.999 – 0.523X_1 + 0.390X_2 + 0.012X_1^2 + 0.021X_2^2 – 0.024\ X_1^2\ X_2^2$

X_1= Extraction temperature
X_2= Methanol concentration

These equations are the alternative ways to calculate the antioxidant activity yielded with the parameters used throughout the 52 experiments.

The post ANOVA statistic for antioxidant activity is obtained to show whether the model is good or not based on the R-squared values as shown in the table below:

Table 1.3: Post ANOVA statistics for antioxidant activity

Standard Deviation	9.94	R-squared	0.5224
Mean	68.75	Adj R-squared	0.4335
C.V.	14.46	Pred – squared	0.3036
Press	6195.47	Adeq Precision	7.938

The value of "Pred R-Squared" of 0.3036 is in reasonable agreement with the "Adj R-Squared" of 0.4335. The values of coefficient of determination, R-squared should be more than 0.8 for the optimum result. The value obtained is 0.5224 which is lower than the targeted value of 0.8 and considered not really good. There are several errors that maybe occurred during the experiments.

D. *Diagnostics graphs*

There are two different graphs generated from the Design Expert software in the diagnostic section. The graphs plotted are the comparisons between the actual lines the predicted lines for d responses are shown below:

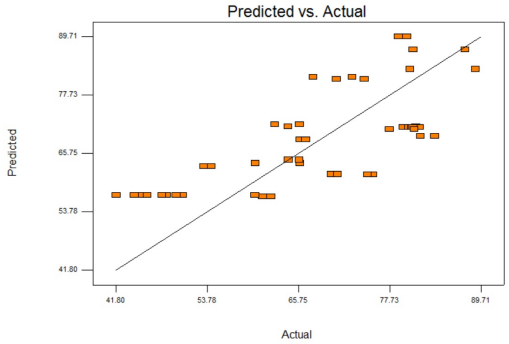

Figure 1.1: Predicted vs. Actual graph for antioxidant activity response

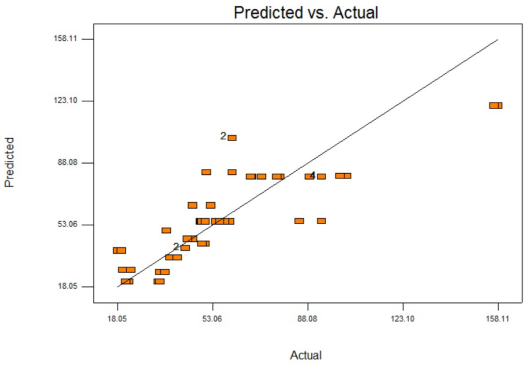

Figure 1.2: Predicted vs. Actual graph for total phenolic content response

The plots above show significant differences between the predicted and the actual values obtained. The relationship between concentrations and temperature are observed to be linear. From Figure 1.1, the highest peak for the antioxidant activity of the predicted yield reached the maximum value with the actual point at 88.71 but the points scattered away from the linear line. Thus, the graph shows a moderate plot because some of the points are very close with the actual linear line but some points are scattered too far. From the total phenolic content plot in Figure 1.1, it is observed that the highest value for the predicted is much lower than the actual, but only on certain point. The points are scattered in between the linear line with only few points far from the line compared to the antioxidant activity plot. However, the predicted and actual values for both responses and factors have high effectiveness of fit with each other.

E. *3D Model graphs*

The 3D model graph surface plot were generated from the same software, but with a better angles to observed the curves and differences between the concentrations and temperatures. There are four difference conditions of 3D graph model plotted which are from response surface methodology (RSM) in order to optimize the system to get the relationship between the variables and responses. The minimum and maximum values of each parameters were observed at the tip of the 3D curve and all the data is in the targeted values. Thus, the relationship between responses and factors can be observed clearly.

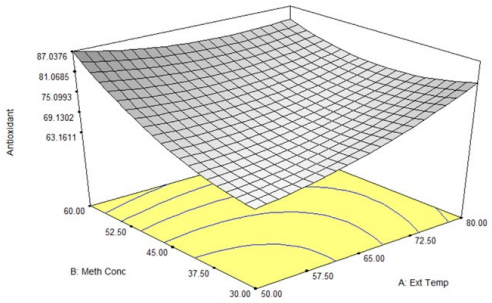

Figure 1.3: Potato skin antioxidant activity 3D graph

From the graph above, the plot shows a slow constant increase in antioxidant activity of potato skin with methanol concentration and extraction temperature. It can be observed that the highest value antioxidant activity value is at temperature of 50°C with methanol concentration of 60%. The value of antioxidant is at the peak of 87.0376% with peak amount of methanol concentration. Therefore, the higher the methanol concentration, the higher the amount of antioxidant extracted. The plot shows the same relationship of the antioxidant activity with the extraction temperature Rapid changes of the response with factors is observed as the response increases with temperature and methanol concentration.

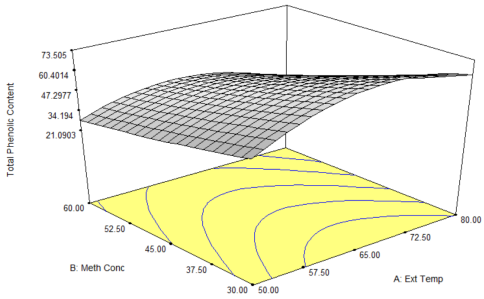

Figure 1.4: Potato skin total phenolic content 3D graph

From the graph above, the plot shows a uniform increase in total phenolic content yield of potato skin as the methanol concentration decreases. The curve shows an increase of yield when the extraction temperature increases until it reached the peak at 73.505 mg/g but decreases slighly as the temperature gets higher to 80°C with methanol concentration of 50%. Therefore, TPC response shows different plot changes with different factors.

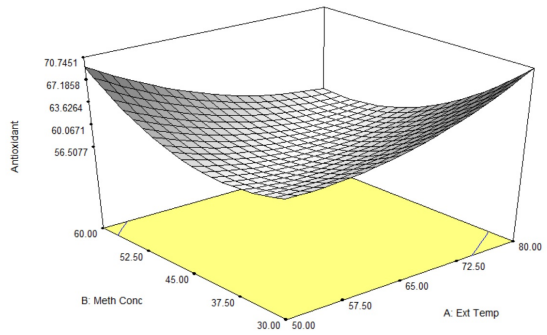

Figure 1.5: Onion skin antioxidant activity 3D graph

From the graph above, the plot shows a drastic changes in the antioxidant activity of onion skin on both methanol concentration and extraction temperature responses. The antioxidant activity decrease from 70.7451% to 62.2117% when the methanol concentration used decreases from 60% to 30%. Therefore, the lower the methanol concentration, the lower the amount of antioxidant extracted. The plot shows the same relationship of the antioxidant activity with the extraction temperature. The antioxidant activity increases as the temperature increase until both axis reaches the maximum value at the yield of 70.7451% at 80°C extraction temperature. Therefore, rapid changes of the response with factors are observed as the response increases with temperature and methanol concentration.

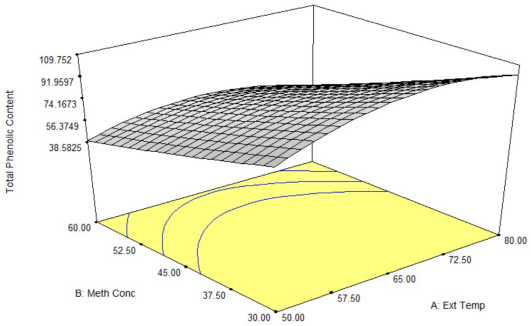

Figure 1.6: Onion skin total phenolic content 3D graph

The plot shows that the amount of total phenolic content of onion skin yielded is lower over an increase of methanol concentration. However, significant changes with extraction temperature can be observed as TPC increases up to 91.9597 mg/g at the highest temperature of 80°C. Significant changes of the response with factors as the TPC increases with lower values of methanol concentration but increases with temperature are observed.

F. Optimization

In the optimization part, the targeted yield amount TPC and antioxidant activity for both potato and onion skin are obtained. The targeted values are set and the optimum conditions for each reponses are generated which are 88.08 mg/g and 69.39%. The optimum condition of the onion skin for the determination of total phenolic content and antioxidant activity is at the extraction temperature of 80.00°C with 35.52% methanol concentration. The amount of total phenolic content and antioxidant activity yielded at this condition for the onion skin are 89.85 mg/m and 65.39%. For the potato skin, the optimum condition for is at the extraction temperature of 60.38°C with 30.00% methanol concentration. The amount of total phenolic content and antioxidant activity yielded at this condition are 69.05 mg/g and 66.89%. The predicted value of antioxidant activity is 71.20% and 55.10 mg/g for the TPC as stated in the point prediction section.

IV. Conclusion

In conclusion, the best condition of the onion skin for the determination of total phenolic content and antioxidant activity were at the extraction temperature of 80.00°C and 35.52% methanol concentration yielding 89.85 mg/mg of total phenolic content and 65.39% of antioxidant activity. For the potato skin, the best condition wad at the extraction temperature of 60.38°C and 30.00% methanol concentration yielding 69.05 mg/g of total phenolic content and 66.89% of antioxidant activity. The onion skin shows slightly higher total phenolic content compared to potato skin but in vice versa on antioxidant activity yield. However, both herbs extraction amount achieved the targeted values. All the graphs obtained and equations generated from Design Software were discussed thoroughly. Therefore, the results obtained were valid and had been justified. All in all, the main objectives which are to study the antioxidant properties in onion and potato skin and to study the effect of extraction temperature and methanol concentration on antioxidant properties of onion and

potato dried skin were achieved the research was successfully done.

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References

[1] Singh, P.P., & Saldana, M.D.A. (2011).Subcritical water extraction of phenolic compounds from potato peel. Food Researh International, 10 , 35–38

[2] Corzo-Martinez, M., Corzo, N., & Villamiel, M. (2007). Biological properties of onions and garlic. Trends in Food Science and Technology, 18, 609–625.

[3] Moreno FJ, Corzo-Martínez M, Castillo del MD and Villamiel M, Changes in antioxidant activity of dehydrated onion and garlic during storage. Food Res Intern 39: 89-897 (2006)

[4] S. G. Dmitrienko, V. A. (2012). Methods of Extraction, Preconcentration,and. Journal of Analytical Chemistry, Vol. 67, 299

[5] Al-Weshahy,A.&Rao , A.V.(2009).Isolation and characterization of functional components from peel samples of six potatoes varieties gro wing in Ontario . Food Resear chInternation al, 42, 1062–1066.

[6] Shahidi, F., & Naczk,M.(1995).Antioxidant properties of food phenolics. In F.Shahi di & M.Nacz k (Eds.), Food phenolics: Sources,chemistry , effects and applications (pp.235–277)

[7] Toyokuni et al., 2003. S. Toyokuni, T. Tanaka, W. Kawaguchi, N.R.R. Fang, M. Pzeki, S. Akatsuka, H. Hiai, O.I. Aruoma, T. Bahorun
Effects of the phenolic contents of Mauritian endemic plant extracts on promoter activities of antioxidant enzymes Free Radical Res., 37 (2003), pp. 1215-122

[8] Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technology 1995; 28:25-30

[9] Box, G. E. P., and Draper, N. R., (1987), Empirical Model Building and Response Surfaces, John Wiley & Sons, New York, NY

[10] Peterson, G.L., Anal. Biochem. 100, 201-220 (1979)

[11] Sotillo, R.D., M. Hadley and E.T. Holm, 1994. Potato peel waste: Stability and antioxidant activity of a Freeze- Dried Extract. Journal of Food Science, 59(5): 1031-1033.

[12] Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Byrne H.D., 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Anal. 19, 669-675.