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Optimisation of mechanically deboned chicken meat (MDCM) aromatic herbal sausage formulations using simplex-lattice mixture design

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Abstract

Nowadays, consumers are demanding for more natural foods, obliging the industry to include natural antioxidants instead of synthetic antioxidants to retard lipid oxidation and control spoilage bacteria in foods in order to improve their quality and nutritional value. Thus, various types of herbs and spices have been added in a variety of foods to improve their quality. Mechanically Deboned Chicken Meat (MDCM) sausage which is very susceptible to microbial growth and oxidation due to the extensive stress during the machine-deboning process and frozen storage resulted in decrease functionality of the product. This study was aimed to determine an optimum formulation of MDCM herbal sausage incorporated with three dried herbs using mixture design (Design Expert 8.0.1) software. Thirteen formulations of MDCM herbal sausages consisted of three independent variables: *Persicaria hydropiper* (X_1), *Murraya koenigii* (X_2) and *Etlingera elatior* (X_3) were developed. The effects of these aromatic Malaysian herbs on the total phenolic content (TPC), antioxidant activity and sensory qualities (aroma, colour, firmness, herbal taste, juiciness, after taste and overall acceptability) of sausages were determined using Folin-Ciocalteu colorimetric method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging assay and Quantitative Descriptive Analysis (QDA), respectively. The three independent variables had significant ($p<0.05$) effects on all response variables. The optimum formulation of sausage with the highest desirability (0.93) consisted of 59.46% (X_1), 0% (X_2) and 40.54% (X_3) produced 23.25 mg GAE/g EW of TPC value, 132.84 mg/mL of EC_{50} value and moderate score for overall acceptability (8.7). The predicted response values and the actual obtained response values for the optimised products were not significant at 95% confidence level.

Introduction

In the food industry, the use of mechanically deboned chicken meat (MDCM) in sausage formulations has recently increased in popularity due to its lower price compared to other meats, the strong tendency to replace red meat for healthier white meat and provide uniform textures to the end products (Babji *et al.*, 2005; Daros *et al.*, 2005). However, MDCM is susceptible to rapid oxidative rancidity due to the extreme machine-deboning process, which causes extensive stress and aeration (cell breakage and protein denaturation) as well as increases the extraction of lipids and heme components from the bone marrow (Hassan and Swet-Fan, 2005; Daros *et al.*, 2005). The synthetic antioxidants such as butylated hydroxylanisole (BHA) and butylated hydroxyltoluene (BHT) have been widely used in the food industry. Due to the poor safety and high toxicity of synthetic antioxidants, consumers are demanding more natural foods, thereby obliging the industry to include natural antioxidants and antimicrobials instead of synthetic preservatives to retard lipid oxidation and control spoilage-inducing bacteria in foods to improve their quality and prolong their storage shelf life.

Herbs and spices have been used for thousands of centuries, and the medicinal values of their phenolic components have been documented. Phenolic compounds present in herbs and spices possess bioactive properties such as antioxidant and antimicrobial properties (Wojdylo *et al.*, 2007; Chen *et al.*, 2008; Sengul *et al.*, 2009). In previous work, three aromatic Malaysian herbs, namely *Persicaria hydropiper* (kesum leaves), *Murraya koenigii* (curry leaves) and *Etlingera elatior* (torch ginger flower) showed that they have high total phenolic content (TPC), antioxidant activity (based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), β-carotene

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bleaching assays) and antimicrobial activity (based on a disc diffusion assay, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)) among six aromatic Malaysian herbs (Nurain *et al*., 2013; Nurain *et al*., 2014). Moreover, these aromatic herbs are well known and commonly used in Malay dishes, such as asam pedas, laksa and curry, to provide a unique flavour and aroma to foods.

Up to date, there are no studies on the optimisation of dried aromatic Malaysian herbs in MDCM sausage using mixture design. Therefore, the objective of this study was to determine an optimum formulation of MDCM sausage incorporated with three dried aromatic Malaysian herbs using mixture design (Design Expert 8.0.1) technique based on TPC, antioxidant activity and sensory acceptability. The MDCM sausage incorporated with these dried Malaysian aromatic herbs was produced to explore the synergistic antioxidative effects of these aromatic herbs in meat product, and these herbs also used as flavour and aroma enhancers in foods. In addition, product optimisation studies involved multiple formulations and various approaches that may be used to identify the range of optimal formulations and sensory qualities that are critical to consumer acceptance. The main practical application of this study was to obtain the optimised formulation of MDCM aromatic herbal sausage using simplex-lattice mixture design. The results of this study will develop new formulation of nutritional MDCM sausage.

Materials and Methods

Chemicals and raw materials

Ethanol, Folin-Ciocalteu reagent, sodium carbornate were purchased from Merck (Darmstadt, Germany). Gallic acid and DPPH were obtained from Sigma-Aldrich Co. (St. Louis, USA). Ascorbic acid was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Isolated soy protein (ISP), sodium nitrite, sodium tripolyphosphate (STPP), liquid smoke, BHT and BHA were food grade and purchased from Sigma-Aldrich Co. (St. Louis, USA).

Three aromatic Malaysian herbs used in the present study, namely *Persicaria hydropiper* (L.) H. Gross, *Murraya koenigii* Spreng. and *Etlingera elatior* (Jack) R.M. Sm. were collected from Kuala Selangor, Selangor, Malaysia. The herbs were identified by a botanist from Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The voucher specimens for each herb were preserved at the herbarium of Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia under the reference number SK 2030/12, SK 2031/12 and SK 2034/12, respectively. MDCM was purchased from Lucky Frozen Food Company (Penang, Malaysia). Chicken fat, salt, sugar, potato starch, white pepper, black pepper and chicken flavour were purchased from Giant supermarket (Shah Alam, Malaysia).

Experimental design

A three-component, constrained simplex-lattice mixture design was performed using Design Expert software (Stat-Ease, Inc., Version 8.0.1.0, Minneapolis, USA). The experimental design consisting of thirteen points including three replicates of percentage dried aromatic herbs were developed to determine the combined effect of *P. hydropiper* (X_1), *M. koenigii* (X_2) and *E. elatior* (X_3) as three factors of independent variables. The experimental domain consisted of different proportions of independent variables between zero and hundred ($0 \leq X \leq 100$). To allow error estimation, all mixtures were prepared in three independent replications. Table 1 shows the experimental design for percentage of *P. hydropiper, M. koenigii* and *E. elatior* obtained from simplex-lattice mixture design. As dependent variables, the TPC, DPPH radical scavenging activity assay (EC_{50} value) and sensory qualities (aroma, colour, firmness, juiciness, herbal taste, after taste (metallic taste) and overall acceptability) were chosen.

Preparation of dried aromatic herbs

The three herbs were washed thoroughly with tap water and visibly damaged leaves were removed before rinsing. The herbs were stripped and dried at 50^{o}C in a cabinet dryer (Vision Scientific CO. Ltd, Daejon, South Korea) until a constant weight was obtained. The fully dried aromatic herbs were ground into a fine powder using an ultra-centrifugal mill (Restch, ZM 200, Haan, Germany) to maximum size of 0.5 mm. The dried aromatic herbs were vacuum-packed using a table-top vacuum pack machine (Clarity Excel, CE-400/2F, Selangor, Malaysia) and stored until further use.

Processing of MDCM aromatic herbal sausage

Sausage formulations were formulated based on method by Babji *et al.* (2001). Thirteen formulations of MDCM aromatic herbal sausages were processed, starting with the preparation of pre-emulsion. The frozen MDCM was thawed at 4°C in a chiller (Saintifik Maju, Selangor, Malaysia) for 30 mins. Then, the meat was chopped into small cubes (2 cm^3)

Table 1. Experimental Design for percentage of *P. hydropiper, M. koenigii* and *E. elatior* obtained from Simplex-Lattice mixture design

Sample No.	Factors (%)		
	X_1	X_2	X_3
1	0	100	0
2	0	100	0
3	100	0	0
4	17	17	66
5	0	50	50
6	17	66	17
7	33.3	33.3	33.3
8	66	17	17
9	100	0	0
10	50	0	50
11	50	50	0
12	0	0	100
13	0	0	100

Note: X_1: Dried *P. hydropiper*; X_2: Dried *M. koenigii*; X_3: Dried *E. elatior.*

and kept cold prior to processing. The meat was ground for 1 min using a bowl mixer (Robot Coupe, South Carolina, USA). All ingredients were added and ground to form a homogeneous emulsion and kept at temperature less than 10°C to prevent emulsion breakdown. The amount of synthetic and natural antioxidants was added to the formulations based on the total weight of the meat (USDA, 1999). The batters were then manually stuffed in cellulose casings (2 cm in diameter) using a manual stainless steel sausage stuffer (Friedr. Dick, Deizisau, Germany) and were hand-linked to form approximately 10 cm links in length. The sausages were cooked using a combi oven (Fagor, Onati, Spain) at 55°C (20 mins), 65°C (20 mins), 75°C (20 mins) and 80°C (15 mins). The cooked sausages were sprayed with water for 5 mins to remove any adhering oil and were cooled down by subsequently immersed in ice. The casing was removed manually. All sausages were vacuum-packed in polyethylene bags using a table-top vacuum pack machine (Clarity Excel, CE-400/2F, Selangor, Malaysia) and stored in a freezer (Sanyo, San Diego, USA) at -18°C for further analysis.

Total phenolic content (TPC)

TPC of aqueous and ethanolic dried herbal extracts was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Gallic acid standard was prepared in various concentrations (0.1 – 0.5 mg/ml). Approximately 0.5 ml of Folin-Ciocalteu reagent (50%) was added to the 0.1 ml of sample (0.5 mg/ml) and mixed using a vortex (Vision Scientific CO. Ltd, Daejon, Korea) for 3 mins. The mixture was added with 7.9 ml of distilled water and left for 5 mins at room temperature. After 5 mins, 1.5 ml of sodium carbonate (7.5%) was added and this made up the volume to 10 ml. The mixture was allowed to stand for 2 hr in dark place and room temperature with intermittent shaking. The absorbance of samples and standard were measured at 765 nm using an ultraviolet-visible (UV-Vis) spectrophotometer (Perkin Elmer, Lambda 35, California, USA). TPC of samples was expressed as mg of gallic acid equivalents (GAE) per g of dried herbal extract weight (DEW) (mg GAE/g DEW) using an equation obtained from the standard gallic acid calibration curve.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

The ability of antioxidant in sample to scavenge DPPH radical by hydrogen donor was determined according to Braca *et al.* (2001). Ascorbic acid and combination of BHA/BHT were used as standards. A solution of 0.1 mM DPPH in ethanol was prepared. Approximately 0.6 ml of various concentrations (0.2 – 1 mg/ml) of sample and standards were added to 4.5 ml of ethanolic DPPH solution. The mixture was shaken vigorously and allowed to stand in dark place for 20 mins. The absorbance of samples, standards and control (water and ethanol) were measured at 517 nm using an UV-Vis spectrophotometer (Perkin Elmer, Lambda 35, California, USA). The percentage of scavenging effect of samples was determined. Graph of scavenging effect (%) was plotted against the corresponding concentration of the extract to obtain efficiency concentration (EC) values. EC_{50} is defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50% (Azizah *et al*., 2007). The lowest EC_{50} values give the highest scavenging activity of sample.

Sensory evaluation

In order to find an optimum MDCM aromatic herbal sausage formulation, sensory evaluation was conducted using the QDA trained panellists at Laboratory of Food Sensory, School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.

Descriptive analysis panels: The QDA evaluation consisting of eight trained panellists (aged between 28 to 32 years old) from the Food Technology Program of Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia. Panellists were recruited based on the following criteria: (1) had no food allergies; (2) had satisfied gender balance requirement consisting of 50% male and 50% female; and (3) had eaten sausage or other related products at least once a month to make sure the panellists familiar with the characteristics of products.

Descriptive analysis training: The panellists were trained in six training sessions of two hours to discuss, clarify each attribute, familiarise with the characteristics of the sausages and train in scaling of attributes intensities. The attribute terms, scorecard and scale for evaluation of sausage sample were generated by the panellists. This procedure was repeated until panel consensus was achieved. Testing was initiated after the panellists agreed on the specifications.

Descriptive analysis testing: Each replication of the experimental design was evaluated within three sessions. Therefore, each panellist rated four treatments per evaluation. Samples were prepared 15 mins before evaluation and coded with three-digit random numbers. The scale used is an interval scale consisting of a horizontal line 15 cm long. Panellists were instructed to evaluate the aroma, colour, firmness, juiciness, herbal taste, after taste (metallic taste) and overall acceptability of sample using scoresheet provided and requested to rate samples relative to intensity scores of reference sample established during training session. At every session, each panellist was provided with standard references. Intensity scores for each attribute were analysed statistically to determine significant differences among the sausage formulations evaluated and to monitor panellist performance.

Statistical and data analysis

The Design Expert software (Stat-Ease, Inc., Version 8.0.1.0, Minneapolis, USA) software was used for the experimental design and statistical analysis system (SAS) program (SAS Institute, Version 9.0, 2009, North Carolina, USA) was used for data analysis. The effect and regression coefficients of individual linear, interactive, and cubic terms were determined. The software was used to fit a predictive model to the responses described by Scheffe (1958) as follows:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X2 + \beta_{13}X_1X3 + \beta_1X_1X_3 \quad (1)$$

where Y is a predicted dependent variable (either TPC, EC_{50}, aroma, colour, firmness, juiciness, herbal taste, after taste (metallic taste) and overall acceptability); β is corresponding parameter estimates for each linear and cross product term produced from the prediction models; and X_1 is the dried *P. hydropiper*, X_2 is the dried *M. koenigii*, and X_3 is the dried *E. elatior*.

All models with $R^2 > 0.70$ were chosen (San-Juan *et al*., 2006). The stastical significance of all terms in the regression equations was examined by one-way analysis of variance (ANOVA) for each response and the significance test level was set as a probability ($p<0.05$). Model significance, significance of lack of fit and R^2 values were used to judge the adequacy of model fitness. The interaction effects of herbs mixture on the sausages were determined using a contour plot. The significant predictive models were used to generate contour plots for each response by setting a response goal area. To achieve the optimum region, contour plots of the predicted equations for each parameter were superimposed represented all combinations of mixtures that would meet pre-set criteria for an acceptable prototype product. This region of overlap was defined as the optimum region. Model verification was performed on two replicates of predicted values and actual values. The percent accuracy between the predicted and actual values was determined, and t-test was performed to determine whether a significant difference existed between the predicted and actual values.

Results and Discussion

TPC, DPPH assay (EC_{50} Value) and sensory evaluation

The relationship between the proportions of each dried herb towards the TPC, DPPH assay (EC_{50} Value) and sensory evaluation of the MDCM sausages are summarised by using a trace plot as shown in Figure 1.

The trace plot shows that the sausages incorporated with three dried aromatic Malaysian herbs or high percentage of *P. hydropiper* and *M. koenigii* exhibited the highest values of TPC compared to the sausage incorporated with single herb. As discussed in the earlier works (Nurain *et al*., 2014) several phenolic compounds including flavonoids were detected in *P. hydropiper, M. koenigii* and *E. elatior* using RP-HPLC instrument. The high amount detected in *P. hydropiper* and *M. koenigii* may have given the highest TPC in the sausage formulations. Therefore, the combination of these three herbs in sausage formulations gave the high value of TPC. Likewise, Maizura *et al*. (2011) reported that the mixture of *P. minus* (family: polygonaceae) and *C. longa* (turmeric) extracts showed significantly increased ($p<0.05$) in TPC with value of 1.03 mg GAE/g EW compared to the single turmeric extracts. Up to date, there is no available data on phenolic content of MDCM sausage.

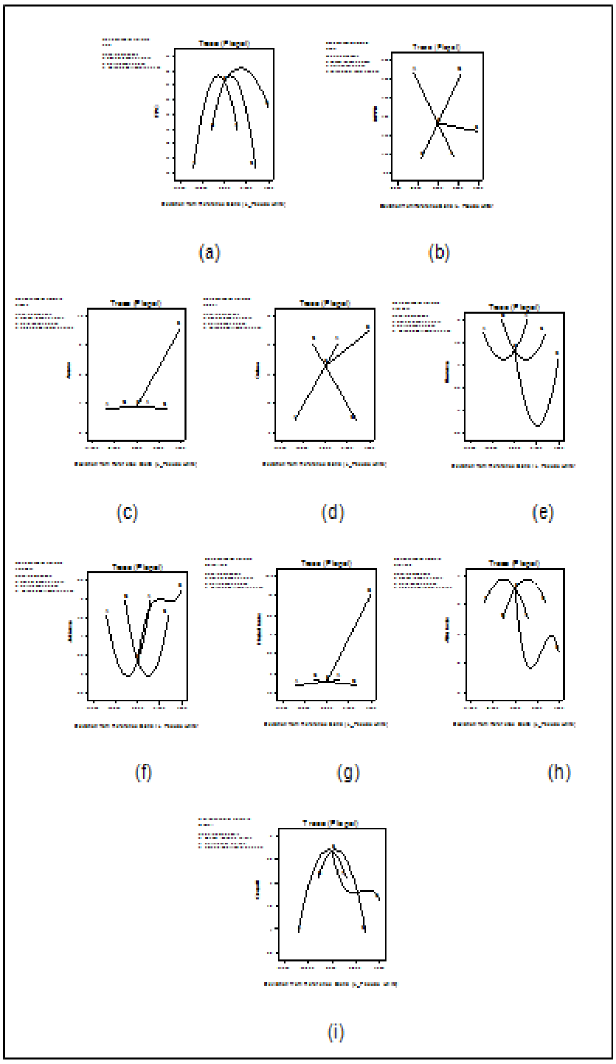


Figure 1. Trace Plot of (a) Total Phenolic Content, (b) EC_{50} Value, (c) Aroma, (d) Colour, (e) Firmness, (f) Juiciness, (g) Herbal Taste, (h) After Taste (metallic taste) Attributes and (i) Overall Acceptability of MDCM Aromatic Herbal Sausage

Note: A: *P. hydropiper*; B: *M. koenigii*; C: *E. elatior*.

Based on the trace plot, the scavenging activity of samples was influenced by the combination of herbs. However, the formulations incorporated with single herb of *E. eliator* showed the lowest of scavenging activity. Additionally, the MDCM sausage formulations incorporated with two or three dried aromatic Malaysian herbs exhibited the highest DPPH radical scavenging activity compared to the others. In comparison with the TPC values of thirteen formulations, the formulation with the highest TPC value exhibited the highest scavenging activity.

Several studies had been conducted to determine the correlation between phenolic compounds and antioxidant activity. As reported by Nurain *et al.* (2013), the TPC of herbal extracts had positive correlations with the scavenging activity. In addition, the *P. hydropiper* and *M. koenigii* extracts exhibited high antioxidant activity as well as ascorbic acid and BHA/BHT combination standards, followed by *E. elatior* extracts using DPPH radical scavenging activity (Nurain *et al*., 2013). Apart from that, this study also suggests that there may be synergistic effects of phenolic compounds detected in the herbs to scavenge free radical. This is supported by Maizura *et al*. (2011) who found that mixture of *P. minus* (family: polygonaceae) and *C. longa* (turmeric) showed a higher ($p<0.05$) DPPH radical scavenging activity when compared to the single turmeric extract. Furthermore, Fuhrman *et al*. (2000) suggested that the natural antioxidants in plants and combination with other antioxidants may have synergistic effect which is defined as an effect that is greater than individual or sum of the combination. Other studies have also been carried out to determine the synergistic effect of antioxidants (Liu *et al*., 2008; Romano *et al*., 2009). Therefore, the high antioxidant activity of herbal extracts was also reflected in MDCM sausages incorporated with these dried aromatic Malaysian herbs.

Furthermore, the aroma increased as the amount of *M. koenigii* increased. The high score (dark colour) of MDCM sausages was also influenced by increased amount of *M. koenigii*. In addition, *P. hydropiper* and *E. elatior* singly, or combination of them exhibited good firmness of MDCM sausages, while the juiciness was influenced by amount of the three aromatic Malaysian herbs. Furthermore, the trace plot shows that the single *M. koenigii* gave the strong herbal taste, however the combination of dried aromatic Malaysian herbs gave the moderate score of herbal taste. Besides, the amount of *P. hydropiper* and *E. elatior i*nfluenced the low score of after taste (metallic taste). The low score of after taste (metallic taste), gave the good sensory. In addition, the trace plot shows that the acceptability is at maximum when the amount of *M. koenigii* reduced. This implies that the highest score of colour and herbal taste of formulations 1 and 2 which incorporated with 100% of *M. koenigii* may contribute to the lowest score of their overall acceptability.

Model fitting

Modelling was necessary for each response to analyse the interaction effect on each dried herbal mixture (Yoon *et al*., 1997). In the present study, the maximum values of TPC and overall acceptability, while the minimum values of EC_{50} were set as goals. The TPC, EC_{50} value and sensory data were subjected to mixture design analysis. Only significant attributes with correlation coefficient (R^2) values of $\geq$ 0.85 were included in the optimisation model (Aminah and Cheng, 2001). Results of the predicted model equation, regression coefficients and probability values are presented in Table 2. The effects of the three independent variables: dried *P. hydropiper* (X_1), dried *M. koenigii* (X_2) and dried *E. elatior* (X_3) on the TPC, EC_{50} value and sensory attributes of MDCM aromatic herbal sausages were determined.

Analysis of variance (ANOVA) was used to assess how well the model represented the data. The analysis of variance indicated the predictive models adequately represented the data for TPC, EC_{50} value and sensory attributes (aroma, colour, firmness, juiciness, herbal taste, after taste (metallic taste), overall acceptability) of MDCM aromatic herbal sausages. Both independent and dependent variables were fitted to quadratic, linear and special cubic models. The predicted regression coefficients showed that the quadratic model was appropriate for TPC and firmness. Linear model was appropriate for EC_{50} value, aroma, colour and herbal taste, meanwhile the special cubic model was appropriate for juiciness, after taste (metallic taste) and overall acceptability. To evaluate the goodness of fit of the model, F-value tests were conducted. The F-values for all responses were significant at the 95% confidence level ($p<0.05$). Lack of fit for all responses was greater than 0.1, indicating they were not significant, less error and the model is fit. Moreover, the R^2 for all responses were high for a response surface ($R^2 \geq 0.85$). Therefore, all responses in the present study were found to be best fitted.

Optimisation of MDCM aromatic herbal sausage formulation

According to the predictive models for each dependent variable in Table 2, three solutions of optimisation were carried out by generating contour plots for nine variables. The simplex-lattice mixture design was able to predict all possible proportions of dried aromatic Malaysian herbs in MDCM sausages which have TPC, antioxidative effect and similar sensorial attributes to the commercial product.

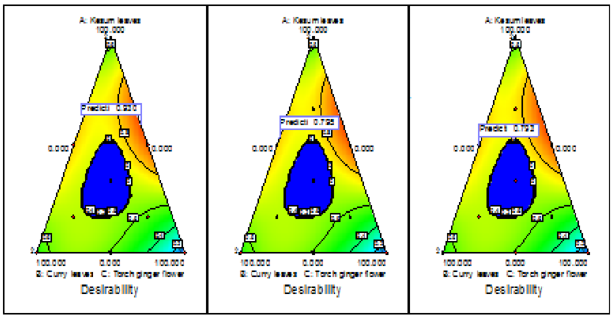
In order to determine the optimum formulation, the regions of acceptability in the three solutions of contour plot for each variable were superimposed. Superimposition of contour plot regions of interest resulted in optimum regions for MDCM aromatic herbal sausage formulation. The three solutions with one optimum values were obtained. The area of overlapping obtained is represented as the shaded region in MDCM aromatic herbal sausage formulations in Figure 2.

The shaded regions indicated that any point within this area represents a combination of three dried aromatic Malaysian herbs that would result in

Table 2. Predicted Model Equation, regression coefficients and probability values of MDCM Aromatic Herbal Sausage for TPC, EC_{50} and Sensory Attributes

Dependent Variable (Y_1)	Model	Predictive Model (Equation in terms of pseudo component)	[a] Prob>F (p<0.05)	[b] Lack of Fit	[c] R^2
TPC	Quadratic	$Y_1 = 15.85X_1 + 18.93X_2 + 10.69X_3 + 29.61X_1X_2 + 39.39X_1X_3 + 2.57X_2X_3$	0.0022	0.1818	0.8993
EC_{50} Value	Linear	$Y_1 = 96.68X_1 + 124.28X_2 + 185.88X_3$	0.0001	0.1566	0.9089
Aroma	Linear	$Y_1 = 6.90X_1 + 9.58X_2 + 6.85X_3$	0.0042	0.9929	0.8655
Colour	Linear	$Y_1 = 12.06X_1 + 12.49X_2 + 9.41X_3$	0.0004	0.1075	0.8873
Firmness	Quadratic	$Y_1 = 7.99X_1 + 7.15X_2 + 7.70X_3 - 6.51X_1X_2 - 2.84X_1X_3 - 7.43X_2X_3$	0.0414	0.1558	0.8545
Juiciness	Special cubic	$Y_1 = 7.94X_1 + 8.22X_2 + 7.57X_3 - 0.54X_1X_2 - 7.12X_1X_3 - 6.79X_2X_3 + 35.55X_1X_2X_3$	0.0458	0.3879	0.9169
Herbal Taste	Linear	$Y_1 = 7.83X_1 + 10.03X_2 + 7.69X_3$	0.0073	0.9156	0.9258
After Taste (metallic taste)	Special cubic	$Y_1 = 5.54X_1 + 4.40X_2 + 6.07X_3 + 5.43X_1X_2 + 4.25X_1X_3 + 5.40X_2X_3 - 98.10X_1X_2X_3$	0.0042	0.1526	0.9222
Overall Acceptability	Special cubic	$Y_1 = 8.10X_1 + 7.62X_2 + 6.93X_3 + 0.56X_1X_2 + 4.42X_1X_3 + 5.41X_2X_3 - 24.47X_1X_2X_3$	0.0453	0.1521	0.9175

Note: X_1: *P. hydropiper*; X_2: *M. koenigii*; X_3: *E. elatior*. [a] Prob>F: must be less than 0.05 for it to be significant, indicates the suitability to use the model and how many percent the results occur due to noise. [b] Lack of fit: Must be greater than 0.1 for it to be not significant. Higher lack of fit indicates less error and the model is fit. [c] R^2: Close to 1 means more significant.



(1) (2) (3)

Figure 2. Optimum Regions (Shaded Areas) Obtained by Superimposing Contour Plots for All Nine Variables of MDCM Aromatic Herbal Sausage Formulation
Note: (1) Solution 1; (2) Solution 2; (3) Solution 3.
A: *P. hydropiper* (kesum leaves); B: *M. koenigii* (curry leaves); C: *E. elatior* (torch ginger flower).

high TPC, low EC_{50} value and consumer acceptance for all sensory attributes. Furthermore, Table 3 shows predicted values for TPC, EC_{50} value and seven sensory attributes of three solutions of MDCM aromatic herbal sausage formulation obtained from the software.

From the three solutions of numerical optimisation results, the optimum MDCM aromatic herbal sausage formulation was determined as combination of 59.46% *P. hydropiper* and 40.54% *E. elatior* with the highest desirability (0.930). The predicted TPC and EC_{50} values for this optimum formulation were 23.25mg GAE/g EW and 132.84 mg/ml, respectively. Moreover the predicted mean score for aroma, colour, firmness, juiciness, herbal taste, after taste (metallic taste) and overall acceptability were 6.88, 10.99, 7.19, 6.07, 7.77, 6.78 and 8.7, respectively. However, another two solutions of formulation exhibited low desirability (0.795 and 0.793, respectively). The interaction between potato starch, locust bean gum and k-carageenan in low-fat sodium-reduced sausages and their effect on cooking yield, expressible moisture, texture and colour were optimised and determined using mixture design (Garcia-Garcia and Totosaus, 2008).

Model verification

In order to distinguish the efficiency of the generated formulations, the three optimum formulations were verified. The MDCM aromatic herbal sausage using these three optimisations of dried aromatic Malaysian herbs percentages were analysed, and the results were statistically compared to the predicted values of the mathematical model.

The comparisons were expressed in percent accuracy (%) and t-test values was used to determine the significant difference (p-value) between the predicted and actual values. Based on the verification

Table 3. Predicted Value for TPC, EC_{50} Value and Sensory Attributes from Optimisation of MDCM Aromatic Herbal Sausage Formulation

Criteria	Solution		
	1	2	3
Dried Aromatic Malaysian Herbs (%):			
P. hydropiper (kesum leaves) (X_1)	59.46	53.32	50.00
M. koenigii (curry leaves) (X_2)	0.00	46.68	50.00
E. elatior (torch ginger flower) (X_3)	40.54	0.00	0.00
TPC (mg GAE/g EW)	23.25	24.66	24.79
EC_{50} Value (mg/ml)	132.84	109.57	110.48
Sensory Attributes (mean score):			
Aroma	6.88	8.15	8.24
Colour	10.99	12.26	12.28
Firmness	7.19	5.98	5.95
Juiciness	6.07	7.94	7.95
Herbal Taste	7.77	8.85	8.93
After Taste (metallic taste)	6.78	6.36	6.33
Overall Acceptability	8.70	8.02	8.00
Desirability	0.930	0.795	0.793

analysis, the percent accuracy between predicted and actual values obtained for the three optimised sausage formulations were more than 86.7% for all variables. The t-test indicated that the predicted and actual values were not statistically different at the 95% confidence levels (p>0.05). Therefore, all the models obtained in the present study were experimentally checked as valid and the three solutions of percentage of dried aromatic Malaysian herbs were accepted for further study. However, the optimum MDCM aromatic herbal sausage formulation was determined as combination of 59.46% *P. hydropiper* and 40.54% *E. elatior* based on their high desirability.

Conclusion

In this study, simplex-lattice mixture design with regression modeling using Design Expert software (Stat-Ease, Inc., Version 8.0.1.0) was successfully used to optimise the best combination of dried aromatic Malaysian herbs for MDCM sausage. According to experimental results, trace plots and contour plots, an increase in *P. hydropiper* and *M. koenigii* gave the high TPC and antioxidative effects. However, the sample with *M. koenigii* did not exhibit high sensory scores. Therefore, a mixture with *E. elatior* should be used to improve sensory properties. From the optimisation, three solutions were obtained. The most suitable dried aromatic Malaysian herbs combination for MDCM sausage production with the highest desirability (0.93) consists of 59.46% of

P. hydropiper and 40.54% of *E. elatior* which produced 23.25 mg GAE/g EW of TPC, 132.84 mg/ml of EC_{50} value and moderate score for overall acceptability (8.70). The predicted response values and the actual obtained response values for the optimised products were more than 88% of accuracy and not significant at 95% confidence level, thus the three solutions of percentage of dried aromatic Malaysian herbs were accepted. This study showed the importance of dried aromatic Malaysian herbs addition in MDCM sausage which significantly affects the TPC, DPPH radical scavenging activity and sensory acceptability of the product.

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