Application of response surface methodology on sensory properties in the development of mushroom-based patties from grey oyster mushroom (*Pleurotus pulmonarius*)

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Abstract. Fast food especially meat patty has undeniably been a part of the culinary landscape across the globe. However, most of patties found in the market and fast food chains nowadays are made up with addition of fat and by-products which are considered unhealthy. Therefore, the objective of this study is to develop a healthy mushroom patty by optimizing the amount of mushroom and all-purpose flour in the formulation. Mushroom contains high protein and low fat content which can be an alternative for meat. Response Surface Methodology (RSM) was used to investigate the effects of different amount of mushroom and all-purpose flour on the consumer acceptance towards sensory properties such as appearance, aroma, taste, texture and overall acceptability by using a 7-point hedonic scale sensory test. The optimized conditions suggested by RSM were found to be 60% of mushroom with 17% of all-purpose flour. At the selected optimum conditions as given above, the highest preference of appearance (5.97), aroma (6.13), taste (6.07), texture (5.87) and overall acceptability (6.10) were obtained. The high sensory analysis scores for all sensory properties indicated the success of this study which will benefit the research on healthier meat alternative products and provide wide opportunities for new food developments in the future.

1. Introduction

Meat-based patty is well known as one of the most favoured processed meat products globally including Malaysia. Meat patty is originally made of ground beef which is also known as red meat. Red meat has been considered as a nutrient-rich food with nutrients such as protein, vitamins and also minerals such as zinc, iron and selenium. However, red meat has also been associated with health disease such as increased risk of colon cancer [1]. Today, most of the commercial patties found in the supermarkets and fast food chains are made of ground beef with additional fats and by-products such as animal’s muscles and leftovers. The commercially available patties have also undergone several stages during the production process, and thus known as processed meat products. Generally, processed meat products such as burgers, nuggets and sausages contain high calories, saturated fats [2] and sodium but low in dietary fibres [3]. High saturated fats found in processed food can increase cholesterol levels resulting in health problems such as hypertension and hypercholesterolemia [4]. Additionally, production of processed meat products has developed higher sensitivity compared to other food products in terms of food and nutrition security. In spite of the widely accepted processed meat products, it has always been debated and criticized by the health specialist due to the established adverse effects such as colon,
prostate and breast cancers, cardiovascular diseases and diabetes [5]. This has created a great challenge to the food industry in producing innovative products with high nutritional values and desirable sensory properties.

During the past years, there were several changes in consumer’s acceptance towards the food choices, giving a large impact to food manufacturers. Currently, the food industry is focusing on making new, creative, innovative and value-added products with high quality and functionality. Additionally, consumers today demand for healthy diets especially highly nutritious, organic and low calorie foods [6]. Therefore, this study aims to develop a healthy patty from grey oyster mushroom which could help to minimize the consumption of processed meat in daily diets. Mushroom is a potential meat alternative due to their high protein content and also low fat and sodium content. Besides, mushrooms also consist of fibres which offer several benefits to the human body. It also provides a meaty and umami flavour making them taste similar as meat [7]. Unbleached all-purpose flour was used as the second ingredient in the development of mushroom patty formulation. All-purpose flour was selected due to the starch content which is important to improve the food characteristics such as enhanced colour, specific volume, crumb structure and texture [8]. All-purpose flour also contains moderate protein content which can be used to give combination effect with mushroom in order to replace the protein from meat. There were various mushroom-based products formulated by previous researchers which focused on meat patty substitute with mushroom. In a study by [9], Agaricus bisporus mushroom was used in the formulation of beef burgers. In another study, meat patty had been formulated with shiitake mushroom due to its high vitamins, potassium and amino acid contents [10]. Also, white jelly mushroom was added as meat substitute in a meat patty formulation and the result showed that the white jelly mushroom provided high oil binding capacity, thus reduced the greasiness of the patty [11]. In this study, the patty was made up of grey oyster mushroom (Pleurotus pulmonarius) with the addition of all-purpose flour. This type of mushroom is low in sodium, fat and energy but high in dietary fibre, protein and carbohydrate [12]. In this study, response surface methodology (RSM) and central composite design (CCD) were used to optimize the amount of mushroom and all-purpose flour used in the formulation of mushroom patties. RSM is a powerful mathematical and statistical method that are beneficial for modelling and analysing problems in which the responses of interest are influenced by several different variables [13]. Optimization using RSM provides a number of advantages as compared to the traditional one factor at a time method (OFAT) in which it allows the description of interactive effects among the variables tested and help reduce the number of experiments needed [14]. RSM and CCD have been successfully applied in a number of food product developments research. One of the examples was the application of RSM in the development of barnyard millet bran incorporated bread which had successfully optimized the use of refined wheat flour and barnyard millet bran [15]. Therefore, the main objective of this study is to evaluate the effects of amount of mushroom and all-purpose flour on consumers’ acceptance using sensory analysis method on several sensory properties such as appearance, aroma, taste, texture and overall acceptability.

2. Materials and method

2.1. Raw materials preparation

Fresh grey oyster mushroom, all-purpose flour, black pepper, oil, garlic and onion were purchased from a local market in Padang Besar, Perlis. The grey oyster mushrooms were washed under running tap water and left for 15 minutes for the water to drain. The mushrooms were then ground to small sizes [±5 mm] using a food processor followed by drying in a lab dryer at 70°C for 1 hour.

2.2. Mushroom patties formulation

All of the ingredients such as dried mushroom, all-purpose flour, chopped onions (17%), minced garlic (6%) and flavoured black pepper (2%) were mixed together to formulate mushroom patties. After homogenous mixing of all ingredients, the batter was spread into thin sheets and round shape mould was used to shape the mushroom patties into small pieces. The raw mushroom patties were then frozen in a freezer at -18°C. In this research, the different combinations of mushroom and all-purpose flour were
developed based on RSM as explained in Section 2.4. Meanwhile, the percentage of other ingredients such as onion, garlic and flavoured black pepper were fixed.

2.3. Cooking procedure
Mushroom patties were thawed at 4°C for 12 hours. The patties were then cooked on a frying pan for 4 minutes on each side until an internal temperature of 74 ±1°C was achieved. The cooked patties were used for further analyses.

2.4. Experimental design and optimization
Formulation of mushroom patties were optimized based on three levels face centred-central composite design (CCD) with 13 different combinations carried out in random orders. This experimental design was generated using Design Expert 10.0.1 (STAT-EASE Inc. Minneapolis, USA). The different combinations were prepared by varying the percentage of grey oyster mushroom and all-purpose flour. The upper and lower limits for two independent variables were established after trial and error as shown in Table 1. The amount of mushrooms and all-purpose flour used in all runs were generated by Design Expert software with actual and coded variable levels as shown in Table 2. The mixtures for all 13 combinations were shaped into patties. The patties were then stored in the freezer before frying process for sensory analysis to evaluate the consumers’ acceptance towards the products.

Table 1. Formulation of mushroom patties.

| Factor | Parameter              | Unit | Low limit | High limit |
|--------|------------------------|------|-----------|------------|
| A      | Amount of mushroom     | %    | 60        | 80         |
| B      | Amount of all-purpose flour | % | 13        | 17         |

Table 2. Combination of mushroom patty formulations with actual and coded variable levels for experimental design.

| Run | Actual and coded levels          |
|-----|----------------------------------|
|     | Amount of mushroom (%) | Amount of all-purpose flour (%) |
| 1   | 70 (0)                          | 17 (1)                          |
| 2   | 80 (1)                          | 13 (-1)                         |
| 3   | 70 (0)                          | 15 (0)                          |
| 4   | 60 (-1)                         | 15 (0)                          |
| 5   | 60 (-1)                         | 17 (1)                          |
| 6   | 70 (0)                          | 15 (0)                          |
| 7   | 70 (0)                          | 15 (0)                          |
| 8   | 70 (0)                          | 15 (0)                          |
| 9   | 70 (0)                          | 13 (-1)                         |
| 10  | 70 (0)                          | 15 (0)                          |
| 11  | 80 (1)                          | 17 (1)                          |
| 12  | 80 (1)                          | 15 (0)                          |
| 13  | 60 (-1)                         | 13 (-1)                         |
2.5. Sensory Evaluation
A total of 30 untrained sensory panels were recruited. The panels were instructed to evaluate the mushroom patty based on the aroma, colour, taste, texture and overall acceptability. A 7-point hedonic scale was used to evaluate all the samples. Six samples were evaluated on the first day and another seven samples on the second day with the same sensory panels.

2.6. Statistical analysis
Data obtained for sensory analysis were analysed using Design Expert 10.0.1. A 2-factor and 3-level face-centred CCD experimental design with five centre points was used to investigate the effect of independent variables on the responses. The percentage of mushroom (X1) and percentage of all-purpose flour (X2) were the independent variables while the sensory properties such as appearance, aroma, texture, taste and overall acceptability were the responses measured in this study. In order to quantitatively define the ranges for independent variables, preliminary experiments were carried out to obtain more effective ranges for X1 and X2 prior to the experimental runs. After obtaining the design value ranges, the upper and lower limits for two independent variables were established as shown in Table 1. The two variables (amount of mushroom and all-purpose flour), levels and also the experimental design in terms of actual and coded levels are as shown in Table 1. The experiments were carried out in random order to optimize the effect of unexplained variability in the responses caused by extraneous factors. The five replicates at the centre were used to allow for pure error sum of square and lack-of-fit estimation. The significance of the results obtained was analysed using analysis of variance (ANOVA) followed by regression analysis and lack-of-fit-test. Then, the response surfaces in 3-dimensional form were analysed to understand the interaction effects between two variables on all responses.

3. Results and discussion
3.1. Model fitting and analysis of variance
This study was conducted to develop a healthy mushroom patty and to optimize the process variables (% of mushroom and all-purpose flour) using RSM and face-centered CCD. The mean response values for sensory analysis are presented in Table 3.

Table 3. Mean sensory analysis data for the sensory evaluation of mushroom patties

| Std | Run | Mushroom content (%) | All-purpose flour content (%) | Appearance | Aroma | Taste | Texture | Overall acceptability |
|-----|-----|----------------------|-------------------------------|------------|-------|-------|---------|----------------------|
| 8   | 1   | 70                   | 17                            | 5.33       | 5.23  | 5.23  | 5.20    | 5.37                |
| 2   | 2   | 80                   | 13                            | 5.03       | 5.03  | 4.47  | 4.80    | 4.80                |
| 9   | 3   | 70                   | 15                            | 5.47       | 5.33  | 5.40  | 5.33    | 5.53                |
| 5   | 4   | 60                   | 15                            | 5.47       | 5.33  | 5.07  | 5.27    | 5.23                |
| 3   | 5   | 60                   | 17                            | 5.50       | 5.67  | 5.50  | 5.20    | 5.53                |
| 10  | 6   | 70                   | 15                            | 5.33       | 5.03  | 5.47  | 5.47    | 5.33                |
| 11  | 7   | 70                   | 15                            | 5.47       | 5.23  | 4.70  | 5.27    | 5.03                |
| 12  | 8   | 70                   | 15                            | 5.50       | 5.23  | 5.20  | 5.30    | 5.33                |
| 7   | 9   | 70                   | 13                            | 5.47       | 5.07  | 4.77  | 5.30    | 5.03                |
| 13  | 10  | 70                   | 15                            | 5.33       | 5.33  | 5.30  | 5.17    | 5.03                |
| 4   | 11  | 80                   | 17                            | 5.23       | 5.10  | 4.80  | 4.83    | 5.37                |
| 6   | 12  | 80                   | 15                            | 5.23       | 5.33  | 5.03  | 5.07    | 5.00                |
| 1   | 13  | 60                   | 13                            | 5.33       | 5.20  | 5.40  | 5.57    | 5.53                |
Based on the results in Table 3, several analyses were conducted. The independent variables and responses were fitted to the model equation and the goodness of fit was examined using ANOVA, the coefficient of determination ($R^2$), lack of fit test, and also analysis of residual. The final equations for the predictive models in terms of coded factors for sensory properties such as appearance, aroma, taste, texture, and overall acceptability are presented in Equation (1) to (5), respectively. $X_1$ and $X_2$ refers to the contents (%) of mushroom and all-purpose flour, respectively.

Appearance = +5.36 – 0.13 * $X_1$ + 0.039 * $X_2$  

Aroma = +5.24 – 0.12 * $X_1$ + 0.12 * $X_2$ – 0.100 * $X_1 X_2$  

Taste = +5.10 – 0.28 * $X_1$ + 0.15 * $X_2$  

Texture = +5.21 – 0.22 * $X_1$ – 0.072 * $X_2$  

Overall acceptability = +5.24 – 0.19 * $X_1$ + 0.15 * $X_2$  

There are a few types of models in experimental design: mean, linear, 2FI (factor interaction), quadratic, and cubic model. Based on the results obtained on sensory evaluation, it was found that the data fit the linear and 2FI (factor interaction) model compared to other models. The ANOVA result used to evaluate the significance of the model are presented in Table 4. The analysis started with the Fischer’s F-test. In this study, the ANOVA results indicated that the linear model was significant for almost all sensory properties including appearance, taste, texture and overall acceptability since the p-values obtained (0.0279, 0.0476, 0.0128 and 0.0288, respectively) were less than 0.05. Meanwhile, the 2FI model was found to be significant for aroma with p-value of 0.0353.

The coefficient of determination ($R^2$) results for all sensory properties ranged between 45.6 to 59.7%, which were generally acceptable. The $R^2$ is used to analyse how difference in a variable can be explained by a difference in the second variable and also to measure the degree of fitness of a regression model [16]. In other words, it measures how close the data were to the fitted regression line. $R^2$ is measured in the range of 0 to 100%. In pure science field, a high $R^2$ of above 60% is required since the behaviour of molecules or particles can be predicted with high accuracy. However, $R^2$ below than that is generally accepted for research related to humans, because human behaviour is difficult to predict [17]. Additionally, the low $R^2$ value was found to be common on evaluation of food appearance in several previous research due to the inconsistent analysis by consumers [18].

Next, lack of fit test that indicated the significance of the replicate errors in comparison with model dependence was conducted since there were repeated measurements during data collection. Based on ANOVA results, the lack of fit F-values for appearance, aroma, taste, texture, and overall acceptability were found to be 0.2336, 0.4771, 0.7168, 0.1833 and 0.7669, respectively which indicated lack of fit values were not significant relative to pure errors. A significant test showed that there could be contributions in the regression response relationship that were accounted for by the fitted models [19]. Therefore, the non-significant values are desirable.
Table 4. ANOVA table for sensory analysis.

| Source of variance | Appearance | Aroma | Taste | Texture | Overall acceptability |
|--------------------|------------|-------|-------|---------|-----------------------|
| Model              | 0.0279     | 0.0353| 0.0476| 0.0128  | 0.0288                |
| R²                 | 0.5113     | 0.5974| 0.4560| 0.5815  | 0.5543                |
| Linear             |            |       |       |         |                       |
| A                  | 0.0111     | NR    | 0.0289| 0.0053  | 0.0306                |
| B                  | 0.3865     | NR    | 0.2001| 0.2761  | 0.0735                |
| 2FI                |            |       |       |         |                       |
| A                  | NR         | 0.0412| NR    | NR      | NR                   |
| B                  | NR         | 0.0492| NR    | NR      | NR                   |
| AB                 | NR         | 0.1463| NR    | NR      | NR                   |
| Lack of fit        | 0.2336     | 0.4771| 0.7168| 0.1833  | 0.7669                |

*Not related.

3.2. Effect of selected variables on sensory properties

The effect of different combinations of mushrooms and all-purpose flour content (%) on sensory properties is presented in Table 4. The mean values for appearance, aroma, taste, texture, and overall acceptability ranged from 5.03 to 5.50, 5.03 to 5.57, 4.80 to 5.57 and 4.80 to 5.53, respectively. The three-dimensional surface plots for some of the effects on sensory properties are shown in Figure 1 to 5.

Figure 1 shows the interaction of both variables on appearance. Based on the figure, it can be seen that increasing the amount of mushroom to 80% and decreasing the amount of all-purpose flour to 13% resulted in the low sensory acceptance for mushroom patty appearance with sensory score of 5.03. This is due to the soft patty texture obtained at high mushroom content. Fresh mushrooms were known to have high moisture content between 85 – 95% [20] which might contribute to the soft patty texture when its amount was increased. In a similar study by [21], the appearance of the patty at high amount of mushroom was found to be undesirable for the panels since the high moisture content of mushroom contributed to soft texture. Inversely, at lower amount of mushroom added (60%), and higher amount of all-purpose flour (15-17%), the sensory acceptance for appearance was found to increase with the score of 5.5. This was due to the functional properties of all-purpose flour that provide the basic structure to food products [22] which helped the patty to retain its shape.

Similar interactions can be found for both variables on all other sensory properties which were aroma, taste and overall acceptability as can be seen from Figures 2 to 5. For aroma, the highest score of 5.67 was obtained at 60% mushroom and 17% all-purpose flour (Figure 2). The highest acceptance on mushroom patty aroma at higher addition of all-purpose flour might be due to the aroma from the crusts formation. All-purpose flour exhibited water absorption ability which caused the formation of crusts that contributed to the aroma of the patty. Vice versa, the low acceptance on patty aroma at higher amount of mushroom was observed. As the amount of mushroom increased, the moisture content also increased, causing the aroma to be involatile [23]. The sensory acceptance for taste was observed to have a similar trend due to the relationship between these two sensory properties. The highest score of 5.5 for taste was observed at 60-65% mushroom content and 15-17% all-purpose flour content (Figure 3). According to [24], it was reported that the combination of aroma and taste induced greater satiation and increased the perceived taste intensity.
Analysis on texture had shown a different trend compared to the other sensory properties as shown in Figure 4. As the amount of both mushroom and all-purpose flour were decreased, the texture was most preferred by food sensory panels. This might be due to the chewy texture obtained at a lower mushroom content. Theoretically, an acceptable biting properties and meaty texture were obtained at lower amount of mushrooms and it can be related to the juiciness provided by the mushroom itself [25]. Lower amount of mushroom and higher amount of all-purpose flour obtained the highest score on overall acceptability based on Figure 5. A high amount of all-purpose flour provided structure, strength and aroma to the patties making them generally acceptable by consumers [26]. When the amount of mushroom was increased and the amount of flour was decreased, the overall acceptability achieved the lowest score because higher amount of mushroom contributed to high moisture content, and causing the loss on the patty texture [21] and also resulted in unexciting appearance, aroma, and taste.

Figure 1. Interaction between variables for appearance.

Figure 2. Interaction between variables for aroma.

Figure 3. Interaction between variables for taste.

Figure 4. Interaction between variables for texture.
3.3. Optimization
The conditions for the chosen parameters were successfully optimized. The results obtained were 60% and 17% for the amount of mushroom and all-purpose flour, respectively. The sensory attributes result was also predicted and is shown in Table 5. An optimum condition validation test was done in order to compare the value predicted by the software. The percentage error was calculated to determine the reliability of this study and is presented in Table 6. Based on previous study, the percentage errors between the range of 4.88% to 27.29% was considered valid. Thus it can be concluded that optimization achieved in the present study were reliable as the percentage error obtained from this study was within range [27].

Table 5. Predicted and observed value of sensory attributes.

| Sensory attributes       | Observed value | Predicted value |
|-------------------------|----------------|-----------------|
| Appearance              | 5.97           | 5.53            |
| Aroma                   | 6.13           | 5.58            |
| Taste                   | 6.07           | 5.53            |
| Texture                 | 5.87           | 5.36            |
| Overall acceptability   | 6.10           | 5.58            |

Table 6. Percentage error calculated between predicted and observed value.

| Run | Parameter | Errora (%) |
|-----|-----------|------------|
|     | X1 (%)    | X2 (%)     | Appearance | Aroma | Taste | Texture | Overall acceptability |
| 1   | 60        | 17         | 7.81       | 9.92  | 9.10  | 9.39    | 9.31                |

a Error = \(((X_{NO})_{exp} - (X_{NO})_{DOE})/(X_{NO})_{DOE}) \times 100
4. Conclusion

The formulation of grey mushroom patties had been successfully developed and optimized with the addition of all-purpose flour using RSM. Generally, it was found that by decreasing the amount of mushroom from 80 to 60% and increasing the amount of all-purpose flour from 15 to 17% resulted in higher consumer acceptance for all of the sensory properties such as appearance, aroma, taste, texture, and overall acceptability. The optimized conditions based on the Design of Expert (DoE) were 60% of mushroom and 17% of all-purpose flour. At this optimized condition, the score of appearance (5.97), aroma (6.13), taste (6.07), texture (5.87) and overall acceptability (6.10) had increased compared to the predicted score. From the result, it can be concluded that the formulations of mushroom patties were successful due to the improvement in all sensory scores which indicated increased consumers’ acceptance towards the products. Mushroom patties can be considered as a healthy fast food product compared to the commercially available meat patties due to the healthier ingredients used. Further studies should be conducted to determine the nutritional compositions and possible addition of ingredients such as protein isolates to improve the meaty texture of mushroom patties.

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