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Determining the Minimum Contamination Percentage of Chicken Plasma Protein in Surimi-Based Products using Response Surface Methodology

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ABSTRACT

The purpose of this research is to determine the minimum amount of plasma that could be added into surimi during the production without reducing its protein solubility, cohesiveness, and whiteness of surimi using response surface methodology (RSM) using MINITAB software version 19. The percentage of chicken plasma protein (CPP) range from 0.5 to 2.5%, (X_1, w/w) and percentage of sorbitol range from 2 to 6%, (X_2, w/w) were investigated as parameters that influence the dependent factors that were included in this analysis were protein solubility (Y_1), cohesiveness (Y_2), and whiteness (Y_3). The results shows that optimal conditions for protein solubility, cohesiveness, and whiteness of surimi were attained at a CPP concentration of 0.79% and a sorbitol concentration of 4.68%, with the predicted protein solubility around 49.09 mg/ml, cohesiveness at 0.65 and whiteness value of 75.58. The optimal protein solubility, cohesiveness, and whiteness were determined to be 46.56 mg/ml, 0.65, and 75.55 correspondingly. As there was no statistically significant difference ($p>0.05$) between the predicted and measured values for protein solubility, cohesiveness, and whiteness, the optimal conditions predicted by RSM can be accepted. The result presented here is useful for analysis laboratory to set lowest detection limit when detecting blood plasma in surimi-based products.

Keywords: Contaminant, Cryoprotectant, Gelling agent, Plasma, RSM, Sorbitol, Surimi

1 Introduction

Surimi was first introduced in Japan and nowadays surimi is well-known all around the world through its based products such as crab meat, fish balls as well as fish cake. In the production of surimi, several types of fish that are commonly used as surimi raw material includes Pacific whiting, Alaska pollock, threadfin bream etc. According to [1], surimi is recovered from mechanically deboned fish flesh that passed through several processes which are mincing, washing, mixing with cryoprotectant and freezing. [2] stated that the major determinants for quality of surimi and its related products are gel properties of surimi which included its texture and gel-forming ability. Hence, food additives such as Bovine Protein Plasma (BPP), Pig Protein Plasma (PPP), Chicken Plasma Protein (CPP), Egg White (EW) and potato powder

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are often used in surimi production as gelling agent [3-4]. The addition of protein additive has been long practiced since 1991 with utilization of beef plasma protein and porcine plasma protein in Alaskan pollack surimi [5]. The incorporation of plasma protein will help in enhanced the gelling properties of the surimi. Among the functional properties of the plasma protein are the capacity to form a gel on heating and emulsifying properties which are important in food processing [6-7]. The development of surimi derived from blood plasma protein of chicken aims to avoid the cost of blood disposal or treatment from slaughtering process [8-10]. Response Surface Methodology (RSM) is one of optimisation tools used to optimise formulation in a variety of food products such as modification of chicken gelatin [11]. However, in this research we use RSM to determine the lowest plasma contaminants that could be presence in surimi. The utilization of waste from slaughtering industry can be sold at a cheaper price to the consumer is not acceptable for halal food.

2 Materials and Methods

2.1 Materials

The fish paste was obtained from a local market in Selangor, Malaysia and kept at -20 ℃ until used. CPP was collected at a local slaughterhouse (Selangor, Malaysia). Sorbitol, sucrose, sodium tripolyphosphate, and salt (NaCl) all in food grade were commercially available. The chemicals used were Tris-HCl, Sodium dodecyl sulfate, β-mercaptoethanol, urea, trichloroacetic acid (TCA), sodium hydroxide, bovine serum albumin (BSA) and biuret reagent. All chemical reagents which were used for analysis are of analytical grade.

2.1.1 Extraction of CPP and preparation of CPP powder

Blood was collected directly from slaughtered chickens into a vacuum blood collection tube containing 3.2% sodium citrate (Vacutube) with a 9:1 ratio of blood to anticoagulant and vigorous shaking to prevent blood clotting. During collection and transport, the blood was kept at a temperature of 4 ℃ in ice box. The blood was then centrifuged for 20 minutes at 2,300 g (Kubota, Japan) to remove cells and cellular debris. The blood plasma supernatant was collected in a sterile glass bottle and frozen at -35 ℃ for 24 hours. The frozen plasma is then freeze-dried until a constant weight is attained using a freeze dryer (ALPHA 11-4 LD plus, Christ, Germany) at -47 ℃ and 0.133 bar. The powdered chicken plasma protein was kept at 4 ℃ until further usage.

2.1.2 Surimi gel preparation

The surimi was prepared in accordance with the method [12], with a slight modification, the fish paste was thawed for 12 hours at a chilled temperature (4 ℃) before being minced and mixed with the CPP, sorbitol, NaCl (2%, w/w), sucrose (4%, w/w), and sodium tripolyphosphate (0.3%). Crushed ice was used to regulate the moisture level to between 80-85% in order to produce a uniform slurry [7]. The surimi paste was put into a 2cm×2cm×2cm

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ice cube mould and properly wrapped before being frozen at -18 °C for at least 12 hours. Prior to examination, the surimi cubes were boiled at 90 °C for 20 minutes and cooled to room temperature prior to experiment [13].

2.2 Experimental design for Response Surface Methodology (RSM)

The RSM designed the experiment findings of two factors, five levels, single block with upper and lower levels. The factorial design was created using central composite design (CCD) in Minitab ver. 19. CCD conducted 13 experiments with two independent variables, the percentage of CPP and sorbitol, at five levels ($-\alpha$ -1, 0, 1, and $+\alpha$) (Table 1). The following is the generalized second-order polynomial model used in the analysis.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \varepsilon \quad (1)$$

where Y expressed the predicted response variables, β_0 is the constant value of response variables at the central point of the model, β_1 known as the linear coefficient and β_2 is the quadratic coefficient for the main factor, meanwhile, β_{12} is the second-order interaction coefficient. The response variable was set at 0 and 1 respectively, which is low and high to observe overall desirability values.

Table 1: *The coded and uncoded values used in optimisation of surimi production.*

Independent variable	Code	Factor Level				
		-α	-1	0	1	α
Percentage of CPP	X_1	0.5	1.0	1.5	2.0	2.5
Percentage of sorbitol	X_2	2	3	4	5	6

2.3 Analysis of protein solubility

Prior to analysis, the surimi was thawed for 24 hours until its core temperature reached 4 °C before being boiled at 90 °C for 20 minutes. After draining the surimi, it was minced in a food processor for 5 minutes. Protein extraction and analysis according to method by [10] with a few adjustments. Instead of 8 M urea, 6 M urea was used in this study.

2.4 Textural properties analysis (cohesiveness)

After defrosting for 12 hours, the surimi was heated for 20 minutes at 90 °C and cooled before analysis. The samples' cohesiveness was measured using the TA.XT. T2i (Stable Micro System, United Kingdom) texture analyser's compression platen (P/75). The return speed was 10 mm/sec and the compression was measured to 50% strain with contact force was 10 g [14]. Each was measured in triplicate.

2.5 Colour analysis (Whiteness)

The colour of cooked surimi samples evaluated in a dimly light room, using Chromameter (CR400, Konica Minolta, Japan). A Chroma meter measured L^*, a^*, and b^* of the sample as it was wrapped in clear plastic. The chromaticity diagram determined the sample's brightness and hue. Each was measured in triplicate. This formula calculated whiteness index. [15]

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$$\text{Whiteness} = L^*-3b^* \quad (2)$$

2.6 Statistical analysis

The model's validity and fitness were evaluated using an ANOVA to determine the lack of fit and optimization's impact on surimi response variables. The regression coefficient (R^2) and probability value (p-value) are also statistical indicators of the response variable polynomial. Table 2 compares experimental response verification values to predicted values. The data were analysed using the t-test method using SPSS 26th version. The statistically calculated p-value at 95.0% confidence level indicated a significant difference at $p<0.05$.

By employing multiple regression analysis, the empirical relationship between the independent variables and the response variables can be described in terms of uncoded values by the following quadratic, second-order polynomial equations:

$$Y_1 = -11.2 + 6.3X_1 + 22.61X_2 + 19.25X_1^2 - 0.163X_2^2 - 15.89\ X_1X_2 \quad (3)$$

$$Y_2 = 0.8580 - 0.1089X_1 - 0.0744X_2 + 0.1327X_1^2 + 0.01863X_2^2 - 0.0702X_1X_2 \quad (4)$$

$$Y_3 = 85.41 - 2.54X_1 - 3.594X_2 - 3.804X_1^2 - 0.0510X_2^2 + 2.560X_1X_2 \quad (5)$$

where Y_1, protein solubility, Y_2, cohesiveness and Y_3, whiteness and X_1, percentage of CPP and X_2, percentage of sorbitol.

3 Results and discussions

3.1 Optimisation of surimi production and validation of models

The validity of the response variables Y_1, Y_2, and Y_3 was established by employing the predicted optimal conditions (X_1: 0.79% and X_2: 4.68%). The experimental findings of Y_1, Y_2, and Y_3 are presented in Table 2 with the following values: 46.56, 0.65, and 75.55. Comparatively, the model predicted results were 49.09, 0.654, and 75.58, respectively. Overall, 0.79% of CPP and 4.68% of sorbitol were found to produce surimi with acceptable protein solubility, cohesiveness and whiteness.

Table 2: *The difference values of experimental and predicted values of response variables under the optimal conditions.*

Independent variable:	**X_1: 0.79%, X_2: 4.68%**		
Responses	**Experimental value**	**Predicted value**	**p-value**
Protein solubility (mg/ml)	46.56[a]	49.09[a]	0.63
Cohesiveness	0.65[a]	0.65[a]	0.96
Whiteness value	75.55[a]	75.58[a]	0.88

Values with different superscripts within the same row are significantly different ($p<0.05$).

where Y_1, protein solubility, Y_2, cohesiveness and Y_3, whiteness and X_1, percentage of CPP and X_2, percentage of sorbitol.

4 Conclusions

Optimisation of protein solubility, cohesiveness and whiteness were obtained as surimi was incorporated with 0.79% of chicken plasma protein and 4.68% of sorbitol and shows that the

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quality is good at 0.79% plasma. Any intention to incorporate plasma can be done down to 0.79%. This minimum amount will help analysis laboratory to set lowest detection limit when detecting blood plasma in surimi-based products.

5 Declarations

5.1 Acknowledgments

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5.2 Competing Interests

The authors declare no conflict of interest.

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