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Antioxidant increase by response surface optimization and Bayesian neural network modelling of pumpkin *(Cucurbita moschata* Duch*)* freezing

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Abstract

Pumpkin antioxidants have been found to benefit diabetics. This current study was attempted to optimize slow freezing treatment for a pumpkin to obtain maximum antioxidant gain using response surface methodology (RSM) and Bayesian regularized neural network (BRANN) approaches. A central composite design was used to generate the freezing experiment and to examine response change as a function of temperature and freezing time. Feedforward neural networks with a 2-15-1 structure were developed and trained using the Bayesian regularization algorithm. The results showed that the freezing data were well fitted to quadratic models generating R^2 for total phenolic compounds (TPC), flavonoid of 0.850 and 0.857 respectively. The RSM optimized freezing of -20^{o}C for 9 hrs were well confirmed to produce an increase in TPC and flavonoid by 54.44% and 60.4% respectively. The BRANN performances were found to be similar to that of RSM. While overfitting was mitigated during the supervised training, the BRANN model served excellent predictive and confirmatory tool for the optimization. In conclusion, slow freezing at -20^{o}C for 9 hrs significantly increases TPC and flavonoid of pumpkin. This novel process may be adopted to provide healthier pumpkins food products for targeted consumers.

1. Introduction

Phenolic compounds have been the focus of research due to their valuable natural antioxidant health benefit. Epidemiological studies have consistently revealed that dietary antioxidant consumption significantly decreases the risk of degenerative diseases including stroke among women with or without cardiovascular disease history and heart failure (Rautiainen *et al*., 2012; Rautiainen *et al*., 2013). On a cohort study, dietary antioxidant has also been demonstrated to exhibit protective effects on glucose tolerance of diabetics (Okubo *et al*., 2014). In particular, pumpkin antioxidants have been confirmed to improve glucose profile in both animal models (Sedigheh *et al*., 2011; Sharmin *et al*., 2012; Dyshlyuk *et al*., 2017) and human clinical trials (Bayat *et al*., 2016; Mahmoodpoor *et al*., 2018). The antioxidants contained in pumpkin *(Cucurbita moschata* Duch*)* include phenol, flavonoid, and anthocyanin with an average value of 23.7 mg, 4.4 mg, 0.14 mg per 100 g produce respectively (Oloyede *et al*., 2012). Pumpkins are highly potent antioxidant sources as they are easy to grow in different variety of climates, and low production cost (Dragovic-Uzelac *et al*., 2005; Provesi and Amante, 2015).

An excellent preservation method for long term application widely used in the food industry is freezing (Xu *et al*., 2014). The most notable advantage of the method is the ability to retain nutrition values particularly that of the heat-sensitive. Another benefit of freezing is it exhibits an extraction effect for some foods (Leong and Oey, 2012). Therefore, an increase in certain nutrients could be expected. Freezing of food is commonly practised for long periods which could increase energy demand. It is very important to establish a processing method that offers optimum gain for nutrients of interest by optimizing the processing factors.

Response surface methodology (RSM) is mathematical methods used to analyse the relationship between interested factors and response in a system and to develop a suitable model by which process optimization can be achieved (Montgomery *et al*., 2011). Another mathematical tool that is designed to have the structure and working principle similar to that of a biological brain is the artificial neural network (ANN).

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An ANN consists of components called neurons that are connected to each other and can be trained using a certain algorithm to serve a useful function (Hagan *et al*., 2014). Compared to conventional statistical computation, ANNs are superior in term of the capability to handle limited data sets with large variation and missing data, to produce a more robust model for the non-linear response variable, and to develop models without prior knowledge of the relationship between variables (Das *et al*., 2016). In the food industry, ANNs have been proven to be powerful approaches to model and optimize a particular process of interest. For drying application, ANN has demonstrated the accurate prediction of phenolic compounds and antioxidant activities of banana (Guiné *et al*., 2015) and apple (Winiczenko *et al*., 2018).

In order to obtain the best possible model, the addition of neurons during ANN training may decrease error. However, at the same time overfitting may occur, thus decreasing the prediction power of the network. Bayesian regularized artificial neural networks (BRANNs) effectively calculate and train the networks hyperparameters by turning off those that are not relevant to avoid overfitting (Burden and Winkler, 2008). Overfitting occurs when the difference of the observed data and prediction values almost zero which result in poor generalizability for new data which have not been recognized before. During network training, the occurrence of overfitting can be observed when the training data set errors decrease while at the same time the errors of the test data increase (Okut, 2016). To the best of our knowledge, there has been no RSM nor BRANN modelling for the slow freezing of pumpkin in relation to its antioxidant changes. This current study was therefore aimed at filling this gap of knowledge and practice. The information of the process would offer an improved freezing method to provide a natural ingredient for healthier food product developments.

2. Materials and methods

2.1 Pumpkin samples preparation

Pumpkin samples were procured from a local market using the following selection criteria: at the stage of commercial maturity (deep and solid of orange skin; hard rind, resisted nail punctures, and sound hollow when thumped), fresh, free from defects, and of relatively same weights of 3-4 kg each fruit. Before the experiment, they were kept at the recommended temperature of 10-11^{o}C and RH of 50-70% (Maynard and Hochmuth, 2007; Raju *et al.*, 2011). The fruits mesocarp were obtained after peeling and discarding the seed and fibrous materials. Approximately 250 g sample consisting of 2×2×3 cm pieces of pumpkin were then frozen at conditions according to the experimental design. Upon freezing completion, the samples were thawed for 2 hrs at room temperature, sliced at approximately 1 mm thick and dried at 55^{o}C to avoid peroxidase and lipoxygenase activities (Suvarnakuta *et al.*, 2005) for 24 hrs in a drying oven (FDH6, Maxindo, Indonesia). The dried slices were ground (Philips HR2116, Indonesia) to obtain flour of 300 µm using a sieve at 50 mesh (Retsch 5657, Germany) before vacuumed-sealed in embossed plastic bags. The samples were stored for not more than 1 month in a dark container at room temperature before further analysis.

2.2 Response surface design of freezing

A central composite design (CCD) of RSM was chosen to generate an experiment of two numeric process factors, namely temperature and freezing time. The factors levels were determined based on the previous fuzzy logic-controlled slow freezing experiment which suggested that maximum increase in total phenolic was resulted from freezing at -18^{o}C for 6 hrs (Kristianto *et al.*, 2020). Therefore, the temperature level in this current experiment was set between -20^{o}C and -10^{o}C, whereas the freezing time was 5 and 9 hrs. The rotatable CCD design with $\alpha = 1.414$ was set to conduct the experiment with the following parameters; 4 factorial points, 4 axial points, and 3 centre points. With twice replication for each point, the setting generated 22 runs (Table 1). The value of temperatures and times in the table was accurate in two decimal places. Meanwhile, the control equipment used in the experiment can merely accept numbers with no decimal fraction. Therefore, the temperatures and times were entered into the system after they were rounded to their closest values. The decimal fractions of the hrs were translated to minutes before the rounding. The number of fruits was assigned to experiment blocks whereas total phenolic compounds (TPC), flavonoid, and antioxidant activities as the response variables.

The collected data were fitted to a second-order polynomial model to obtain the coefficient of determination between the factors and the response. The generalized polynomial used in the RSM study was:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x^2 + \sum \sum_{i<1}^{k} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the predicted response; β_0, β_i, β_{ii}, and β_{ij} represent the regression coefficients for intercept, linear, quadratic and interaction terms respectively; x_i and x_j are the factors; k is the number of factors, and ε is the standard random error. The design of the freezing experiment, model evaluation, statistical analysis and RSM study was carried out using Design-Expert®

Table 1. The design experiment and responses of pumpkin slow freezing

Run	Block	Factor[a]		Response variable			
		Temperature (°C)	Time (hrs)	TPC (mg GAE/g)	Flavonoid (mg QE/g)	DPPH (%)	ABTS (%)
1	1	-10 (1)	9 (1)	13.17	9.58	45.09	41.10
2	1	-15 (0)	7 (0)	8.60	6.19	35.63	27.67
3	1	-20 (-1)	5 (-1)	11.70	8.39	42.63	36.35
4	1	-15 (0)	7 (0)	10.27	7.39	39.41	31.08
5	1	-20 (-1)	9 (1)	15.84	11.28	52.27	58.46
6	1	-10 (1)	5 (-1)	12.89	9.22	45.18	42.51
7	1	-20 (-1)	9 (1)	14.36	10.34	48.87	48.00
8	1	-20 (-1)	5 (-1)	12.20	8.67	44.33	40.36
9	1	-15 (0)	7 (0)	8.31	5.85	33.74	26.85
10	1	-10 (1)	9 (1)	10.71	7.72	39.98	32.12
11	1	-10 (1)	5 (-1)	10.99	8.42	39.79	35.91
12	-1	-7.93 (1.41)	7 (0)	10.12	7.99	39.32	30.71
13	-1	-15 (0)	7 (0)	8.44	6.05	34.40	27.08
14	-1	-22.07 (-1.41)	7 (0)	11.28	8.04	42.63	35.91
15	-1	-15 (0)	4.17 (-1.41)	13.54	9.73	46.41	46.51
16	-1	-15 (0)	7 (0)	10.41	7.43	39.79	31.68
17	-1	-22.07 (-1.41)	7 (0)	12.47	8.93	44.71	41.17
18	-1	-15 (0)	4.17 (-1.41)	12.71	9.07	44.80	41.99
19	-1	-15 (0)	9.83 (1.41)	15.22	10.86	51.32	55.86
20	-1	-7.93 (1.41)	7 (0)	10.95	7.86	41.21	35.01
21	-1	-15 (0)	9.83 (1.41)	15.42	10.99	51.61	56.75
22	-1	-15 (0)	7 (0)	11.08	7.87	41.68	35.31

[a]Numbers in parentheses are coded symbols for levels of the factors.

software version 10.0.1 (Stat-Ease Inc., Minneapolis, USA). ANOVA tests were used to evaluate the statistically significance of the model terms.

2.3 Bayesian regularization network

The freezing experiments were also modelled using multi-layer artificial neural networks. The time and temperature of freezing were used for the inputs whereas antioxidants as the targets. A feed-forward network was built and trained using the Bayesian regularization training function. The final BRANN model was obtained by tuning the network parameters during trial and error of the network training. The parameters adjustments involved changing the number of hidden layers, neurons, and the transfer functions. A model with the highest correlation between observed and predicted data without sign of overfitting was selected as the most representative. The initialization, configuration and training of the neural networks were carried out using Matlab® 2017 for the Linux platform. The model performances of RSM and BRANNs were compared using the following parameter: R^2, MSE (mean squared error), and AAD (relative average absolute deviation). The AAD was calculated as:

$$AAD = \frac{100}{N_{exp}} \sum_{i=1}^{N_{exp}} \left| \frac{X_{i,\ exp} - X_{i,\ pred}}{X_{i,pred}} \right| \quad (2)$$

where N_{exp} is the number of the experiment data, $X_{i,\ exp}$ is the i^{th} experiment value, and $X_{i,\ pred}$ is the respective predicted value. The predicted values for the performance measurements were obtained from three training sessions.

2.4 Total phenolic and flavonoid analysis

For the total phenolic analysis, the dried pumpkin samples of 0.5 g were mixed with 20 mL of 95% ethanol and macerated for 24 hr in the darkroom at ambient temperature. The obtained extracts were filtered through Whatman paper and filled into brown glass bottles. TPC and flavonoid content in pumpkins were estimated using the spectrophotometric technique (Alara *et al.*, 2018) with slight modification. The prepared extracts of 1 mL were mixed with 200 µL Folin-Ciocalteu reagent, then 0.6 mL of 0.2 mM Na_2CO_3 solutions were added and mixed thoroughly. The mixtures were allowed to stand for 2 hrs before measurement of their absorbance at 765 nm. A regression line of $\hat{y} = 0.009 + 0.004x$ ($R^2 = 0.975$) was obtained from six points of 0 to 50 mg/L gallic acid standard concentration range and their respective absorbances. The equation was then used to estimate the TPC content of the samples and the results were reported as gallic acid equivalent in milligrams per gram of dried samples (mg GAE mg/g).

For the flavonoid estimation, 100 µL extracts were mixed with 100 µL of 2% $AlCl_3$ and left at room temperature for 1 hr before their absorbance measurement at 420 nm. The absorbance from 6 points of 0 – 100 mg/L quercetin standard concentration range were also obtained to build a regression line of $\hat{y} = 0.058$

+ 0.005x (R^2 = 0.908). The amount of flavonoid in the samples was calculated based on the equation and expressed as quercetin equivalent in mg/gram of dried samples (mg QE/g).

2.5 Antioxidant activity analysis

The pumpkin antioxidant activities were measured using DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,20-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) methods (Alara *et al.*, 2018). Each point from four concentration range of 50 to 500 ppm from the prepared samples was mixed with 0.1 mM DPPH. After 30 mins of the reaction in the dark at room temperature, the absorbances of the mixtures were measured at 517 nm. Similar procedures were carried out for ABTS analysis in which four points of differing sample concentrations from 50 to 500 ppm were mixed with 285 µL of 2.45 mM potassium persulfate and 7 mM ABTS solutions. The mixtures were reacted at room temperature for 2 hrs before their absorbance reading at 734 nm. The ability to donate electron (DPPH assay) and to scavenge free radical of the samples (ABTS assay) was calculated from the following formula:

$$Inhibition\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} * 100 \quad (3)$$

where $Abs_{control}$ and Abs_{sample} were absorbances of the blank and pumpkin samples respectively. Results were reported as the percentage of inhibition at 500 ppm.

3. Results and discussion

3.1 Total phenolic, flavonoid, antioxidant activity

The TPC content of fresh pumpkins was 8.02 and 9.98 mg GAE/g for each block respectively, whereas the corresponding flavonoid contents were 5.73 and 7.11 mg QE/g. The respective antioxidant activities of the samples as measured by DPPH were 32.14 and 38.75%. These findings were close to resulting from other studies which reported a value of 6.5 mg GAE/g and 40.82% for TPC and antioxidant activity respectively (Sopan *et al.*, 2014). Another study found a slightly higher TPC content of 12 mg GAE/g (Sedigheh *et al.*, 2011). The variation of pumpkin antioxidant contents could be attributed to the different factors related to the cultivar used in the experiment (Kulczyński *et al.*, 2020), planting practice including the use of fertilizer and season (Oloyede *et al.*, 2014).

The freezing treatment caused an increase in both TPC and flavonoid from 8.31 to 15.84 mg GAE/g and from 5.85 to 11.28 mg QE/g, respectively. The maximum increase was as a result of freezing at -20°C for 9 hrs which was the factorial point in the design of the experiment. The antioxidant activities also increased following the phenolic change which very likely due to the antioxidant capability of the compounds. The antioxidant increase might be caused by the extractable effect of the freezing (Leong and Oey, 2012). The resulting frozen pumpkins with high antioxidant could be incorporated into the production of healthy foods, such as pumpkin cookies targeted for obese individuals (Mustika and Kartika, 2020). It is worth noting that providing foods carrying specific health function should be accompanied by nutrition education because consumer health interest can be negatively correlated to their eating habits (Santoso *et al.*, 2019).

3.2 Response surface modelling

Response surface modelling was applied to examine the effect of the slow freezing on pumpkin antioxidant changes. Statistical properties of the polynomial model of the proposed design were evaluated before the collection of response data. It was found that the model was not aliased ensuring that the design point used in the experiment were sufficient. There was also no problem related to the model adequacies based on the number of degrees of freedom for lack of fit and pure error, standard error of coefficients, and the fractional design space plot. Therefore, the next step of data collection could proceed subsequently. The CCD result of the experiment on target responses is shown in Table 1. The blocking was aimed to observe variability due to different pumpkin fruits and necessary to control nuisance factor in an experiment (NIST/SEMATECH, 2012). The responses for centre points between block 1 and 2 (coded zero for both factors in Table 1) show that there are not many differences indicating that the blocking exhibits less impact on the results.

The most suitable freezing model was selected based on the highest R^2 and the insignificance for lack of fit. For all responses, the selected model to fit the data was quadratics as presented in Table 2. The ANOVA tests suggested that all models were highly significant ($p<0.0001$), indicating that only 0.01% of the *F-values* occurred due to noise. The lack of fit assessments confirmed that there were no problems attributed to the model's fitness. The coefficient of determination R^2 (Table 3) and the respective adjusted R^2 for all models differed only slightly (less than 0.2) suggesting a good indication for the models. Based on this assessment, it can be concluded that the models fitted the data quite well. As indicated by the R^2, the RSM models were able to explain 83.3 - 87.7% variation for the data. In the freezing process, the temperature of the freezing medium along with the heat transfer coefficient are important environmental factors that govern freezing time and heat load (Pham, 2014). In this current study, other influencing factors related to food properties were

Table 2. ANOVA for quadratic model of pumpkin slow freezing

Source	TPC (mg GAE g)		Flavonoid (mg QE/g)		DPPH(%)		ABTS(%)	
	F-values	*p-value*	*F-values*	*p-value*	*F-values*	*p-value*	*F-values*	*p-value*
Model	16.836	< 0.0001[a]	17.922	<0.0001[a]	14.959	<0.0001[a]	21.381	<0.0001[a]
A	6.414	0.0223	3.775	0.07103	7.653	0.0144	9.382	0.0079
B	9.779	0.0067	9.699	0.00711	9.502	0.00758	14.244	0.0018
AB	4.961	0.0407	6.396	0.02314	3.957	0.06522	10.118	0.0062
A^2	8.122	0.0118	12.094	0.00337	8.275	0.01153	5.032	0.0404
B^2	62.732	< 0.0001	68.549	<0.0001	53.096	<0.0001	73.082	<0.0001
Lack of Fit	0.404		0.4874		0.404		0.4687	
Adjusted R^2	0.8	0.7527[b]	0.8088	0.6974[b]	0.777	0.753[b]	0.8359	0.710[b]
Predicted R^2	0.692		0.7095		0.664		0.722	
AP	10.795		11.1035		10.326		12.1138	

A = freezing temperature (°C), B = freezing time (hrs), [a] = significant, [b] = not significant, AP = adequate precisions

Table 3. The optimized slow freezing conditions, predicted and confirmed responses

Optimized conditions	Response	Confirmation experiments[c]	RSM		BRANN prediction
			95 % PI (low – high)	Predicted mean	
A = -20°C	TPC[a]	13.90±1.18	13.34 - 16.52	14.93	15.01
B = 9 hrs	Flavonoid[b]	10.13±0.98	9.58 – 11.77	10.67	10.79
	DPPH (%)	48.23±2.89	46.43 - 54.43	50.43	49.39
	ABTS (%)	47.96±6.44	47.22 - 59.64	53.43	53.12

A = freezing temperature, B = freezing time, [a] = mg GAE/g, [b] = mg QE/g, [c] = mean and standard deviation of four runs, PI = prediction interval

assumed to be constant. Due to the absence of a published model in this particular area of research to date, it was not possible to perform a model comparison. However, for future purpose when comparing models, instead of using R^2 the use of adjusted R^2 is recommended as this measure takes the number of independent variables into account during the model development, hence bias due to difference predictor number would be avoided. Adjusted R^2 increases only when the added predictor generates sufficient reduction in the residual sum of square (Montgomery *et al.*, 2011).

The effect of freezing time was greater than freezing temperature. For TPC, the coefficient estimates were 0.78 ($p<0.01$) and -0.63 ($p<0.05$), respectively. The effect of the two factors on flavonoid, DPPH, and ABTS followed a similar pattern. The reproducibility of the model as represented by CV (coefficient of variation) ranged from 5.83% to 9.96% with DPPH being the lowest. A model having a CV not greater than 10% was considered capable (Xu *et al.*, 2014). The responses for any given levels of freezing temperature (A) and time (B) in term of actual factors could be predicted using the following equations:

$$\text{TPC (mg GAE/g)} = 49.654 + 1.442A - 9.039B - 0.079A*B + 0.034A^2 + 0.589B^2 \quad (4)$$

$$\text{Flavonoid (mg QE/g)} = 37.416 + 1.213A - 6.559B - 0.061A*B + 0.028\,A^2 + 0.422B^2 \quad (5)$$

$$\text{DPPH (\%)} = 129.723 + 3.456A - 20.666\,B - 0.176A*B + 0.086A^2 + 1.356B^2 \quad (6)$$

$$\text{ABTS (\%)} = 198.455 + 5.575A - 39.307B - 0.437A*B + 0.104A^2 + 2.470B^2 \quad (7)$$

The effect of temperature and time on the targeted variables are presented in three-dimensional graphs for more clarity (Figure 1). The contour and three-dimension surface clearly shows that higher antioxidants gain could be obtained by freezing at a lower temperature for a longer time. As freezing does not inactivate enzymes (Fellows, 2009), freezing at a higher temperature may result in more degradation of bioactive compounds, hence lower antioxidant gains. The use of temperature lower than -22°C which was the low axial value of -1.41 in the design might not be recommended as the freezing system would operate at the area of high load. Moreover, a similar study has shown that a lower freezing temperature of -32°C applied to berries had not improved total phenolic retention and antioxidant activity significantly as compared to the processing at -18°C (Khattab *et al.*, 2015).

3.3 Optimization and confirmation

The adequate precision measure in Table 2 indicates a strong signal of the process model to be optimized. Therefore, optimization was conducted by setting all response variables at their maximum values while the freezing temperature and time at their default ranges.

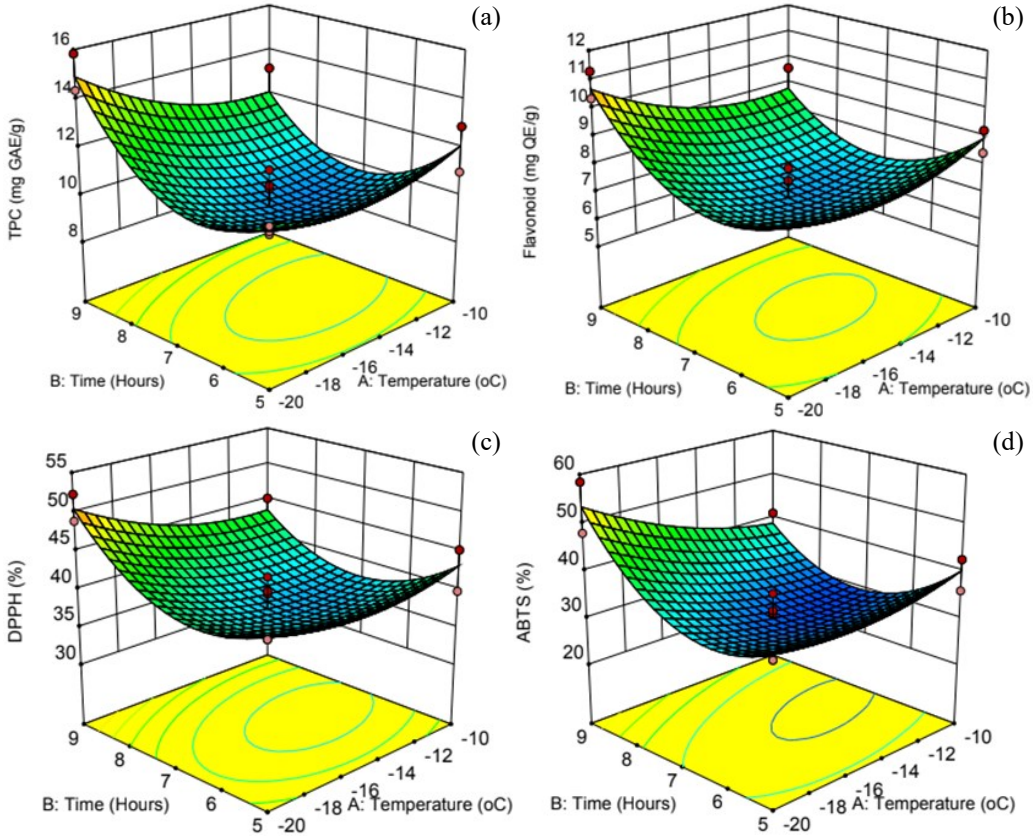


Figure 1. The surface plot of TPC (a), flavonoid (b), DPPH (c) and ABTS (d) of pumpkin as a function of freezing temperature and time

Four confirmation runs were then conducted following the optimized condition of freezing at -20°C for 9 hrs having a desirability of 87.7%. The confirmation results (Table 3) show that response values fall between the prediction interval, indicating that the model has been well confirmed. Based on the confirmation data, the increase in TPC and flavonoid resulted from the optimized process were 54.44% and 60.4%, respectively. The RSM prediction interval indicated a quite broad range of possible response values which could be expected from the confirmation runs. As the antioxidant compounds of pumpkin may vary due to agricultural-related practices (Oloyede *et al.*, 2014), the higher antioxidant gain could likely be expected from the prospective freezing implementation. Compared to other published data, the increase in antioxidant compounds in this current study was higher. An increase in total phenolic and antioxidant capacity of strawberry by individual quick freezing at -20°C for 24 hrs has been previously reported to be less than 10% (Oliveira *et al.*, 2015). An industrial freezing treatment; by which asparagus, green beans, and zucchini were blanched, frozen at -40°C, and maintained at -18°C in a thermostatic chamber for 2 months; resulted in total flavonoids increase from 19 to 50% (Mazzeo *et al.*, 2015).

3.4 Neural network modelling

The development of the neural network for the freezing was started with small shallow network architecture. The network composed of freezing temperature and time as an input, one hidden layer, and an output layer of neurons. The number of neurons in the hidden layer was increased in each training session aiming at the minimum possible to avoid overfitting (Hagan *et al.*, 2014) until the best performance was achieved. Based on the trial and error experiments, the most suitable network structure was a two-layer feed-forward neural networks type (Figure 2). The most suitable transfer functions were logarithmic sigmoid and linear for the hidden layer and output layer respectively. The Bayesian regularization back-propagation algorithm used in the training sessions offers reduction or elimination of the exhaustive cross-validation and is more robust than regular back-propagation (Burden and Winkler, 2008). The Bayesian performance is also better than the early stopping method in the effort to achieve network generalization especially for small data set (Beale *et al.*, 2012). The final models were selected from those with high R^2 and no sign of overfitting during the training. The performances for the chosen models are

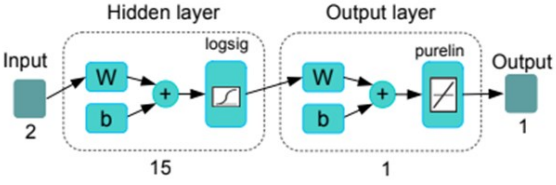


Figure 2. Neural network architecture of the slow freezing of pumpkin

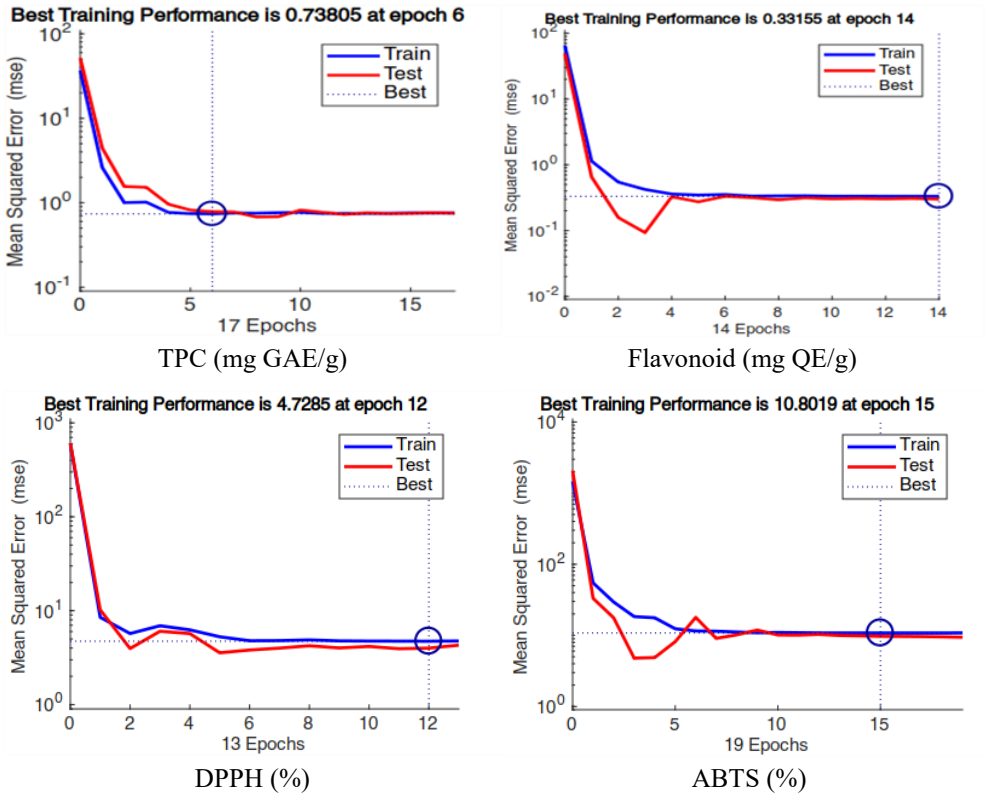
shown in Table 4.

Table 4. Performance of RSM and BRANN

Measure	RSM	BRANN
R^2:		
- TPC (mg GAE g)	0.850	0.850
- Flavonoid (mg QE/g)	0.857	0.858
- DPPH (%)	0.833	0.834
- ABTS (%)	0.877	0.881
MSE:		
- TPC (mg GAE g)	0.674	0.681
- Flavonoid (mg QE/g)	0.32	0.324
- DPPH (%)	4.269	4.317
- ABTS (%)	10.289	10.125
AAD:		
- TPC (mg GAE g)	6.442	6.545
- Flavonoid (mg QE/g)	5.957	5.914
- DPPH (%)	4.188	4.340
- ABTS (%)	7.251	7.669

In general, the performance of BRANN for the freezing for all responses was similar to that of RSM based on the R^2, MSE, and AAD measures. A very slight improvement might be observed on the model for ABTS response which indicated higher R^2 and lowers MSE, and for flavonoid for which ANN exhibited slightly lower AAD. Other studies reported that ANN performance is better as compared to RSM (Sinha *et al.*, 2013; Pilkington *et al.*, 2014; Simić *et al.*, 2016). As a good practice, before concluding a network performance, a condition that should be satisfied in the absence of overfitting as to avoid poor generalization (Okut, 2016). In this current research, overfitting did not likely to occur. From the training performance graph (Figure 3), it can be seen that the error for training data sets for all responses decreased steadily and at the same time the error for test data sets followed a similar trend. This indicated that the network had learnt sufficiently. This could be the evidence that over fittings might be problems during the network learning. Therefore, the developed models could be used satisfactorily. A higher correlation coefficient might be obtained by tuning network parameter or adding more neurons, but this would also increase the chance to overfit, hence decreasing model generalizability. The use of the small network along with Bayesian regularization seems to work well with the experiment data to mitigate overfitting in this research (Burden and Winkler, 2008; Hagan *et al.*, 2014).

Furthermore, in order to enable assessment of how well the model fits the experimental data the residuals were calculated (Figure 4). Overall, as assessed by the distance from the points to the zero lines the RSM and ANN prediction capabilities were quite accurate. The graphs also show that the distribution of the residuals across the experiment run number might not be a notable problem. The residual analysis should be emphasized high R^2 alone is not a guarantee that the obtained model fit data well and a model which does not fit the data does not provide an accurate solution to the problem being investigated (NIST/SEMATECH, 2012).



TPC (mg GAE/g) Flavonoid (mg QE/g)

DPPH (%) ABTS (%)

Figure 3. Training performance of BRANN of pumpkin slow freezing

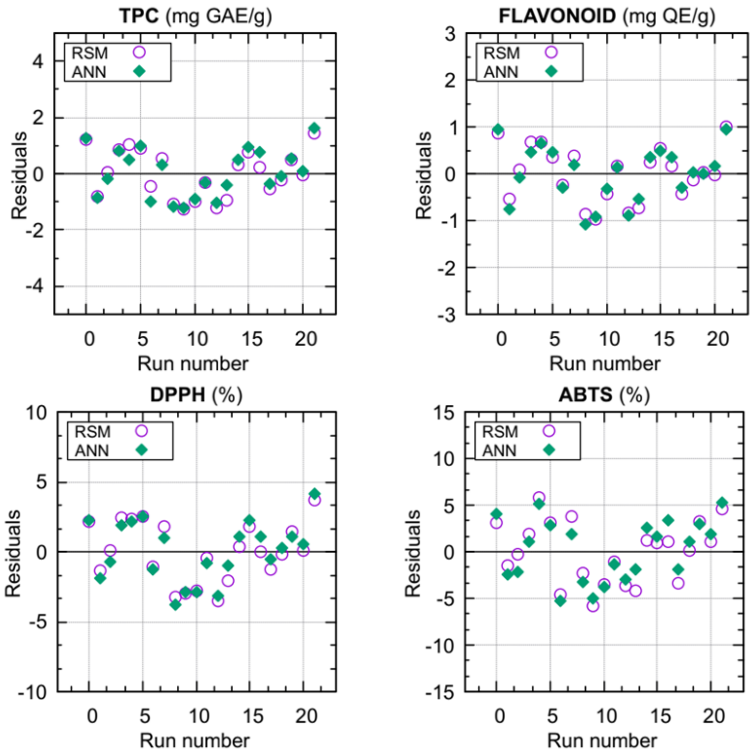


Figure 4. Comparison of RSM and BRANN residuals depicting similar performances

4. Conclusion

The process modelling of the slow freezing of pumpkin follow a quadratic model which results in R^2 for TPC, flavonoid, DPPH, and ABTS of 0.850, 0.857, 0.833, and 0.877, respectively. The optimized and confirmed freezing process is treatment at -20°C for 9 hrs which causes marked increases in TPC and flavonoid contents by 54.44% and 60.4% respectively. Higher increases are likely to be obtained when pumpkins with more antioxidant contents are employed as the raw materials. The freezing can also be modelled using small 2-15-1 neural networks trained with a Bayesian regularization algorithm. Mitigation of overfitting is successfully attempted during the training hence good generalization can be obtained. Based on the R^2, MSE, and AAD the BRANN performance is similar to that of RSM. The resulted process model may be used to produce frozen pumpkin products with the additional benefit of higher natural antioxidant.

Conflict of interest

The authors declare no conflict of interest.

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