eISSN 2550-1453 http://eproceedings.chemistry.utm.my/

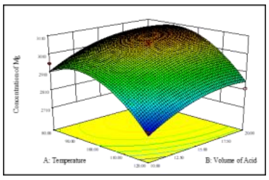
# Comparison between Univariate and Multivariate Optimisations on Wet Digestion of Almond Nuts Prior To Determination of Magnesium

Tan Jun Hao and Azli bin Sulaiman*

[a]*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia*
**Corresponding Author: azlisulaiman@utm.my*

***Article history:***
*Received 15 June 2019*
*Accepted 12 July 2019*

**GRAPHICAL ABSTRACT**



3D graph of temperature versus volume of acid obtained from multivariate optimisation.

**ABSTRACT**

Optimisation is the act of improving the efficiency of a certain product, process or system to obtain the best response for measurement. There are two types of optimisation method, which are univariate and multivariate optimisation, for the latter is more effective and efficiency as it considers interactions between parameters. However, univariate optimisation is still being use more frequently than multivariate in certain cases. The purpose of this study is to compare univariate optimisation and multivariate optimisation, hence, to determine the presence of significant difference between both methods. The comparison was done using the analysis of magnesium in almond nuts where the parameters of temperature, volume of nitric acid and time of digestion were optimised. A two-sample assuming unequal variances t-test was then used to determine the difference between both univariate and multivariate optimisation. It was found that there was a significance difference between both methods, whereby based only on the best responses, univariate optimisation method was superior. However, taking all the factors and interactions, multivariate optimisation was, overall, the more efficient and superior method.

*Keywords: Univariate, multivariate, optimization, Almond nuts, determination of Magnesium*



## 1. INTRODUCTION

Optimisation generally refers to the act of improving the efficiency of a certain product, process or system to obtain the maximum gain available. “Optimisation” has been frequently used in the field of analytical chemistry as a means to obtain optimum conditions for a procedure or process which will produce the best response achievable [1].

In the field of analytical chemistry, optimisation has been used through two different methods which are the univariate and the multivariate optimisation. In the past, optimisation in analytical chemistry has been done using the univariate optimisation method or commonly known as the one-variable-at-a-time (OVAT). The discipline of this method is that only one parameter is changed while others are kept constant at a certain value in order to monitor the former’s influence on the experimental response [2]. However, its major disadvantage is that this method does not take in account of the interaction between the parameters studied. Besides that, it is also time and cost consuming due to the increasing number of experiments to be conducted and increasing consumption of materials and chemicals [3].

Hence, in order to overcome the problems above, multivariate optimisation has been discovered and used in modern analytical chemistry field. There are many multivariate optimisation methods and one of the most relevant method is response surface methodology (RSM). Response surface methodology is a mathematical and statistical methods collection used in optimising processes based on the polynomial equation’s fit to the experimental responses with the interest of making statistical previsions whilst describing a set of data’s behaviour [2, 4, 5]. As such, the objective of the RSM is to simultaneously optimise the parameters studied in order to obtain the best performance of the process without disregarding the interactions between the studied parameters [2].

Although RSM has a better advantage than OVAT method in terms of time, effort and resources, many still uses the latter method in routine method development [6]. Engineers tend to disregard the notion that OVAT is less efficient than RSM until it is explained to them. As another example, based on the research and projects done by UTM Faculty of Science’s chemistry department final year student, it can be seen that many of them used OVAT method when doing optimisation in their final year project rather than using RSM methodology [7].

A distinct explanation between both methods is needed to be stated numerically and statistically even though there are many literature works reporting the efficiency of RSM higher than OVAT in the form of theory. Hence, in this research, comparison will be done between the two optimisation methods based on the optimum responses obtained under the same factors. The research will be done by doing optimisation on the sample preparation of the analysis of magnesium in almond nuts using flame atomic absorption spectroscopy (FAAS) since analysis of magnesium in almond nuts is a common and easy analysis.

## 2. EXPERIMENTAL

Sample preparation method of almond nuts was adopted and modified from the literature done by Moodley et al [8]. An amount of 0.5 ± 0.0005 g powder of nuts was weighed in a conical flask. Then, 15 mL of concentrated nitric acid was added. Then, the solution was heated at 100°C for 30 minutes using a hotplate through a made-shift reflux system made by putting a filter funnel at the flask's mouth. After that, the solution was left to cool to room temperature before transferring it into a 100 mL volumetric flask and diluting it using deionised water. Then, 1 mL aliquot of the sample solution was obtained and transferred into another 100 mL volumetric flask. The aliquot was diluted using deionised water.

The experiment was divided into three main stages. The first stage involved the optimisation of three digestion parameters, which were temperature, digestion time and volume of nitric acid through univariate optimisation. The second stage was the parameters' optimisation by multivariate optimisation method, through the usage of RSM software with the basis of the Box-Behnken Design. The third stage was the comparison of both optimisation methods to determine significant difference. For univariate optimisation, every parameter's optimised value was used in the subsequent optimisation until the experiment ended. For the multivariate optimisation, the optimisation was done with the help of RSM software, Design Expert 7.1.6, where experimental parameter values were computerised with the basis of Box-Behnken Design. For the last stage, the comparison was done using a two-sampled assuming unequal variance t-test. Method validation on precision, percentage recovery, and limit of detection and limit of quantitation were done as well.

## 3. RESULTS AND DISCUSSION

### 3.1. Standard Calibration Graph of Magnesium

A series of magnesium standard solution of 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm and 0.5 ppm was used to plot a calibration graph of magnesium as shown in Figure 1. Based on the graph, the calibration curve correlation coefficient is found to be 0.9964, with a straight-line equation of y = 0.8277x + 0.0072.

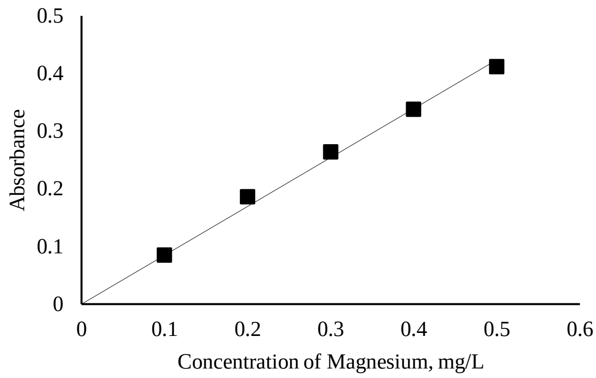



**Figure 1.** Standard calibration graph of magnesium

### 3.2. Univariate Optimisation

Univariate optimisation on digestion of almond nuts was done on three parameters (temperature, time of digestion, volume of nitric acid) prior to determination of Mg by FAAS. Optimum values for each parameter will be based on the highest response.

#### 3.2.1. Univariate Optimisation: Temperature Parameter

In this experiment, 7 samples of almond nuts were digested with the temperature parameter values of 60, 70, 80, 90, 100, 110 and 120°C with controlled values of volume of nitric acid at 15 mL and time of digestion at 30 minutes, as shown in Table 1. Based on the results in Table 1 and Figure 2, it can be seen that almond nuts sample digested at 90°C showed the highest response of 3172.39 ± 25.94 mg $kg^{-1}$ of magnesium. Figure 2 showed that as the temperature increased from 60 to 90°C, the response increased as well. As the temperature increases, the kinetic energy of the molecules in the system increases as well, allowing faster digesting reaction.

However, as the temperature increases from 90 to 120°C, the response decreases. This contradicts with the notion that acid strength increases with temperature. This may be due to the water percentage of the acid decreasing as there is water evaporation in an open system. As acid strength comes from the concentration of hydronium ions, $H_3O^+$ or hydrogen ions, $H^+$, when water concentration decreases, there is less $H^+$ being dissociated which induces the low concentration of hydronium ions. So, the acid cannot function effectively at higher temperature in an open system even with a reflux system. Based on research using acid digestions, it is advised to use a close system for acid digestion method, by using an acid digestion block. So, the optimum value for the temperature parameter is 90°C as it yielded the highest response.

**Table 1.** Concentration of magnesium in sample under different temperature values

| Sample | Temperature (°C) | Mg Concentration (mg kg$^{-1}$) |
|---|---|---|
| 1 | 60.00 | 2373.83 ± 23.94 |
| 2 | 70.00 | 2674.12 ± 13.97 |
| 3 | 80.00 | 3034.54 ± 21.96 |
| 4 | 90.00 | 3172.39 ± 25.94 |
| 5 | 100.00 | 3113.77 ± 17.96 |
| 6 | 110.00 | 2913.59 ± 19.96 |
| 7 | 120.00 | 2834.33 ± 15.97 |

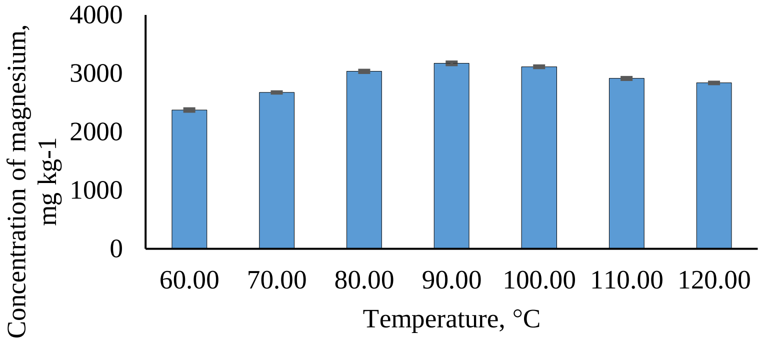



**Figure 2** Graph of temperature versus concentration of magnesium

3.2.2. Univariate Optimisation: Time of Digestion Parameter

In this experiment, 7 samples of almond nuts were digested with time of digestion parameter values of 10, 20, 30, 40, 50, 60 and 70 minutes with controlled values of volume of nitric acid at 15 mL and temperature at 90°C, as shown in Table 2 with their respective Mg concentration. The value of temperature was obtained from the previous optimisation.

According to Figure 3, samples showed an increase in concentration of magnesium detected as the time of digestion rose from 10 minutes to 30 minutes. Increasing the time allows the acid to digest more almond nut samples so that more magnesium is released into the solution. For digestion time beyond 30 minutes (40, 50, 60 and 70 minutes), the responses remained constant in the range of 3156.21 mg kg$^{-1}$ to 3172.39 mg kg$^{-1}$. This is because the maximum amount of detectable magnesium in 0.5 g of almond nut for these parameters has been reached. So, based on the best response, the optimum value for the time of digestion is 40 minutes.

**Table 2.** Concentration of magnesium in sample under different time of digestion values

| Sample | Time of Digestion (mins) | Mg Concentration (mg kg$^{-1}$) |
|---|---|---|
| 1 | 10.00 | 2635.26 ± 10.38 |
| 2 | 20.00 | 2895.37 ± 11.78 |
| 3 | 30.00 | 3134.36 ± 12.78 |
| 4 | 40.00 | 3174.92 ± 15.97 |
| 5 | 50.00 | 3153.69 ± 9.58 |
| 6 | 60.00 | 3154.95 ± 17.37 |
| 7 | 70.00 | 3174.29 ± 13.97 |

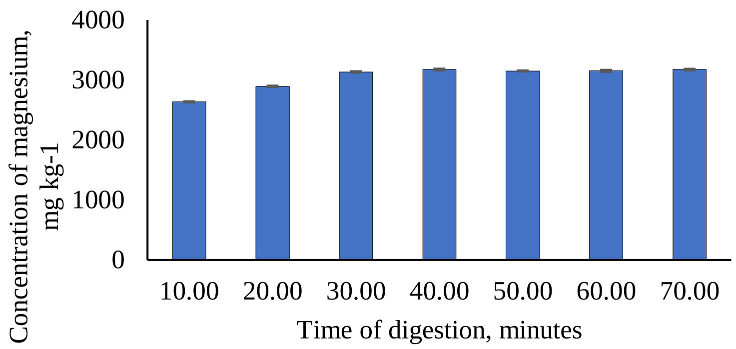



**Figure 3**. Graph of time of digestion versus concentration of magnesium

3.2.3. Univariate Optimisation: Volume of Nitric Acid Parameter

In this experiment, 7 samples of almond nuts were digested with time of digestion parameter values of 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, and 25.0 mL with controlled values of digestion time at 40 minutes and temperature at 90°C, as shown in Table 3 with their respective Mg concentration. The value of digestion time and temperature were obtained from previous optimisations.

According to the results shown in Figure 4, it can be seen that almond nuts sample digested with controlled values (Temperature: 90°C and time of digestion: 30 minutes) showed an increase in response as the volume of $HNO_3$ increases, which is similar to the trend in the optimisation of digestion time parameter. The responses then reached a constant range of 3155.58 mg kg$^{-1}$ to 3176.19 mg kg$^{-1}$ due to possible maximum detectable magnesium at these parameters (from 17.50 mL to 25.00 mL) being reached. So, the optimum value for this parameter is 20.00 mL as it yielded the highest response of 3176.19 ± 13.56 mg kg$^{-1}$.

**Table 3.** Concentration of magnesium in sample under different volume of nitric acid values

| Sample | Volume of Nitric Acid (mL) | Mg Concentration (mg kg$^{-1}$) |
|---|---|---|
| 1 | 10.00 | 2417.58 ± 14.67 |
| 2 | 12.50 | 2896.52 ± 11.96 |
| 3 | 15.00 | 3115.64 ± 19.64 |
| 4 | 17.50 | 3155.58 ± 15.98 |
| 5 | 20.00 | 3176.19 ± 13.56 |
| 6 | 22.50 | 3156.84 ± 10.39 |
| 7 | 25.00 | 3175.55 ± 11.98 |

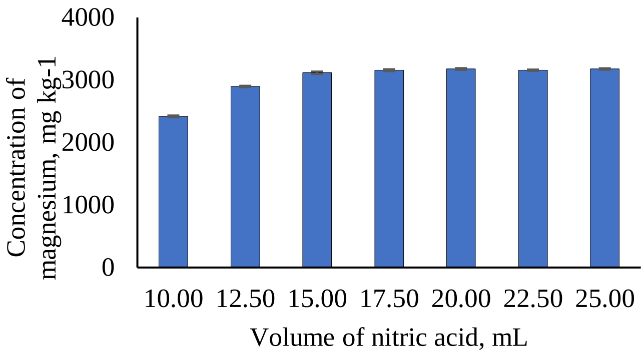



**Figure 4.** Graph of volume of nitric acid versus concentration of magnesium

3.2.4. Univariate Optimisation of Magnesium Analysis in Almond Nuts

The optimum values of each parameter was obtained from univariate method, which were 90°C (temperature), 40 minutes (time of digestion) and 20.00 mL (volume of 65% nitric acid). Based on Table 3, these parameter values yielded a concentration of 3176.19 mg $kg^{-1}$. However, to reassure this response, three replications of almond nut sample were digested using the optimum parameters and yielded an average magnesium concentration of 3170.16 ± 10.99 mg $kg^{-1}$.

3.3. Multivariate Optimisation

The conditions for digestion were optimized using response surface methodology, which is essentially used to design experiments, evaluate the variables' effects, and determine the variables' optimum conditions in order to predict targeted responses. By using Design Expert 7.1.6, a set of 15 experimental data, were computerised on two bases, the initial controlled parameters and Box-Behnken Design (BBD) as shown in Table 4. Based on those data, experiments were done and responses (concentration of Mg) corresponding to each experiment are shown in Table 5.

**Table 4.** Levels of variables tested in the Box-Behnken design (BBD)

| Level | Temperature (°C) | Volume of Nitric Acid (mL) | Time of Digestion (mins) |
|---|---|---|---|
| -1 | 80.00 | 10.00 | 20.00 |
| 0 | 100.00 | 15.00 | 30.00 |
| 1 | 120.00 | 20.00 | 40.00 |

**Table 5.** Samples' parameter values of multivariate optimisation and analysis responses of magnesium concentration

| Sample | Temperature (°C) | Volume of Nitric Acid (mL) | Time of Digestion (mins) | Mg Concentration (mg $kg^{-1}$) |
|---|---|---|---|---|
| 1 | 80.00 | 10.00 | 30.00 | 2959.41 ± 24.00 |
| 2 | 120.00 | 10.00 | 30.00 | 2738.36 ± 31.98 |
| 3 | 80.00 | 20.00 | 30.00 | 3038.78 ± 17.99 |
| 4 | 120.00 | 20.00 | 30.00 | 2818.87 ± 19.87 |
| 5 | 80.00 | 15.00 | 20.00 | 2939.41 ± 35.97 |
| 6 | 120.00 | 15.00 | 20.00 | 2780.00 ± 28.00 |
| 7 | 80.00 | 15.00 | 40.00 | 2998.20 ± 29.65 |
| 8 | 120.00 | 15.00 | 40.00 | 2799.44 ± 22.13 |
| 9 | 100.00 | 10.00 | 20.00 | 2778.89 ± 31.56 |
| 10 | 100.00 | 20.00 | 20.00 | 2958.82 ± 29.96 |
| 11 | 100.00 | 10.00 | 40.00 | 2918.25 ± 25.45 |
| 12 | 100.00 | 20.00 | 40.00 | 3138.74 ± 33.65 |
| 13 | 100.00 | 15.00 | 30.00 | 3077.54 ± 23.78 |
| 14 | 100.00 | 15.00 | 30.00 | 3039.39 ± 20.15 |
| 15 | 100.00 | 15.00 | 30.00 | 3058.78 ± 17.96 |

The results were then analysed using Design Expert and the approximation quadratic function of Mg was obtained and expressed as in Equation 1. $C_{Mg}$ is the predicted concentration of magnesium while A, B and C are temperature of digestion, volume of nitric acid and time of digestion respectively.

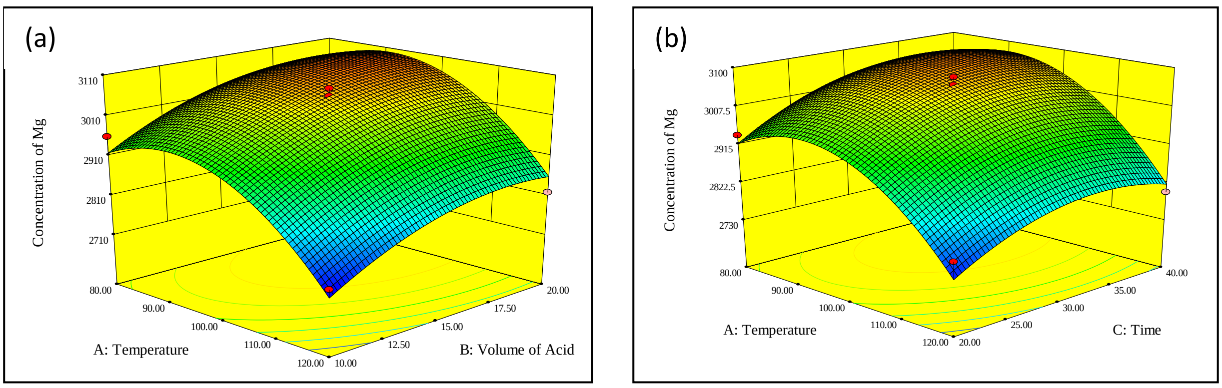
$$C_{Mg} = 3058.27 - 99.89A + 70.04B + 49.69C + 0.28AB - 9.84AC + 10.14BC - 119.56A^2 - 50.15B^2 - 59.74C^2 \quad \text{(eqn. 1)}$$

The adequacy of the quadratic model was confirmed by analysis of variance (ANOVA) and the results are presented in Table 6. When any mathematical model's ANOVA has high statistical significance with F-values at 95% confidence level and p-values less than 0.05, it can be accepted. The test of F-values allows the possibility of testing the data's variation from the fitted model while a small p-value causes the rejection of the null hypothesis, which signifies that the factor is significant [9]. So, based on the ANOVA in Table 6, a "model" F value of 7.16 indicates that the model is significant since there is only 2.16% (model's p-value) of probability that this result may occur due to noise. Furthermore, by testing the regression model, the model can be further confirmed for its adequacy. The "lack of fit" F value of 14.07 indicates that the lack of fit is not significant. This implies that there is only a 6.71% chance that a lack of fit F value this large may occur due to noise. Thus, the insignificant lack of fit indicates that the model is adequate.

**Table 6.** ANOVA of results obtained using response surface quadratic model for concentration of Mg

| Source | Sum of Squares | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|
| **Model** | $2.071\times10^5$ | 23011.21 | 7.16 | 0.0216 | **Significant** |
| **A-Temperature** | 79827.34 | 79827.34 | 24.82 | 0.0042 | |
| **B-Volume of Acid** | 39244.16 | 39244.16 | 12.20 | 0.0174 | |
| **C-Time** | 19752.54 | 19752.54 | 6.14 | 0.0560 | |
| **AB** | 0.32 | 0.32 | $1.009\times10^{-4}$ | 0.9924 | |
| **AC** | 387.08 | 387.08 | 0.12 | 0.7428 | |
| **BC** | 411.43 | 411.43 | 0.13 | 0.7352 | |
| **$A^2$** | 52782.09 | 52782.09 | 16.41 | 0.0098 | |
| **$B^2$** | 9286.62 | 9286.62 | 2.89 | 0.1500 | |
| **$C^2$** | 13178.77 | 13178.77 | 4.10 | 0.0988 | |
| **Residual** | 16080.04 | 3216.01 | | | |
| **Lack of Fit** | 15352.42 | 5117.47 | 14.07 | 0.0671 | **Not significant** |
| **Pure Error** | 727.62 | 363.81 | | | |
| **Cor Total** | **$2.232\times10^5$** | | | | |

After generating the quadratic model, the relationship between the responses and the experimental variables was illustrated graphically through 3D plots shown in Figures 5 (a)-(c). These plots were obtained by plotting response values versus experimental parameter values simultaneously.



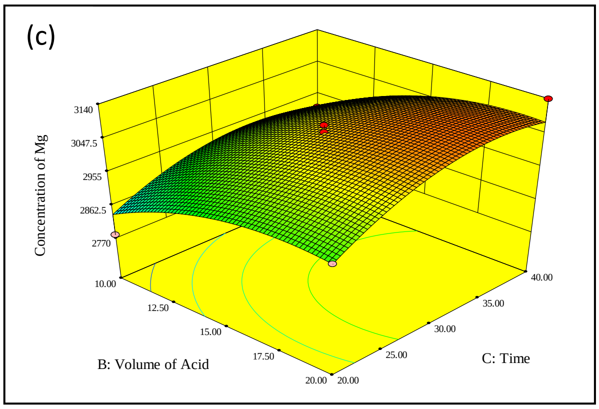



**Figure 5.** Combined effects of (a) temperature and volume of nitric acid, (b) temperature and time of digestion, and (c) volume of nitric acid and time of digestion.

According to Figure 5(a), an increase in temperature from 80°C to 120°C at low volume of nitric acid showed that the concentration of magnesium increases until it peaked at around 90 to 100°C and started to decrease as the temperature increases further. The trend was also true as the volume of nitric acid increased, with the concentration of Mg at larger acid volume being slightly higher than that of lower acid volume. This situation can be related to the issue of using an open reflux system where at higher temperature, evaporation of water reduces the efficiency of strong acids since acids need water to dissociate $H^+$ ions for the digestion reaction.

Based on Figure 5(b), it can be seen that as the temperature increased (80 – 120°C) at short digestion time, Mg concentration increased until it peaked at the range of 90 – 100°C, and started to decrease as temperature increased further. This was also true for all digestion time, with Mg concentration at digestion time ranging around 30 to 35 minutes being higher than that of the minimum and maximum value of digestion time. Both Figures 5 and 6 showed similar trends whereby Mg concentration increases until it peaks before decreasing as temperature increases. It can be deduced that in this method, the temperature parameter is a very significant parameter.

According to Figure 7, at all digestion time, the Mg concentration showed an increase as the volume of nitric acid increased. The same trend can also be observed at all acid volume values as digestion time increases. However, based on the graph, it can be seen that digestion time has a higher effect on the increase of Mg concentration than acid volume. This indicates that the method does not need a large amount of acid for complete decomposition of sample as the effect of increasing acid volume has little effect on the concentration of Mg. So, in RSM, the acid volume parameter can be minimized as much as possible without causing significant decrease in the concentration of Mg.

The main purpose of RSM was to optimize the yields (concentration of magnesium) using the most favourable response. Through RSM, it is found that the optimum values of the parameters were at temperature of 91.30°C, nitric acid volume of 12.56 mL and digestion time of 34.09 minutes as the final optimum conditions. These optimum parameters were generated based on the factor of minimizing resource (nitric acid) while maximizing the response yield. The predicted response based on the optimum parameters was an Mg concentration of 3043.49 mg $kg^{-1}$. However, to prove and validate the predicted optimum response, samples were needed to be prepared under these optimum parameters and then analysed. So, three replicates of almond samples were conducted under the optimum conditions and yielded an average Mg concentration of 3050.28 ± 12.07 mg $kg^{-1}$, which was in good agreement with the predicted response.

3.4. Comparison of Univariate and Multivariate Optimisation

Both univariate and multivariate optimization have yielded each of their optimum parameters for a favourable response as shown in Table 7. The presence of difference between both methods was determined by a statistical test, t-test, with a hypothesis, $H_0$ as shown below:

(a) $H_0$ = There is no significant difference between univariate and multivariate optimisation based on the analysis of magnesium in almond nuts.

**Table 7.** Optimum parameters for each optimisation method

| Parameter | Univariate Optimisation | Multivariate Optimisation |
|---|---|---|
| Temperature (°C) | 90.00 | 91.30 |
| Volume of Nitric Acid (mL) | 20.00 | 12.56 |
| Time of Digestion (min) | 40.00 | 34.69 |

A set of 6 replicates were done for each optimisation method with their respective optimum parameters which yielded results shown in Table 8 along with mean and standard deviation.

**Table 8.** Concentration of magnesium under optimum parameters from both univariate and multivariate optimisation

| Sample | Univariate Optimisation's Mg concentration (mg kg$^{-1}$) | Multivariate Optimisation's Mg concentration (mg kg$^{-1}$) |
|---|---|---|
| 1 | 3176.19 ± 18.93 | 3056.94 ± 19.63 |
| 2 | 3157.47 ± 15.97 | 3036.36 ± 14.26 |
| 3 | 3176.82 ± 23.68 | 3057.55 ± 18.23 |
| 4 | 3136.86 ± 20.36 | 3076.92 ± 17.63 |
| 5 | 3158.10 ± 19.97 | 3038.18 ± 14.98 |
| 6 | 3156.21 ± 17.43 | 3057.55 ± 16.57 |

By using Microsoft Excel's Data Analysis function, the responses in Table 8 were analysed through a two-sample assuming unequal variances t-test. According to the t-test result in Table 9, it was found that the p-value (two-tailed) of $2.23 \times 10^{-7}$ was smaller than the alpha value of 0.05 and the t-value of 12.35 was larger than t-critical value (two-tailed) of 2.23. So, based on the findings of p-value < α, and t-value > t-critical, the hypothesis, $H_0$ was rejected. Thus, it can be concurred that there is a significant difference between both optimisation methods in the analysis of magnesium in almond nuts. Based only on the optimum responses produced by both method, it can be seen that univariate optimisation is the better method, which is not relevant compared to other researches that stated univariate method is inferior to multivariate [10, 11].

**Table 9.** Two-sample assuming unequal variances t-test results from Microsoft Excel's Data Analysis software

| Source | Univariate Optimisation | Multivariate Optimisation |
|---|---|---|
| **Mean** | 3160.277765 | 3053.917919 |
| **Variance** | 220.8513211 | 224.2023587 |
| **Observations** | 6 | 6 |
| **Hypothesized Mean Difference** | 0 | |
| **df** | 10 | |
| **t Stat** | 12.3494361 | |
| **P(T<=t) one-tail** | 1.11464E-07 | |
| **t Critical one-tail** | 1.812461123 | |
| **P(T<=t) two-tail** | 2.22927E-07 | |
| **t Critical two-tail** | 2.228138852 | |

However, univariate optimisation only focused on the responses rather than the interactions and other factors such as resource and time. According to the optimum parameters of both methods, in terms of resource and time, multivariate optimisation is far more superior to univariate optimisation since the former takes less nitric acid and less time to produce the optimum response (3050.28 ± 12.07 mg kg$^{-1}$) which is not that significantly inferior to that of latter (3170.16 ± 10.99 mg kg$^{-1}$).

Unlike univariate optimisation, the optimum parameters of multivariate optimisation can be achieved due to the usage of Design Expert software which allows user to determine the degree of importance for the response and parameters while choosing which response or parameter to put as maximization, minimization or in range based on the user's specifications, without ignoring interactions between parameters. So, the multivariate method's optimized parameter was chosen with the basis of minimising the volume of nitric acid while maximising the response without any significant negative effect on the response. Also, multivariate optimisation required less runs (15 runs) to produce the optimum parameters while univariate optimisation required a total of 21 runs to produce its optimum parameters. It can be deduced that multivariate optimisation requires less resource and time in its process to obtain optimum parameters.

### 3.5. Method Validation

#### 3.5.1. Linearity

The calibration curve for the determination of magnesium in almond nuts was demonstrated by the determination of 5 standard calibration solutions of Mg in the concentration range of 0 to 0.5 ppm. The regression equation of the calibration curve is $y = 0.8277x + 0.0072$ and the correlation coefficient is found to be 0.9964. So, the curve is considered to have a high linearity and high correlation since its correlation coefficient of 0.9964 is higher than 0.995.

#### 3.5.2. Precision

Precision was measured to validate the closeness of the data obtained from the studied method. Results were obtained in intraday and interday where this is a one-week interval between both days, as shown in Table 10 and Table 11 respectively.

**Table 10.** Concentration of Mg for intraday measurement

| Day | Concentration of Mg (mg kg$^{-1}$) | Relative Standard Deviation (%) |
|---|---|---|
| 1 | 3050.89 ± 11.02 | 0.36 |

**Table 11**. Concentration of Mg for inter-day measurement

| Day | Concentration of Mg (mg kg$^{-1}$) | Relative Standard Deviation (%) |
|---|---|---|
| 8 | 3018.39 ± 19.40 | 0.64 |

According to the results, there is little difference in the concentration of Mg between intraday and interday, along with a low relative standard deviation for both days. Thus, it can be implied that the method has a good precision.

#### 3.5.3. Percentage of Recovery

Percentage recovery of the method was done to assess the method's ability to recover analytes. The recovery of magnesium from the replicate spiked sample was found to be an average of 94.99% with RSD of 0.59%. This indicated that there is minimal loss of detectable magnesium through the method.

#### 3.5.4. Limit of Detection and Limit of Quantitation

Limit of detection (LOD) was found to be 2.803 µg L$^{-1}$ while limit of quantitation (LOQ) was found to be 9.343 µg L$^{-1}$ as shown in Table 12. Both LOD and LOQ values were low. The low LOD value indicated that the lowest amount of magnesium in the sample which can be detected by FAAS is 2.803 µg L$^{-1}$. The LOQ value also implied that the method is sufficiently sensitive in detecting magnesium in samples. So, FAAS method is suitable in detecting and determining concentration of magnesium in almond nuts sample.

**Table 12** Limit of detection and limit of quantification of Mg

| Metal | LOD (µg L$^{-1}$) | LOQ (µg L$^{-1}$) |
|---|---|---|
| Mg | 0.0046 | 0.0154 |

## 4. CONCLUSION

Both univariate optimisation and multivariate optimisation were studied using analysis of magnesium in almond nuts as a basis. Both methods were studied to compare the results of optimisation and to determine any significance difference between the methods using a t-test. The parameters of the magnesium analysis being optimised by both methods were temperature of digestion, time of digestion and volume of nitric acid.

Univariate optimisation method produced optimum values of 90.00°C, 20.00 mL and 40.00 minutes for parameter temperature, volume of nitric acid and time of digestion respectively, which resulted in its optimum response of an average 3170.16 ± 10.99 mg $kg^{-1}$ magnesium concentration. On the other hand, multivariate optimisation method produced optimum values of 91.30°C, 12.56 mL, and 34.69 minutes for the parameter temperature, volume of nitric acid and time of digestion respectively, which resulted in its optimum response of an average 3050.28 ± 12.07 mg $kg^{-1}$ Mg concentration.

Based on the t-test results, it was shown that there was a significance difference between both optimisation methods. When based solely on the response, univariate optimisation was better than multivariate optimisation. However, after considering all factors (resource and time) and interaction between parameters, multivariate optimisation clearly has the higher advantage than univariate optimisation in terms of efficiency. Multivariate optimisation required less resource and time (15 runs) to obtain its optimum parameter compared to univariate (21 runs). In addition, multivariate's optimum parameter requires less volume of nitric acid and time to obtain an optimum response which is not inferior to that of univariate's.So, multivariate optimisation, in this case, was proven to be more efficient and effective than univariate optimisation, even though univariate's response is slightly higher than the multivariate's..

## REFERENCES


[1] Araujo, P.W. and R.G. Brereton, Experimental design III. Quantification. TrAC Trends in Analytical Chemistry. 15(3) (1996) 156-163.

[2] Bezerra, M.A., R.E. Santelli, E.P. Oliveira, L.S. Villar, and L.A. Escaleira, Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 76(5) (2008) 965-977.

[3] Lundstedt, T., E. Seifert, L. Abramo, B. Thelin, Å. Nyström, J. Pettersen, and R. Bergman, Experimental design and optimization. Chemometrics and intelligent laboratory systems. 42(1-2) (1998) 3-40.

[4] Myers, R.H. and D.C. Montgomery, Response surface methodology: process and product optimization using designed experiments. (1995) 1-15.

[5] Carley, K.M., N.Y. Kamneva, and J. Reminga, Response surface methodology. (2004) 13-27.

[6] Vera Candioti, L., M.M. De Zan, M.S. Cámara, and H.C. Goicoechea, Experimental design and multiple response optimization. Using the desirability function in analytical methods development. Talanta. 124 (2014) 123-138.

[7] Tung, C.Y., Spectrophotometric Method for the Determination of Saccharin in Preserved Fruits and Beverages, in Faculty of Science. 2009, Universiti Teknologi Malaysia: Faculty of Science.

[8] Moodley, R., A. Kindness, and S.B. Jonnalagadda, Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia and walnut) consumed in Southern Africa. Journal of Environmental Science and Health Part B. 42(5) (2007) 585-591.

[9] Hormozi-Nezhad, M.R., M. Jalali-Heravi, H. Robatjazi, and H. Ebrahimi-Najafabadi, Controlling aspect ratio of colloidal silver nanorods using response surface methodology. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 393 (2012) 46-52.

[10] Lobo, F.A., D. Goveia, A.P.d. Oliveira, E.R. Pereira-Filho, L.F. Fraceto, N.L.D. Filho, and A.H. Rosa, Comparison of the univariate and multivariate methods in the optimization of experimental conditions for determining Cu, Pb, Ni and Cd in biodiesel by GFAAS. Fuel. 88(10) (2009) 1907-1914.

[11] Caldas, L.F.S., B.B.A. Francisco, A.D.P. Netto, and R.J. Cassella, Multivariate optimization of a spectrophotometric method for copper determination in Brazilian sugar-cane spirits using the Doehlert design. Microchemical Journal. 99(1) (2011) 118-124.